METHICILLIN RESISTANT *Staphylococcus aureus* FROM PATIENTS CARE ENVIRONMENT AT MUHIMBILI NATIONAL HOSPITAL, DAR ES SALAAM

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Department of Microbiology and Immunology

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By

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A Dissertation Submitted in (Partial) Fulfillment of the Requirement for the Degree of Master of Science (Microbiology and Immunology