METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS (MRSA) COLONIZATION AMONG INTENSIVE CARE UNIT (ICU) PATIENTS AND HEALTH CARE WORKERS AT MUHIMBILI NATIONAL HOSPITAL, DAR ES SALAAM, TANZANIA

By

Alfred Geofrey

A Dissertation Submitted in (partial) Fulfillment of Requirements for the Degree of Master of Science in Epidemiology and Laboratory Management of Muhimbili University of Health and Allied Sciences

Muhimbili University of Health and Allied Sciences
October, 2013

CERTIFICATION

The undersigned certify that they have read and hereby recommend for acceptance by Muhimbili University of Health and Allied Sciences a dissertation entitled **Methicillin-Resistant *Staphylococcus aureus* (MRSA) Colonization Among Intensive Care Unit (ICU) Patients and Health Care Workers at Muhimbili National Hospital, Dar es salaam, Tanzania,** in fulfillment of the requirements for the degree of Master of Science in Epidemiology and Laboratory Management of Muhimbili University of Health and Allied Sciences.

_____

Prof. S. Aboud (Supervisor)

Date: ……

_____

Ahmed Abade (Supervisor)

Date:……..

DECLARATION AND COPYRIGHT

I, **Alfred Geofrey,** declare that this **dissertation** is my own original work and that it has not been presented and will not be presented to any other university for a similar or any other degree award.

Signature…….……..Date…….....

This dissertation is a copyright material protected under the Berne Convention, the Copyright Act 1999 and other international and national enactments, in that behalf, on intellectual property. It may not be reproduced by any means, in full or in part, except for short extracts in fair dealing, for research or private study, critical scholarly review or discourse with an acknowledgement, without the written permission of the Directorate of Postgraduate Studies, on behalf of both the author and the Muhimbili University of Health and Allied Sciences.

DEDICATION

I would like to thank my beloved wife, **Zawadi Polla** for encouragement and advice during the entire study period. I really appreciate the guidance and blessings from my father **Geofrey Mwanyika** and my mother **Rose Kilumile**

ABSTRACT

Background: Methicillin-resistant *Staphylococcus aureus* (MRSA) has been recognized as important nosocomial pathogen worldwide. MRSA may induce clinically manifested diseases, or the host may remain completely asymptomatic.

Objective: To determine the magnitude of MRSA colonization among ICU patients and health care workers at Muhimbili National Hospital (MNH) in Dar es Salaam, Tanzania.

Methodology: A cross-sectional hospital-based study was conducted from October 2012 to March 2013 in the two ICUs at MNH. Admitted patients and health care workers were enrolled in the study. Interviewer administered questionnaires, patient history forms, observation charts and case report forms were used to collect data. Swabs (nostrils, axillary or wounds) were collected. MRSA were screened and confirmed using cefoxitin, oxacillin discs and oxacillin screen agar. Antibiotic susceptibility was performed using Kirby-Bauer disk diffusion method. The risk factors for MRSA were determined using the logistic regression analysis and a p - value of <0.05 was considered as statistically significant.

Results: Of the 169 patients and 47 health workers who were recruited, the mean age was 43.4 years SD ± 15.3 and 37.7 years SD ± 11.44 respectively. Among the patients males contributed 108 (63.9%) while in health care worker majority 39(83%) were females. The prevalence of MRSA colonization among patients and health care workers was 11.83% and 2.1% respectively. All (21) MRSA isolates were highly resistant to penicillin and erythromycin, and 18 (85.7%) were highly sensitive to vancomycin. Being male (AOR 6.74, 95% CI 1.31-34.76), history of sickness in past year (AOR 4.89, 95% CI 1.82- 13.12), being sick for more than 3 times in the past year (AOR 8.91, 95% CI 2.32-34.20), being diabetic (AOR 4.87, 95% CI 1.55-15.36) and illegal drug use (AOR 10.18, 95%CI 1.36-76.52) were found to be independently associated with MRSA colonization.

Conclusions: A study identified a high prevalence of MRSA colonization among patients admitted in the ICU. MRSA isolates were highly resistant to penicillin and erythromycin. History of illegal drug use was highly associated with MRSA colonization.

Recommendations: Patients and health care workers should be screened prior to ICU admission and routinely, respectively, to identify individuals carrying MRSA. MRSA

screening should prioritize groups at risk for MRSA colonization. Since MRSA show high resistance to commonly used antimicrobials, antibiotic susceptibility testing is recommended for effective treatment.

ACKNOWLEDGEMENTS

I would like to acknowledge the supervisors, Prof. Said Aboud of Muhimbili University of Health and Allied Sciences (MUHAS) and Ahmed Abade, Laboratory advisor at Tanzania Field Epidemiology and Laboratory Training Programme (TFELTP) for guidance and supervision from conception of research ideas to completion of the dissertation write up. I am grateful to the administration of Muhimbili National Hospital for granting me permission to conduct this study. Indeed I am grateful to the TFELTP under the Ministry of Health and Social Welfare for fully funding this dissertation work. Finally, I appreciate the participation of study participants without whom this study would have not been possible.

TABLE OF CONTENTS

CERTIFICATION FOR EXAMINATION ii

DEDICATION ii

DECLARATION AND COPYRIGHT iii

ABSTRACT iv

ACKNOWLEDGEMENTS vii

TABLE OF CONTENTS viii

LIST OF TABLES AND FIGURES xi

LIST OF ABBREVIATIONS xii

CHAPTER 1: Introduction and Background 1

1.1 Background 1

1.2 Statement of a problem 2

1.3 Rationale and Justification of the study 3

1.4 Research questions 3

1.5 Objectives 3

1.5.1 Broad 3

1.5.2 Specific 4

CHAPTER 2: Literature review 5

2.1 Prevalence of MRSA globally 5

2.2 Prevalence of MRSA in Europe 5

2.3 MRSA in Sub-Saharan Africa 6

2.4 MRSA in East Africa 7

2.5 MRSA in Tanzania 7

CHAPTER 3: Methodology 8

3.1 Study design 8

3.2 Study setting 8

3.3 Study population 8

3.3.1 Inclusion criteria 8

3.3.2 Exclusion criteria 8

3.4 Sample size 8

3.5 Data collection 9

3.6 Laboratory investigations 9

3.6.1 Specimen collection 9

3.6. 2 *S. aureus* isolation 10

3.6.3 MRSA detection 10

3.6.4 Antimicrobial susceptibility testing 10

3.7 Data management and analysis 11

3.7.1 Data management 11

3.7.2 Data analysis 11

CHAPTER 4: Results 12

4.1 Socio-demographic and clinical characteristics of patients admitted at ICU 12

4.2 Socio-demographic characteristics of the health care workers at ICU. 13

4.3 Prevalence of MRSA colonization among patients by socio-demographic characteristics 14

4.4 Prevalence of MRSA colonization among patients by clinical characteristics 15

4.5 Prevalence of MRSA colonization among health care workers 15

4.6 Antimicrobial susceptibility patterns of MRSA isolates 16

4.7 Risk factors for MRSA colonization among patients 18

CHAPTER 5: Discussion 22

CHAPTER 6: Conclusions and Recommendations 24

6.1 Conclusions 24

6.2 Recommendations 24

CHAPTER 7: References 25

8.0 APPENDICES 31

8.1 Appendix 1 Informed consent form (English version) 31

8.2 Appendix 2 Informed consent form (Swahili version) 34

8.3 Appendix 3: Questionnaire 37

8.4 Appendix 4: Case Report Form (Laboratory Results) 40

LIST OF TABLES AND FIGURES

Table 1: Socio-demographic and clinical characteristics of patients (N=169) admitted at ICU, MNH, Nov 2012 - March 2013 (N=169)…..……...….12

Table 2: Socio-demographic characteristics of health care workers (N=47) at ICU, MNH…..13

Table 3: Prevalence of MRSA among patients (N=169) admitted at MNH ICU by socio-demographic characteristics (N=169)……..……..……14

Table 4: Prevalence of MRSA among patients (N=169) admitted at MNH ICU by clinical characteristics……15

Table 5: Multivariate analysis of risk factors (socio -demographic) of MRSA colonization among patients (N=169) admitted at MNH ICU, Nov. 2012 to March 2013……..……...18

Table 6: Multivariate analysis* of risk factors (clinical characteristic) among patients (N=169) admitted at MNH ICU, Nov. 2012 to March 2013 ……...……20

Figure 1: Antimicrobial susceptibility patterns of MRSA isolated from ICU patients and Health workers, MNH, Nov. 2012-Mar 2013 (n=21)……..…….…….16

Figure 2: MRSA resistance to all six antibiotics discs, MNH, Nov. 2012 – March 2013…...17

Figure 3: MRSA susceptibility to Oxacillin and Cefoxitin which used to detect them from Methicillin Susceptible Staphylococcus aureus (MSSA)……..……17

LIST OF ABBREVIATIONS

AOR	Adjusted odds ratio
BMC	Bugando Medical Centre
CA- MRSA	Community associated MRSA
CDC	Centre for disease control and prevention
CI	Confidence interval
COR	Crude odds ratio
HA- MRSA	Health associated MRSA
HIV	Human immunodeficiency virus
ICU	Intensive care unit
LA - MRSA	Livestock associated MRSA
MNH	Muhimbili National hospital
MRSA	Methicillin resistant *staphylococcus aureus*
MSSA	Methicillin susceptible *staphylococcus aureus*
MUHAS	Muhimbili university of health and allied sciences
NCCLS	National committee for clinical laboratory standards
NNIS	National nosocomial infections surveillance
PBP	Penicillin binding protein
SD	Standard deviation
SSTIs	Skin and soft tissue infection(s)

VRE	Vancomycin resistant Enterococci

CHAPTER 1: Introduction and Background

1.1 Background

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a type of *Staphylococcus aureus* species that is resistant to the antibacterial activity of methicillin and other related antibiotics of the penicillin class (Johnson A, 2007). *S aureus* is one of the principal human pathogens that colonize healthy individuals in the community in general, and it is responsible for severe infections such as surgical wound infections, bloodstream infections, and pneumonia in hospitalized patients (Silvana *et al*., 2005). MRSA was initially described in the 1960s and is now endemic in many hospitals and may account for up to 30% of the staphylococcal infections identified in hospital settings of the developed countries (Jones *et al*., 2007). Infection due to *S. aureus* also imposes a high and increasing burden on health care resources, as well as increasing morbidity and mortality (Chu *et al.*, 2005).

MRSA is often carried asymptomatically on the human body (Lee, 2003). The nostrils are the principal site of colonization by both *S. aureus* (80.4%) and MRSA (26.4%), followed by the perineal area, with rates of 27.6% and 12.6%, respectively (Cavalcanti *et al*., 2005). MRSA acquisition in the ICU is associated with reduced numbers of trained nurses and hygiene failures predominantly involving hand-touch sites (Dancer *et al*., 2006).

Highly virulent community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA) with Panton-Valentine leukocidin (PVL) is common worldwide (Weng-Tsung *et al*., 2006). PVL is present in the majority CA-MRSA isolates studied and is the cause of necrotic lesions involving the skin or mucosa, including necrotic hemorrhagic pneumonia(Kaneko *et al*., 2004). CA- MRSA first appeared in high-risk populations such as intravenous drug users, people in nursing homes and people who were chronically ill, but they are now reported even in healthy children (Tenover *et al*., 2009). Factors that facilitate the spread of these MRSA infections include crowding, frequent skin-to-skin contact between individuals, participation in activities that result in compromised skin surfaces, sharing of personal items that may become contaminated with wound drainage, and challenges in maintaining personal cleanliness and hygiene (CDC, 2003). These CA-MRSA infections have become an increasing problem in athletics as well as in the general population during recent years. CA-MRSA

outbreaks has been reported among club teams as well as interscholastic, intercollegiate, and professional teams in different sports around the world, including American football, fencing, soccer, rugby, and wrestling (Gantz *et al*., 2003; Bowers *at al*., 2008)

It is found that, the extra stay in hospital, together with extra days in intensive care, extra medical and nursing care and additional costly antibiotic treatment due to MRSA infections, lead to major cost implications and loss of health service resources in the unit (Watters *et al*., 2004).

Persons 65 years of age or older, women, persons with diabetes, and those who had been in long-term care in the past year were more likely to have MRSA colonization than younger, male, persons without diabetes and those who had been in short – term care in the past year (Graham *et al*., 2006). Antibiotic use within 3 months before admission, hospitalization during the past 12 months, diagnosis of skin or soft-tissue infection at admission and HIV infection have been found to be associated with MRSA colonization (Hidron *et al*., 2005).

Many patients that are not infected on admission may actually become colonized with MRSA while in the ICU. A previous study in a large surgical ICU cohort showed that 8% of patients were colonized with MRSA at the time of admission, and that these individuals could serve as a reservoir for the spread of this pathogen (Warren *et al*., 2006). These MRSA infections are generally seen in individuals who have ongoing interactions with the healthcare system for example dialysis patients and these infections may develop in these individuals as outpatients (Shorr AF, 2006). Critically, colonized healthcare workers may play an important role in cross transmission of MRSA among patients; hence routine screening for MRSA colonization should be reinforced to reduce the number of cross-transmissions (Hall *et al*., 2012).

1.2 Statement of a problem

MRSA is prevalent in many hospitals and often reflects the difficulties in hospitals and the health service generally, in terms of the control and prevention of healthcare-associated infection (Humphreys H, 2008). MRSA on average accounts for 57% of *S. aureus* isolates causing nosocomial infection in intensive care units (NNIS, 2003). There are evidences showing the existence of MRSA colonization and infections in Tanzania. A study conducted at Bugando Medical Centre found the prevalence of MRSA in the surgical wards to be 16.2%

(Mshana *et al*., 2009). Another study conducted at Muhimbili National Hospital (MNH) to assess the incidence of bloodstream infection and risk factors for fatal outcome of children showed that 12% of hospital isolated *S.aureus were* MRSA (Bloomberg *et al.*, 2007). However, there is limited data on existence of MRSA colonization among ICU patients who are at high risk of being colonized due to high interaction with health care workers during treatment procedures. Not only that but also there is limited data on MRSA colonization on ICU health care workers who can play a great role in MRSA transmission to patients and among themselves.

1.3 Rationale and Justification of the study

Patients getting colonized with MRSA either at admission to the hospital or during hospitalization increases the risk for subsequence MRSA infection (Kepler *et al.*, 2004). Since there is high interaction between patients and health care workers in the course of treatment in the ICU, the chance of transmission among them is high. Therefore knowledge on prevalence and risk factors for MRSA colonization is important for effective control and intervention of this problem in this hospital setting. Since MRSA isolates shows resistance to the most of commonly used antibiotics, information on antimicrobial susceptibility patterns is important in developing treatment guidelines for MRSA cases**.**

1.4 Research questions

1. What is the prevalence of MRSA colonization among ICU patients and health care workers at Muhimbili national hospital (MNH)?
2. What are the antimicrobial susceptibility patterns of MRSA isolates at MNH?
3. What are the risk factors associated with MRSA colonization among ICU patients and health care workers?

1.5 Objectives

1.5.1 Broad

1. To determine the magnitude of and risk factors for MRSA colonization among ICU patients and health care workers at MNH, in Dar es Salaam.

1.5.2 Specific

1. To characterize ICU patients and health care workers colonized with MRSA by socio-demographic characteristics.
2. To determine the prevalence of MRSA among ICU patients and health care workers at ICU.
3. To determine antimicrobial susceptibility patterns of MRSA isolated from patients admitted and health care workers at ICU.
4. To determine risk factors for colonization by MRSA among patients admitted at ICU and health workers at ICU

CHAPTER 2: Literature review

2.1 Prevalence of MRSA globally

Globally, MRSA has been a common cause of infection in hospital settings and it now accounts for more than 50% of staphylococcal infections in the community making its existence more important than ever (Kleven e*t al*., 2007). MRSA is not only present but also growing throughout the world with prevalence ranging from 23.3% to 73% (Kleven *at al*., 2007). An international multicenter study of antimicrobial resistance and typing of hospital *S. aureus* isolates from 21 laboratories in 19 countries or states showed that South Africa and Malaysia had the highest rates of MRSA (Zinn *et al*., 2004). A review of recent findings on the global epidemiology of healthcare-acquired/associated (HA), community-acquired/associated (CA) and livestock-associated (LA) MRSA revealed that rates continued to increase rapidly in many regions and there is a dynamic spread of strains across the globe (Stefani *et al*., 2012). HA-MRSA is currently endemic in hospitals in most regions while CA-MRSA clones have been spreading rapidly in the community and also infiltrating healthcare in many regions worldwide (Stefani *et al*., 2012).

2.2 Prevalence of MRSA in Europe

The prevalence of MRSA among patients in the European health settings was reported to be 1% to 24% (Dulon *et al*., 2011). The sentry program, a study which collected 15,439 *S. aureus* isolates from all over the world from 1997-1999, showed that among the regions under investigation, Europe was the region with the most variation of MRSA magnitude (Stefani *et al*., 2003). Apart from demonstrating an increased rate from 12.8% in the early 1990s, that study also showed that MRSA rates were highest in countries from southern Europe such as Greece, Italy, Portugal, and Turkey, although Spain did not follow this trend (Stefani *et al*., 2003). The frequency of MRSA infections continues to grow in hospital-associated settings and more recently in community settings in the United States and worldwide. For CA-MRSA, person-to-person transmission has been reported, and several factors such as children less 2 years old, injection drug users and athletes have been shown to predict disease (Boucher *et al.*, 2008). MRSA still causes significant morbidity and mortality despite huge advances in

medical care in developed countries. Indeed, infections due to *S.aureus* continue to grow in number and complexity as a consequence, ironically, of advances in patient care and of its ability to adapt to a changing environment (Fowler *et al.*, 2005).

A study conducted in Washington University School of Medicine to determine the prevalence and risk factor for MRSA nasal colonization found that 8% of patients were colonized with MRSA at the time of admission, and these individuals could serve as a reservoir for the spread of this pathogen (Warren *et al*., 2006).

Temporary contaminated hands of HCWs are important vectors for MRSA transmission and appropriate hand hygiene is considered the key intervention to minimize this transmission mode (Pittet *et al*., 2006). A report from the United States of America showed that health care workers served as a potential reservoir of MRSA for hospital patients and such MRSA carriers could lead to epidemic spread (Herwaldt *et al*., 2003).

The resistance to methicillin in staphylococci is mediated by the *mecA* gene that encodes a modified penicillin-binding protein (PBP), the PBP2a or 2' which shows reduced affinity to penicillins located at staphylococcal cassette chromosome *mec* (SCC*mec*) (Van Duijkeren *et al*., 2004). SCC*mec* is speculated to be transmissible among staphylococcal species as a mobile element; however, its origin/reservoir has not been clarified yet (Hanssen *et al*., 2006)

2.3 MRSA in Sub-Saharan Africa

The prevalence of MRSA isolates is high in sub-Saharan countries, ranging from 16.8% in CÔte d'Ivoire to 29.6% in Nigeria (Kesah *et al*., 2003). A study conducted in Gaborone to determine the epidemiology of *S aureus* skin and soft-tissue infections (SSTIs) in hospitalized children and adults showed an increase in proportion of MRSA SSTIs in Botswana between 2000 and 2003 and high levels of MRSA resistance to commonly used antibiotics were revealed (Truong *et al.*, 2011). A multinational surveillance project in eight Sub-Saharan African hospitals reported *S. aureus* colonization rate of 57.5% among food handlers in Gaborone, and 11.2% of bacteremia in this setting is caused by MRSA, with neither study stratifying results by HIV status as risk factors for MRSA colonization (Loeto *et al*., 2007).

2.4 MRSA in East Africa

A previous study conducted in Kenya to determine the prevalence of MRSA colonization and infection among patients attending various urban health facilities showed that 84.1 % of all *S. aureus* isolates were MRSA (Kithela ME, 2007). The analyses identified an increase in the proportion of MRSA for both outpatient and inpatient settings in the five health care facilities. Furuncle, abscesses and frequent hospital visits were found to be associated with MRSA infection/colonization, both in skin and wound cultures (Kithela ME, 2007). MRSA was highly prevalent (31.5%) in *S. aureus* isolated from patients with surgical site infections in Mulago National Referral Hospital, Kampala Uganda (Ojulong *et al*., 2009)

2.5 MRSA in Tanzania

A study conducted at MNH to assess the incidence of bloodstream infection and risk factors for fatal outcome in a prospective cohort study of 1828 consecutive admissions of children aged zero to seven years showed that 12% of the total hospital *S.aureus* isolated were MRSA (Bloomberg *et al.,* 2007). Another study conducted at Bugando Medical centre (BMC), to determine the prevalence of clindamycin inducible resistance among MRSA showed that 16.2% of *S. aureus* isolates were MRSA (Mshana *et al*., 2009). A prospective review of 300 cases to determine experiences with surgical treatment of chronic lower limb ulcers at a tertiary hospital in northwestern Tanzanian found that 23 out of 29(79.3%) of *S. aureus* isolates were MRSA(Mbunda *et al*., 2012). A recent study conducted to determine the prevalence of *S. aureus* and MRSA carriage, risk factors of *S. aureus* colonization and antimicrobial susceptibility patterns of *S. aureus* strains in under-5 children in Dar es Salaam, showed the prevalence of MRSA to be 10.5% and none of the factors was found to be significantly associated with MRSA colonization (Moyo et al, unpublished data). The prevalence of MRSA colonization on the skin, infected soft tissues and noses among HIV infected adults attending clinics in Dar es salaam was found to be 1.5% and prior hospital admission, prior use of cotrimoxazole and other antibiotics were found to be associated with MRSA colonization (Maucky et al, unpublished data).

CHAPTER 3: Methodology

3.1 Study design

A cross-sectional hospital-based study was conducted from October 2012 to March 2013.

3.2 Study setting

The study was conducted in two ICUs at MNH in Dar es Salaam, Tanzania. MNH is a tertiary facility which receives referred patients from other health facilities all over the country. The ICUs selected admit seriously sick patients of all ages and various underlying diseases who need close observation and monitoring from the health care workers. Some patients also need special devices for feeding, breathing and treatment procedures.

3.3 Study population

Patients admitted and health care workers attending the patients in two ICUs of MNH were included in the study. The health care personnel included physicians and nurses who have direct contact to admitted patients.

3.3.1 Inclusion criteria

Patients admitted in the ICU for any underlying disease were eligible for enrolment in the study. Only ICU health care workers or patients who gave the informed consent were enrolled in the study. Patient's relatives signed the informed consent on behalf of serious sick patients.

3.3.2 Exclusion criteria

All patients with prior staphylococcal infection were excluded from the study. The exclusion was achieved by referring to patient's history forms and laboratory investigation report.

3.4 Sample size

The estimated sample size for this study was 216 participants, 169 ICU patients and 47 health care workers). The estimation of sample size was calculated by using the following formula

$$n = \frac{z^2 p(1-p)}{\varepsilon^2} \quad \text{or} \quad n = \frac{z^2 p(100-p)}{\varepsilon^2}$$

Where n= sample size

z=level of confidence of the study=2 (for the 95% confidence level)

p=prevalence from previous study=12% -for the patients (Bloomberg *et al.*, 2007)

or, p= "best guess" prevalence=3% (for health care workers). This assumption considered a small number of ICU health care workers available at Muhimbili national hospital.

ε =maximum likely error=5%

3.5 Data collection

Interviewer administered questionnaires were used to collect socio-demographic information about the health care workers and patients through their relatives/guardian/parents. The questionnaires were also used to collect information on risk factors for MRSA colonization for both patients and health care workers, which included duration of stay in the hospital use of antibiotics within the past 3 months, being diabetic, illegal drug use, being sick in the past one year and smoking habit for patients. The risk factors for health care workers included profession, gloves exchange rate, hand washing and wearing surgical masks. Patient's history forms and observation charts were used to obtain more information of the patients. Laboratory result (case report) forms were used to collect laboratory results during diagnosis.

3.6 Laboratory investigations

3.6.1 Specimen collection

Swabs were collected from either of the following sites; anterior nostrils, axillary regions or wounds of the ICU patients and health care workers. Anterior nostrils were the principal sites for swab collection except for the patients inserted with nasal gastric (NG) tubes who were collected from either of the remaining mentioned sites above. It was assumed that MRSA were equally likely to be isolated from any of these sites. The swabs were collected using sterile cotton swabs in the Stuart transport medium and immediately transported to the laboratory for culturing.

3.6. 2 *S. aureus* isolation

All swabs were inoculated on blood agar medium containing 5% blood and 7.5% mannitol salt agar (Oxoid®, England) and incubated at 35ºC for 24 hours (Murray *et al.,* 1995).Mannitol salt medium is a selective medium as it allows the growth of staphylococcus and prevent other microorganisms on the skin which cannot persist a high (7.5%NaCl) concentration of salt. *S aureus* was firstly identified by using colony morphology on 5% blood agar. Creamish to golden yellow colonies with or without hemolysis were further identified using Gram staining, Catalase test, a slide coagulase test, and a DNAse test (Oxoid®, England). In case of discrepancy between the coagulase and the DNAse tests, a latex agglutination test (Slidex Staph Test, England) was carried out (Murray *et al.,* 1995).

3.6.3 MRSA detection

MRSA detection was done using cefoxitin, oxacillin discs (Oxoid®) and oxacillin screen agar (5% NaCl, 6mg/ml oxacillin) **(**Murray *et al.,* 1995; Saxena *et al.,* 2003)**.** Plates were incubated at 37 ºC for cefoxitin disc and at 33 ºC in the case of oxacillin disc and oxacillin agar. All isolates resistant to cefoxitin and oxacillin were considered as MRSA. Disk diffusion technique for detecting MRSA is simple and inexpensive however it not always reliable since phenotypic expression of methicillin resistance is known to be heterogeneous, therefore molecular detection of mecA gene or its product PBP2a can be more accurate.

3.6.4 Antimicrobial susceptibility testing

Kirby Bauer National Committee for Clinical Laboratory Standards (NCCLS) modified disk diffusion technique was used to determine antimicrobial susceptibility for MRSA. The antibiotics were selected based on first line regimen of antibiotics that are commonly used to treat infections at MNH, these included sulfamethoxazole, tetracycline, erythromycin, clindamycin, vancomycin and penicillin. Other antibiotics that could have been tested are Rifampin Rifampcin that is used to treat MRSA in combination with fusidic acid (daptomycin, linezolid, rifampcin, quinupristin/dalfopristin) Ciprofloxacin. Mueller Hinton sensitivity testing agar was inoculated with MRSA, antibiotics discs were placed in a single plate and

incubated at 35 ºC for 16 to 18 hours. Inhibition zone sizes were measured and interpreted using NCCLS standard *S aureus* ATCC 25923.

3.7 Data management and analysis

3.7.1 Data management

An appropriate data sorting between ICU patients and workers was done to facilitate subsequent processing and analysis. Completeness of the data was checked before processing and also double entry was applied to ensure the quality and accuracy of data.

3.7.2 Data analysis

The outcome variable was presence or absence of MRSA while the independent variables included age, sex, education level, occupation and risk factors. Epi Info version 3.5.1 (2008) was used for data entry and analysis. Descriptive statistics summarized the characteristics of patients and staff and their prevalence of MRSA. The risk factors for MRSA were determined using the logistic regression and a p - value of <0.05 was considered as statistically significant.

Only variables significantly associated with outcome and had a $p \leq 0.2$ were included in the final model. Stepwise backward elimination unconditional logistic regression final model was used to come up with the final best model.

3.8 Ethical considerations

The study was conducted according to the existing ethical guidelines. Ethical approval was obtained from the MUHAS Senate Research and Publications Committee. Written informed consents were directly obtained from conscious admitted patients and health care workers at ICU. The written informed consents for unconscious admitted patients were obtained from their relatives/guardians. Only authorized personnel such as physicians and nurses had access to the data. Participants who were found to carry MRSA were managed according antimicrobial susceptibility tests results returned to them and other clinical information since there is no treatment guideline MRSA cases at MNH.

CHAPTER 4: Results

4.1 Socio-demographic and clinical characteristics of patients admitted at ICU

A total of 169 ICU patients were enrolled in the study. Of these, 108 (63.9%) were males (Table 1). The mean age of patients was 43.4 years (SD) ± 15.3. A total of 110 (65.1%) patients were from inside Dar es Salaam. Many, 108 (63.9%), had primary education. Seventy two (42.6%) of patients had respiratory symptoms.

Table 1: Socio-demographic and clinical characteristics of patients(N=169) admitted at intensive care unit, MNH, Nov 2012 - March 2013

Characteristics	Category	Number	%
Age (years)	0 – 15	10	5.9
	16 - 30	22	13
	31 - 45	57	33.7
	46 - 60	63	37.3
	61 and above	17	10.1
Sex	Male	108	63.9
	Female	61	36.1
Education level	Primary	108	63.9
	Secondary	45	26.6
	Tertiary	7	4.1
	No formal education	9	5.3
Occupation	Business	56	35.9
	Farmer	30	19.2
	Health worker	7	4.5
	Retired professionals	29	18.6
	Other*	34	21.8
Area of residence	Inside DSM	110	65.1
	Outside DSM	59	34.9
Clinical information	Fever	9	5.3
	Muscular pain	6	3.6
	Confusion	18	10.7
	Dizziness	4	2.4
	General body malaise	49	29
	Respiratory symptoms	72	42.6

*includes (builders, teachers, house wives etc)

4.2 Socio-demographic characteristics of the health care workers at ICU.

Forty-seven health care workers were enrolled in the study of which 39 (83%) were females. The mean age was 37.7 years (SD) ± 11.44. All health care workers were residents of Dar es Salaam. The majority (55.3%) of health care workers were nurses. Most of them 31 (66%) had tertiary education (Table 2).

Table 2: Socio-demographic characteristics of health care workers (N=47) at intensive care unit, MNH, Nov 2012 - March 2013

Characteristics	Number	Percentage
Age (years)		
20-30	21	44.7
31-40	6	12.8
41-50	13	27.7
51 +	7	14.9
Sex		
Male	8	17
Female	39	83
Education level		
Primary	6	12.8
Secondary	10	21.3
Tertiary	31	66
Profession		
Clinicians	3	6.4
Nurses	26	55.3
Assistant nurse	18	38.3

4.3 Prevalence of MRSA colonization among patients by socio-demographic characteristics

Table 3 summarizes prevalence of MRSA colonization among patients by socio-demographic characteristics. The overall prevalence of MRSA colonization among patients was 11.83%. Of 20 MRSA isolates, 17(85%) were from nares and remaining 3(15%) from axillary parts. Males contributed 18 (90%) of the total MRSA isolates. Patients with 60 years and above had the highest prevalence (23.5%) among the age groups.

Table 3: Prevalence of MRSA among patients(N=169) admitted at MNH ICU by socio-demographic characteristics

Characteristics	Category	n	MRSA		P-value
			+ ve n (%)	-ve n (%)	
Age (years)	0 - 15	10	2 (20.0)	8 (80.0)	0.34
	16 - 30	22	2 (9.1)	20 (90.9)	0.5
	31 -45	57	5 (8.8)	52 (91.2)	0.38
	46 - 60	63	7 (11.1)	56 (88.9)	0.82
	60 and above	17	4 (23.5)	13 (76.5)	0.12
Sex	Male	108	18 (16.7)	90 (83.3)	0.01
	Female	61	2 (3.3)	59 (96.7)	0.01
Education level	Primary	108	12 (11.1)	96 (88.9)	0.7
	Secondary	45	5 (11.1)	40 (88.9)	0.86
	Tertiary	7	2 (28.6)	5 (71.4)	0.19
	No formal education	9	1 (11.1)	8 (88.9)	0.69
Occupation	Business	56	5 (8.9)	51(91.1)	0.41
	Farmer	30	3 (10.0)	27 (90.0)	0.51
	Health worker	7	2 (28.6)	5 (71.4)	0.19
	Retired	29	5 (17.2)	24 (82.8)	0.17
	Other	34	3 (8.8)	31 (91.2)	0.4
Residence(Regions)	Inside DSM	110	12(10.9)	98(89.1)	0.61
	Outside DSM	59	8(13.6)	51(83.3)	0.61

N=total respondents, n=frequency

4.4 Prevalence of MRSA colonization among patients by clinical characteristics

Table 4 summarizes prevalence of MRSA colonization among patients by clinical characteristics (symptoms and clinical features). The clinical characteristics were based on presenting symptoms and clinical diagnosis documented in the case file. MRSA colonization was high among patients with muscular pains (33.3%) followed by fever (22.2%). None had more than one symptom.

Table 4: Prevalence of MRSA among patients(N=169) admitted at MNH ICU by clinical characteristics

Characteristic	n	MRSA + ve n (%)	-ve n (%)	P-value
Fever	9	2 (22.2)	6 (77.8)	0.24
Muscular pain	6	2 (33.3)	4 (66.7)	0.25
Confusion	18	1 (5.6)	17 (94.4)	0.34
Dizziness	4	1(25.0)	3 (75.0)	0.40
Body weakness	49	5 (10.2)	44 (89.8)	0.68
Respiratory symptoms	72	7 (9.7)	65 (90.3)	0.46
Chills	1	0 (0)	1(100)	0.88

N=total respondents, n=frequency

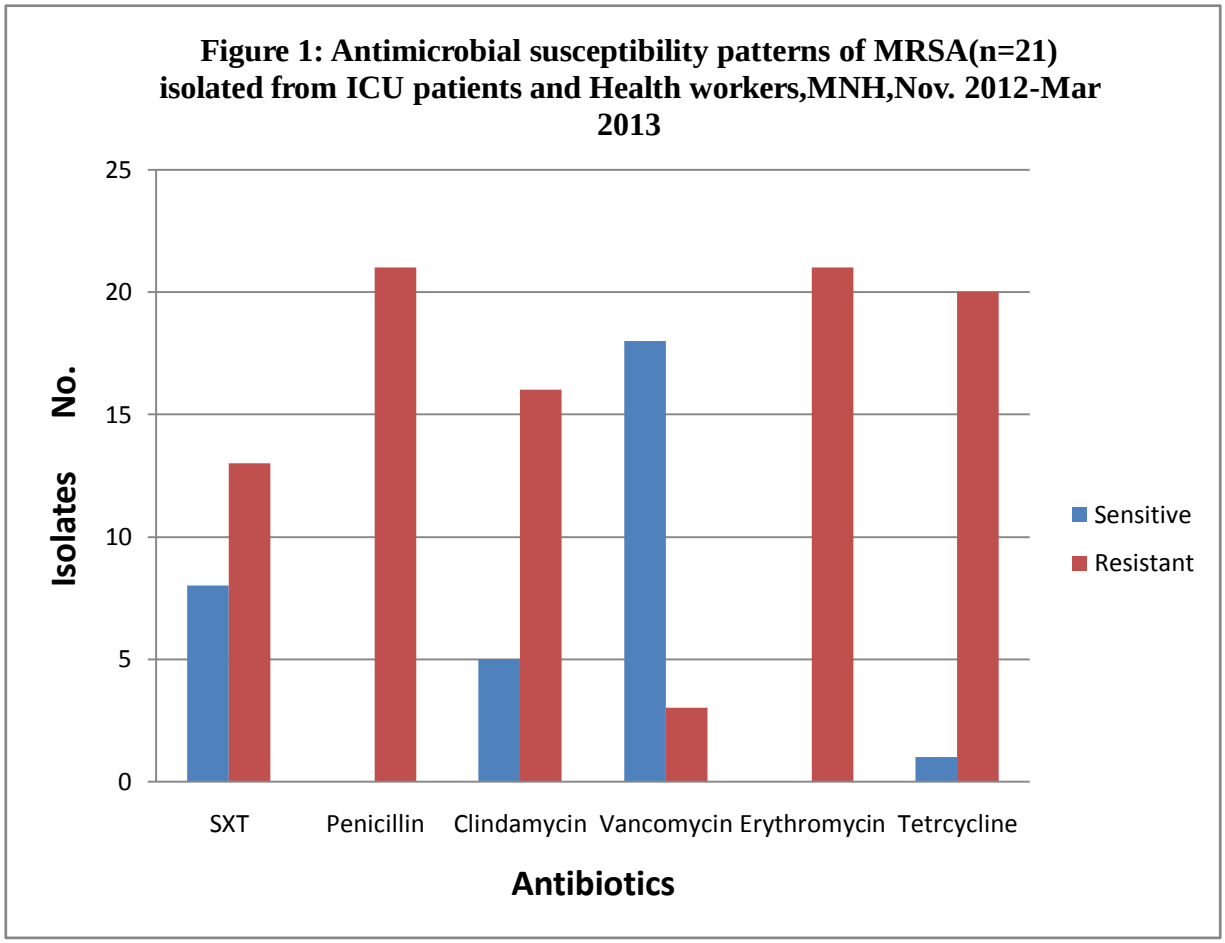
4.5 Prevalence of MRSA colonization among health care workers

Of 47 health care workers, only one was colonized with MRSA. The respondent was a male, 26 years old residing in Dar es Salaam with tertiary educational level and assistant nurse by profession.

4.6 Antimicrobial susceptibility patterns of MRSA isolates

The antimicrobial susceptibility patterns of the MRSA isolated from patients and health care workers from the ICU are shown in figure 1 below. All 21(100%) MRSA isolates were resistant to penicillin and erythromycin, and 16(76.2%) to clindamycin while majority 18(85.7%) were sensitive to vancomycin.

Figure 1: Antimicrobial susceptibility patterns of MRSA(n=21) isolated from ICU patients and Health workers,MNH,Nov. 2012-Mar 2013



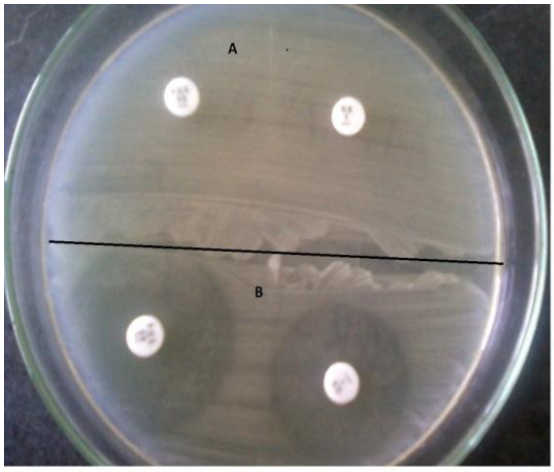
Two (9.5%) MRSA isolates showed resistance to all six antibiotic disks (clindamycin, erythromycin, tetracycline, sulfamethoxazole, penicillin and vancomycin) as shown in figure 2 below

Figure 2: MRSA resistance to all six antibiotics discs, MNH, Nov. 2012 – March 2013



MRSA were responding differently to oxacillin and cefoxitin**.** Some strains showed complete resistance while other were more susceptible (figure 3)

Figure 3: MRSA susceptibility to Oxacillin and cefoxitin which used to detect them from Methicillin Susceptible Staphylococcus aureus (MSSA)



A, MRSA showing resistance to both oxacillin and cefoxitin, **B** MSSA, sensitive to both oxacillin and cefoxitin

4.7 Risk factors for MRSA colonization among patients

Table 5 summarizes the multivariate analysis of risk factors (socio-demographics) of MRSA colonization among patients admitted. Being male and history of illegal drug uses were found to be independently associated with MRSA colonization.

Table 5: Multivariate analysis* of risk factors (socio -demographic) of MRSA colonization among patients (N=169) admitted at MNH ICU, Nov. 2012 to March 2013

Characteristic	COR	95% CI	AOR	95%CI
Age				
0 - 15	1.96	0.39 - 9.95		
16 - 30	0.72	0.15 - 3.33		
31 -45	0.62	0.21 - 1.81		
46 - 60	0.9	0.34 - 2.38		
60 and above	2.62	0.76 - 8.99		
Sex				
Male	5.9	1.32 - 26.38	6.74	1.31-34.76
female	0.17	0.03 - 0.8		
Education				
Primary	0.83	0.32 - 2.15		
Secondary	0.91	0.31 - 2.66		
Tertiary	3.2	0.58 - 17.72		
No formal education	0.93	0.11 - 7.83		
Occupation				
Business	0.64	0.22 - 1.86		
Farmer	0.8	0.22 - 2.91		
Health worker	3.2	0.58 - 17.72		
Other	0.67	0.19 - 2.44		
Retired	1.74	0.38 - 5.23		
Smoking habit				
Current smoker	5.5	0.62 - 41.22		
Ex-smoker	5.08	1.42 - 17.98	0.27	0.05 - 1.37
Non smoker				
History of illegal drug use				
Yes				
No	12.17	2.5 - 59.27	10.18	1.36-76.52

*Only variables significantly associated with outcome were included in the final model**.** COR

=Crude odds ratio, AOR = adjusted odds ratio, CI confidence interval

Table 6 summarizes multivariate analysis of risk factors (clinical characteristics) of MRSA colonization among patients. History of sickness in the past year, being sick more than three times, skin and soft tissue infection and being diabetic were independently associated with MRSA colonization among patients.

Table 6: Multivariate analysis* of risk factors (clinical characteristics) among patients (N=169) admitted at MNH ICU, Nov. 2012 to March 2013

Characteristics	Category	COR	95% CI	AOR	95%CI
Fever	Yes				
	**No	2.65	0.5 - 14.12		
Muscular pain	Yes				
	**No	4.03	0.69 - 23.57		
Confusion	Yes				
	**No	0.41	0.05 - 3.25		
Dizziness	Yes				
	**No	2.56	0.25 - 25.89		
Body weakness	Yes				
	**No	0.8	0.27 - 2.32		
chills	Yes				
	**No	0	undefined		
RS	Yes				
	**No	0.7	0.26 - 1.84		
Sick in past 1 year	Yes				
	**No	4.892	1.82 - 13.12	4.89	1.82 - 13.12
Number of times of sickness in past 1 year(either admitted or not)	**< 3times				
	>3times	10.37	3.38 - 31.79	8.91	2.32 - 34.20
Patient with NG tubes	Yes				
	**No	0.9	0.35 - 2.20		
Source of admission	Hospital				
	**Home	0.66	0.18 - 2.58		
Used antibiotics (past 3 months)	Yes				
	**No	6.76	2.24 - 20.4	1.16	0.63 - 31.32
History of SSTIs	Yes				
	**No	7.9	2.16 - 29.17	7.9	2.16 - 29.17
Diabetes	Yes				
	**No	4.48	1.67 - 12.02	4.87	1.55 - 15.36
Cancer	Yes				
	**No	7.79	0.47 - 129.73		
Kidney	Yes				
	**No	0.86	0.18 - 4.05		
Heart diseases	Yes				
	**No	1.11	0.43 - 2.87		
Hypertension	Yes				
	**No	1.52	0.46 - 5.0		
Asthma	Yes				
	**No	1.25	0.14 - 10.99		
TB	Yes				
	**No	8.17	1.08 - 61.57	5.45	0.87 - 7.89
Bacterial infections(systemic)	Yes				
	**No	2.75	0.68 - 11.14		

*Only variables significantly associated with outcome were included in the final model.

**=reference group, COR =Crude odds ratio, AOR = adjusted odds ratio, CI= confidence interval RS =respiratory symptoms

CHAPTER 5: Discussion

MRSA is the leading cause of nosocomial infections and are usually associated with poor outcomes in ICUs (Borg *et al*., 2007). This study found the prevalence of MRSA colonization of 11.8% among patients admitted and 2.1% among health care workers in the same ICUs. MRSA isolates showed the highest resistance to erythromycin and penicillin. In multivariate analysis, being male, history of sickness in the previous year, illegal drug use, skin superficial infection, being sick more than three times in a past year and being diabetic were found to be independently associated with MRSA colonization among patients.

In the current study we found a high prevalence (11.83%) of MRSA colonization among patients admitted in the ICU. This can be due to already colonized patients on admission in which transient hand carriage of the organisms on the hands of health care workers could account for the major mechanism for the patients-to-patients transmission (Gurieva *et al*., 2012). This prevalence is comparable to a one found under 5-years children (10.5%) in Dar es Salaam, Tanzania (unpublished). A possible explanation for the slight higher prevalence includes differences in socio-demographic characteristics (age, occupation) and health conditions as former study was done to healthier individuals. It is also similar to that reported in previous studies in USA in 2004, 2006 and 2007 which ranged from 11.4% to 15.7% (Boyce JM, 2004, Huang *et al*., 2006 and Ridenour *et al.,* 2007). The finding differs with the prevalence of MRSA in eight countries that was reported to be relatively high (21-30%) in Nigeria, Kenya and Cameroon and low (10%) in Tunisia and Algeria (Kesah *et al.,* 2003). The prevalence is different from 14.9% reported by Wang and his colleague in Taiwan and the difference could be due to differences in study design as they conducted a case control study (Wang *et al*., 2010).This study found low MRSA colonization among health care workers. The reason for this could be due to strict personal hygiene observed while attending patients including washing hands before and after contact with patients, wearing personal protective equipment (PPE) and trained nurses as reported by Dancer and his colleague (Dancer *et al*., 2006).

MRSA isolates showed the highest resistance to penicillin and erythromycin. In the current study, disk diffusion technique was used to detect MRSA among *S. aureus* isolates. Use of

cefoxitin disc have been found to have high correlation (>95%) with the presence of *mecA* gene (Swenson *et al.,* 2005). This technique can be an alternative to PCR for detection of MRSA in resource constraint settings (Mathews *et al.,* 2010). High resistance (76.2%) among MRSA isolates to clindamycin could be due to induction of the resistance by erythromycin (Mshana *et al.,* 2009). The resistances of MRSA to the commonly used antibiotics have impact on extra stay in intensive care, extra medical and nursing care, and additional costly antibiotic treatment and led to major cost implications and loss of health service resources in the unit (Watters *et al.,* 2004). Therefore, antimicrobial susceptibility patterns will be useful in development of treatment guidelines for MRSA.

The current study found that being male, history of sickness in the past year, illegal drug use, skin superficial infection and being diabetic contributed to the MRSA colonization in patients while admitted in the ICU. Being male and diabetes as independent risk factors of MRSA colonization found in this study have been reported in the previous studies (Graham *et al.,* 2006, Furuno *et al.,* 2005). Diabetic patients are prone to MRSA colonization as they have reduced immunity which fails to combat the pathogens. The reason why male were more likely to be colonized with MRSA than female could be due to higher proportion (63.9%) of male than female recruited in the study. History of being sick in the past year and its association to MRSA colonization could be due to increased MRSA exposure in hospital settings when they were seeking treatment. The reason for association between skin superficial infections and MRSA colonization could be due to weakened integrity of skin which reduces skin immunity to fight against MRSA. Patients with history of illegal drug use were more likely to be colonized with MRSA than non user. The reason for this could be due to the contamination of intravenous drug devices (e.g syringe) they share to inject drugs.

The study has some limitations. It was not possible to analyze the risk factors of MRSA colonization among health care workers as only one staff was colonized. A cross sectional study was conducted in which patients were not screened prior to admission and not followed up to determine whether they got colonized before or during their stay in the ICU. It was not possible to conduct molecular typing to characterize MRSA due to limited budget.

CHAPTER 6: Conclusions and Recommendations

6.1 Conclusions

The study found high prevalence of MRSA colonization among patients admitted in the ICU. There was high resistance of MRSA isolates to erythromycin and penicillin. Being male, history of sickness and superficial skin infections in the previous year, being sick more than three times in past year, history of illegal drug use and being diabetic were found to be independently associated with MRSA colonization.

6.2 Recommendations

Patients and health care workers should be screened prior to ICU admission and routinely, respectively, to identify individuals carrying MRSA. MRSA screening should prioritize patients at risk to MRSA colonization. Since MRSA has shown to be resistant the most commonly used antibiotics, the antibiotic susceptibility testing is recommended for effective treatment of MRSA. Further study should be conducted to determine MRSA colonization on inanimate for effective control and prevention.

CHAPTER 7: References

1. Bloomberg B, Manji KP, Urassa WK, et al. Antimicrobial resistance predicts death in Tanzanian children with bloodstream infections: a prospective cohort study. BMC Infect Dis 2007; 7: 43.
2. Borg MA, Kraker M, Scicluna E, et al. Prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) in invasive isolates from southern and eastern Mediterranean countries. Oxford Journals 2007; 60, Issue 6: 1310-1315.
3. Boucher HW, Corey, GR. Epidemiology of Methicillin-Resistant *Staphylococcus aureus.* Clin Infect Dis 2008; 46 (Supplement 5):344-349.
4. Bowers AL, Huffman GR, Sennett BM. Methicillin resistant Staphylococcus aureus infection in collegiate football players. Med Sci Sports Exerc 2008; 40(8):1362–7.
5. Boyce JM. New insights for improving hand hygiene and practice. Infect. Control. Hosp. Epidemiol 2004; 25:187-188.
6. Cavalcanti MM, Emmanuel RF, Carlos C, et al. Prevalence of *Staphylococcus aureus* introduced into intensive care units of a University Hospital. Braz J Infect Dis 2005;9: 56-63. ISSN 1413-8670.
7. Centers for Disease Control and Prevention. Outbreaks of community associated methicillin-resistant *Staphylococcus aureus* skin infections—Los Angeles County, California, 2002–2003. MMWR Morb Mortal Wkly Rep 2003; 52:88.
8. Chu VH, Crosslin DR, Friedman JY, et al. *Staphylococcus aureus* bacteremia in patients with prosthetic devices: costs and outcomes. Am J Med 2005; 118:1416.
9. Dancer SJ, Coyne M, Speekenbrink A, et al. MRSA acquisition in an intensive care unit. Am J Infect Control.2006; 34(1):10-7.
10. Dulon M, Haamann F, Peter, C, Schablon A, Nienhaus A. MRSA prevalence in European healthcare settings: a review. BMC Infect Dis 2011; 11:138. doi: 10.1186/1471-2334-11-138.
11. Fowler VG, Miro JM, Hoen, B. Staphylococcus aureus endocarditis: a consequence of medical progress. JAMA 2005; 293:3012-21.

12. Furuno JP, Perencevich EN, Johnson JA, et al. Methicillin-resistant *Staphylococcus aureus* and Vancomycin-resistant Enterococci Co-colonization. Emerg Infect Dis 2005; 11(10): 1539–1544.
13. Gantz N, Harmon H, Handy J, et al. Methicillin resistant Staphylococcus aureus infections among comptitive sports participants—Colorado, Indiana, Pennsylvania, and Los Angeles county, 2000–2003. MMWR Morb Mortal Wkly Rep 2003;52(33):793–5.
14. Graham P, Lin S, Larson E. A U.S. Population-Based Survey of *Staphylococcus aureus* Colonization. Ann Intern Med 2006; 144:318-325.
15. Gurieva T V, Bootsma CJ and Bonten JM. Decolonization of Patients and Health Care Workers to Control Nosocomial Spread of Methicillin-resistant *Staphylococcus aureus.*BMC Infect Dis. 2012;12(302).
16. Hall IM, Barrass I, Leach S, Pittet D, Hugonnet S. Transmission dynamics of methicillin-resistant Staphylococcus aureus in a medical intensive care unit. J R Soc Interface. 2012;9(75):2639-52
17. Hanssen, A. M., and J. U. Ericson Sollid. SCC*mec* in staphylococci: genes on the move. FEMS Immunol. Med. Microbiol 2006; 46:8-20.
18. Herwaldt LA. *Staphylococcus aureus* nasal carriage and surgical site infections. Surgery, 2003; 134: 2-8.
19. Hidron AI, Kourbatova EV, Halvosa JS, et al. Risk Factors for Colonization with Methicillin-Resistant *Staphylococcus aureus* (MRSA) in Patients Admitted to an Urban Hospital: Emergence of Community-Associated MRSA Nasal Carriage. Clin. Infect. Dis 2005; 41:159–66.
20. Huang SS, Yokoe D S, Hinrichsen V L, Spurchise LS, Datta R, Miroshnik I, Platt R**.** Impact of routine intensive care unit surveillance cultures and resultant barrier precautions on hospital-side methicillin-resistant *Staphylococcus aureus* bloodstream infection. Clin. Infect. Dis 2006; 43:971-978.
21. Humphreys H. Can we do better in controlling and preventing methicillin-resistant *Staphylococcus aureus* (MRSA) in the intensive care unit (ICU)? Eur J Clin Microbiol Infect Dis 2008;27:409-13.

22. Johnson A. Methicillin-resistant Staphylococcus aureus (MRSA) infection.www.mrsasupport.co.uk (2007).
23. Jones SW, Stafford RE. Controlling Methicillin-resistant *Staphylococcus aureus* Infection.Touch briefings (2007).
24. Kaneko J, Kamio Y. "Bacterial two-component and hetero-heptameric pore-forming cytolytic toxins: structures, pore-forming mechanism, and organization of the genes". Biosci Biotechnol Biochem 2004; 68 (5): 981–1003.
25. Kepler A D, Stewart JJ, Crouch HK, Florez C E, Hospenthal1 D R**.** Methicillin-Resistant *Staphylococcus aureus* (MRSA) Nares Colonization at Hospital Admission and Its Effect on Subsequent MRSA Infection. Clin Infect Dis 2004; 39(6):776-82.
26. Kesah CB, Redjeb S, Odugbemi TO. Prevalence of methicillin-resistant Staphylococcus aureus in eight African hospitals and Malta. Clin Microbiol Infect 2003; 9:153-156**.**
27. Kithela ME. Methicillin resistant staphylococcus aureus colonization and infection among patients with skin and soft tissue infections in Nairobi, Kenya (2007) present at Jomo Kenyatta University of Agriculture and Technology Library//THE QR 201.S68 M.
28. Klevens RM, Morrison MA, Nadle J. Invasive methicillin-resistant Staphylococcus aureus infections in the United States. JAMA 2007; 298:763-71.
29. Lee JH. Methicillin (Oxacillin)-resistant *Staphylococcus aureus* strains isolated from major food animals and their potential transmission to humans. Appl Environ Microbiol 2003; 69(11):6489-94.
30. Loeto D, Matsheka MI, Gashe BA. Enterotoxigenic and antibiotic resistance determination of *Staph aureus* strains isolated from food handlers in Gaborone, Botswana. J Food Prot 2007; 70(12):2764-2768.
31. Mathews AA, Thomas M, Appalaraju B, Jayalakshmi J. Evaluation and comparison of tests to detect methicillin resistant S. aureus. Indian 2010 ;53(1):79-82.

32. Mbunda F**,** Mchembe MD**,** Chalya PL et al**.** Experiences with Surgical treatment of chronic lower limb ulcers at a Tertiary hospital in northwestern Tanzania: A prospective review of 300 cases. BMC Dermatology 2012; 12:17.
33. Mshana SE, Kamugisha E, Mirambo M, et al. Prevalence of clindamycin inducible resistance among methicillin-resistant Staphylococcus aureus at Bugando Medical Centre, Mwanza, Tanzania. Tanzan J Health Res 2009; 11:59-64.
34. Murray, PR., Baron EJ, Pfaller MA, Tenover FC, Yolken RH. *Manual of Clinical Microbiology,* ASM Press 1995. 7th ed. Washington DC.
35. National Nosocomial Infections Surveillance (NNIS) System Report: data summary from January 1992 through June 2002. Am J Infect Control 2003*;* 31:481-98.
36. Ojulong J, Mwambu TP, Jolobo M, et al. Prevalence of Methicillin resistant Staphylococcus aureus (MRSA) among isolates from surgical site infections in Mulago hospital- Kampala, Uganda. Internet j. infect. dis 2009; 1528-8366.
37. Pittet D, Allegranzi B, Sax H, et al. Evidence-based model for hand transmission during patient care and the role of improved practices. Lancet Infect Dis 2006; 6:641-652.
38. Ridenour G., Lampen R, Federspiel J, Kritchevsky S, Wong E, Climo M. Selective use of intranasal mupirocin and chlorhexidine bathing and the incidence of methicillin-resistant Staphylococcus aureus colonization and infection among intensive care unit patients. Infect. Control Hosp. Epidemiol 2007.28:1155-1161.
39. Saxena S, Singh K. Talwar V. Methicillin-resistant *Staphylococcus aureus* prevalence in community in the East Delhi area. Japanese Journal of Infectious Disease 2003; 56, 54-56.
40. Sayed SBE, Nasr RA, Shaheen MA. Risk of Colonization of Methicillin-Resistant Staphylococcus Aureus (MRSA) and Vancomycin-Resistant Enterococci (VRE) in Patients admitted to Pediatric Intensive Care Unit of Ain Shams University Hospitals. Egypt. J. Med. Lab. Sci 2005; (ESIC)14, No. 2.
41. Shorr AF. Methicillin-resistant *Staphylococcus aureus* in the Community and the Hospital. Medscape. LLC 2008.

42. Silvana MM, Emmanuel RF, Carlos C, et al. Prevalence of *Staphylococcus aureus* introduced into intensive care units of a University Hospital. Braz J Infect Dis 2005; 134: 2-8.
43. Stefani S, Chung DR, Lindsa JA, et al. Meticillin-resistant *Staphylococcus aureus* (MRSA): global epidemiology and harmonisation of typing methods. Int. J. Antimicrob. Agents 2012; 39(4): 273–282
44. Stefani S, Varaldo PE. Epidemiology of methicillin-resistant staphylococci in Europe. Clin Microbiol Infec 2003; 12:1179-86.
45. Swenson J, Lonsway D, Mcallister S, Thompson A, Jevitt L, Patel J. Detection of *mec* A mediated resistance using cefoxitin disc diffusion (DD) in a collection of *Staphylococcus aureus* (SA), expressing borderline oxacillin MIC. Available at: http://www.bd.com/ds/technicalCenter/whitepapers/lr22306.pdf (2005).
46. Tenover FC, Goering RV. Methicillin-resistant *Staphylococcus aureus* strain USA300: origin and epidemiology. J Antimicrob Chemother 2009; 64(3):441-6.
47. Truong H, Shah SS, Ludmir J, et al. *Staphylococcus aureus* skin and soft-tissue infectionsat a tertiary hospital in Botswana. S Afr Med J 2011; 101:413-416.
48. Van Duijkeren, E.; Box, A.T.A.; Heck, M.E.O.C.; Wannet, W.J.B. & Fluit, A.C. (2004).Methicillin-resistant staphylococci isolated from animals. Veterinary Microbiology 2004; 103:91-97.
49. Wang JT, Liao CH, Fang CT, et al. Incidence of and Risk Factors for Community-Associated Methicillin-Resistant *Staphylococcus aureus* Acquired Infection or Colonization in Intensive-Care-Unit Patients . J Clin Microbiol 2010; 48: 4439–4444.
50. Warren DK, Guth RM, Coopersmith CM. Epidemiology of methicillin-resistant Staphylococcus aureus colonization in a surgical intensive care unit. Infect Control Hosp Epidemiol 2006; 27:1032-1040.
51. Watters K, O'dwyer TP, Rowley H. Cost and morbidity of MRSA in head and neck cancer patients: what are the consequences? J Laryngol Otol. 2004; 118(9):694-9.

52. Wen-Tsung L, Wei-Jen L, Min-Hua T et al. Community acquired Methicillin resistant *Staphylococcus aureus* in Children, Taiwan. Emerging Infectious Diseases. www.cdc.gov/eid 2006 Vol. 12, No. 8.
53. Zinn CS, Westh H, Rosdahl VT, et al. An international multicenter study of antimicrobial resistance and typing of hospital Staphylococcus aureus isolates from 21 laboratories in 19 countries or states. Microb Drug Resist 2004; 10:160-168.

8.0 APPENDICES

8.1 Appendix 1 Informed consent form (English version)

INFORMED CONSENT FORM

Title:

Methicillin-Resistant *Staphylococcus aureus* (MRSA) colonization among Intensive Care Unit (ICU) patients and health care workers in the ICU at Muhimbili National Hospital, Dar es salaam, Tanzania, 2012

Foreword

My name is Alfred Geofrey, a resident from Muhimbili University of Health and Allied Sciences. I am currently conducting a study on Methicillin-Resistant *Staphylococcus aureus* (MRSA) colonization among Intensive Care Unit (ICU) patients and health care workers in the ICU at Muhimbili National Hospital, Dar es salaam, Tanzania.

Research description

The study will take about seven months from October 2012 to April 2013.

Results from this study will be an important source of information to policy makers, hospital workers and other stakeholders on the burden of MRSA colonization and infections in the hospital settings in which patients need intensive care. The information will also raise awareness to health care workers in the ICU on how to reduce or eliminate cross transmission of MRSA between them and patients .The Ministry of Health and Social Welfare will use this information to develop treatment guidelines for MRSA.

We ask for your participation in the study because you are among the health care workers in the ICU/ or you are proxy of the patient admitted in this ICU who are regarded as a group at risk of MRSA colonization. If you agree to participate in this study, you will be required to answer a series of questions that have been prepared for the study through interview in order to obtain the intended information. Also you we will collect either nostril, axillary or wound swabs for MRSA screening.

Benefits

If you agree to participate you will have the following benefits. First you will know if you are or not carrying the bacteria which cause infections once they go inside the body, secondly you will be managed according to existing treatment guidelines at MNH if you will be found carry them.

Risks

Possible risk that can occur during your participation is discomfort during swab collection from the nostril.

Confidentiality

All information obtained from you will be treated confidential and will be used only for the intended purposes of this research. The research team will compile a report that will contain information about all participants while maintaining confidentiality.

Compensation

We do not expect any harm to you as a result of your participation in the study. However if it accidentally occurs there will be no compensations.

Voluntary participation and withdrawal

You have the right to participate or not to participate in the study without giving any reason for your decision. When you have decided to participate you are also free to terminate your participation at any time in the course of the study.

Contacts

If you have any questions about this study you are free to contact, the principal investigator, Alfred Geofrey (0752206899) or geofalfred@yahoo.com. If you have any questions/concerns about your rights as a participant you may contact Prof M. Aboud, Chairman of MUHAS Research and Publications Committee. P.O.BOX 65001 Dar es Salaam. Telephone number: 2150302-6

Documentation

If you agree to participate, please sign this informed consent form.

I …… have read and understood the contents of the informed consent form and my questions have been answered adequately. I therefore consent for participation in this study.

Signature of the interviewee ……..... Date ……

Signature of the interviewer ……....……. Date ……

8.2 Appendix 2 Informed consent form (Swahili version)

FOMU YA RIDHAA KUSHIRIKI KATIKA UTAFITI

Kichwa:

UBEBAJI WA VIMELEA VYENYE UKINZANI KWENYE DAWA AINA YA METICILLIN KWA WAGONJWA WALIOLAZWA KWENYE CHUMBA CHA WAGONJWA MAHUTUTI PAMOJA NA WAFANYAKAZI WA AFYA WAFANYIAO HUMO HAPA HOSPITALI YA MUHIMBILI

Utangulizi

Naitwa Alfred Geofrey, mtafiti kutoka chuo cha afya na sayansi shirikishi Muhimbili. Kwa sasa nafanya utafiti ili kutambua ukubwa wa tatizo na sababu zipelekeazo wagojwa waliolazwa kwenye chumba cha wagojwa mahututi pamoja n wafanyakazi wake kubeba vimelea vyenye ukinzani kwenye dawa methicillin hapa hospitalini Muhimbili. Utafiti huu utafanywa kwa miezi saba, kuanzia mwezi kumi 2012 mpaka mwezi wa nne 2013.

Maelezo ya Utafiti

Matokeo ya utafiti huu yatakuwa ni rasilimali muhimu sana kwa watunga sera, wafanyakazi wa hospitali na wadau wengine katika janga hili la vimelea hivyo. Utafiti huu utasaidia wafanyakazi wa afya wa vyumba vya wagonjwa mahututi na wafanyakazi wengine wa afya kiujumla kutokomeza au kupunguza kwa kiasi kikubwa maambukuzi ya vimelea hivyo kati yao wenyewe na pia kwa wagojwa. Pia utaisaidia wizara ya afya kuandaa mwongozo wa matibabu sahihi wa magojwa wa vimelea hivi.

Tunaomba ushiriki wako katika utafiti huu maana wewe ni mmojawapo wa wafanyakazi wa afya wa chumba ch wagojwa mahututi au ni mtu wa jirani sana wa mgojwa aliyelazwa kwenye chumba cha wagojwa mahatuti ambao wapo kwenye hatari ya kubeba vimelea hivyo hatari.

Ukikubali kushiriki utaulizwa maswali kadhaa yatakayotuwezesha kupata taarifa huska. Pia tutakusanya mabaki ya ndani ya pua, makwanguzi ya kwapa na usaa kwako wewe mfanyakazi au mgojwa wako vitakavyo tuwezesha kutambua kama umebeba/amebeba vimelea hivyo

Faida za kushiriki

Ukikubali kushiriki utafaidika kwanza kujua kama umebeba vimelea hivyo ambavyo huleta magojwa mara baada ya kuingia ndani ya mwili. Pili kama utakuwa umegundulika kubeba vimelea hivyo utapewa dawa ili kuuwa vimelea hivyo.

Hasara za kushiriki

Hasara ya kushiriki katika utafiti huu ni kujisikia vibaya wakati wa kukusanya sampuli puani

Usiri

Taarifa zote utakazotoa zitakuwa siri na hazitatumika kwa matumizi mengine mbali na utafiti huu. Timu ya utafiti itachambua taarifa hizi na kutoa taarifa ambayo haitatoa siri yoyote kuhusu wewe kwa kuwa majina au taarifa inayomlenga mtu binafsi haitatolewa katika taarifa hiyo.

Fidia

Hatutarajii kutokea madhara ya aina yoyote kwako yatakayosababishwa na utafiti huu,hata hivyo kama yatakuwepo,hakutakuwa na fidia ya namna yoyote.

Kushiriki na kujitoa kwa hiari

Unao uhuru wa kushiriki katika utafiti huu au kutokushiriki pasipo kutoa sababu yoyote juu ya hatua utakayokuwa umechukua. Ukishakubali kushiriki katika utafiti huu na ukaona kwamba huwezi kuendelea unao uhuru wa kusitisha ushiriki wako muda wowote wa utafiti.

Mawasiliano

Kama utakuwa na swali lolote kuhusu utafiti huu unaweza kuwasiliana na kiongozi wa utafiti huu ambaye ni Alfred Geofrey simu- 0752206899 au barua pepe- geofalfred@yahoo.com

Kama utakuwa na swali juu ya haki yako kama mshiriki unaweza kuwasiliana na Prof M. Moshi, Mwenyekiti wa kamati ya utafiti ya chuo cha afya Muhimbili kwa anuani S.L.P 65001 Dar es Salaam. Simu ya mezani: 2150302-6

Utayari

Kama umekubali kushiriki, tafadhali saini hapa:

Mimi……., nimesoma na kuelewa kilichoelezwa kwenye fomu hii na maswali yangu yamejibiwa kiufasaha. Hivyo ninakubali kushiriki katika utafiti huu.

Sahihi ya mhojiwa …….. Tarehe ……..

Sahihi ya mhoji…… Tarehe……

8.3 Appendix 3: Questionnaire

Methicillin-Resistant *Staphylococcus aureus* (MRSA) colonization among Intensive Care Unit (ICU) patients and health care workers in the ICU at Muhimbili National Hospital, Dar es salaam, Tanzania, 2012

Questionnaire No…….. Date of interview …….. Interviewer ……

Respondent ☐ Self ☐ Proxy

If proxy, what is the relationship? ☐ Parent ☐ Neighbor ☐ Guardian

☐ Other (Specify) ----------

Category: ☐ Health worker ☐ Patient

A: Demographic Information

1. Sex: Male ☐ Female ☐
2. How old are you? ..... (years)
3. If you are child, what is the occupation of your guardian?.....
4. What is your occupation? .....
5. Place of residence (Region) ……
6. Education level ☐Primary ☐ Secondary ☐ Higher ☐ Never gone to school
7. Are you a staff or a patient? If a staff go to question 25

B: Clinical Information

☐ Fever ☐ chills ☐ Dizziness ☐ Confusion ☐ Muscular pain
☐ Boils and abscesses ☐ Body weakness ☐ Respiratory symptoms
☐ Other specify ……

C: Risk Factors for a patient

8. In the past one year have ever been sick ☐ Yes ☐ No
9. If yes, how many time have been become sick?…….times
10. What was the problem(s)? ☐ Respiratory ☐ Gastrointestinal ☐ Urinary ☐ Skin superficial infection ☐ other systemic symptoms (specify)……
11. For how long have you stayed in the Intensive care units (ICU)?----------days
12. Have you been using antibiotic within the past 3 months? ☐ Yes ☐ No
13. If yes what type of antibiotics (prompt)_____
14. Have you been transferred from another health facility ☐ Yes ☐ No
15. If answer above is yes, from which ward or department?

    A. Surgical ward B. Burns ward C. neurological ward D. othorpediatic E. Emergency
16. Underlying diseases (Tick where appropriate)

    ☐ Diabetes ☐ Cancer ☐ Kidney ☐ HIV ☐ Heart disease ☐ Hypertension ☐ Asthma ☐ TB ☐ Allergic rhinitis ☐ CKD ☐ Biliary system disease ☐ COPD ☐ chronic seizure ☐ Brochiectasis ☐ Bacterial infection
17. Chief complaint ☐ Respiratory ☐ Gastrointestinal ☐ Urinary ☐ skin superficial infection ☐ Other systemic symptoms
18. Source of admission ☐ Hospital ☐ Home
19. Smoking habit ☐ Non smoker ☐ Ex-smoker ☐ Current smoker
20. Were you currently hospitalized or underwent surgery? ☐ Yes ☐ No
21. Is the patient inserted with intravascular devises? ☐ Yes ☐ No
22. Current usage of NG tube

    ☐ Yes ☐ No
23. Use of illegal drugs ☐ Yes ☐ No
24. Family employed in medicine ☐ Yes ☐ No

D: Risk factors for the health care workers

25. For how long have you worked in the ICU?.....
26. Professional category ☐ Doctor ☐ Nurse ☐ Clinical officer ☐ Nursing assistant
27. Do you always wash your hand before contact with the patients? ☐ Yes ☐ No
28. Do you always wear gloves when working with patient? ☐ Yes ☐ No
29. What is your glove exchange rate?

    A. One per patient B. One per two patients C. One per 3 Patients D. One per more than 3 patients E. More than a single pair per one patient F. Not at all

30. Do you always exchange gloves between separated tasks on one patient?
    ☐ Yes ☐ No
31. Do you always wash your hand with disinfectant soap? ☐ Yes ☐ No
32. Do you wear surgical masks when manipulating patients? ☐ Always ☐ sometimes ☐ Not at all
33. Do you undergo routine screening for staphylococcus colonization?
    ☐ Yes ☐ No
34. Do you wear ICU dresses when working with patients in the ICU?
    ☐ Yes ☐ No

8.4 Appendix 4: Case Report Form (Laboratory Results)

Identification number…….. Age……..

Sex…… Residence…… Date collected…….

Type of specimen…… Transport medium used…….. ……...….

Culture report after 24hours……...

……

……

Gram stain results ……

……

Biochemical tests

Catalase test ☐ positive ☐ negative

Coagulase test ☐ positive ☐ negative

DNAse test ☐ positive ☐ negative

MRSA detection

Cefoxitin (disk) ☐ S ☐ I ☐ R

Oxacillin (disk) ☐ S ☐ I ☐ R

Oxacillin (agar) ☐ S ☐ I ☐ R

Final report ☐ MRSA isolated ☐ No MRSA isolated

Sensitivity Results

Sulfamethoxazole	☐	S	☐	I ☐	R
Penicillin	☐	S	☐	I ☐	R
Clindamycin	☐	S	☐	I ☐	R
Vancomycin	☐	S	☐	I ☐	R
Erythromycin	☐	S	☐	I ☐	R
Tetracycline	☐	S	☐	I ☐	R

S= sensitive, I=intermediate, R= resistance

Sign…….Date…….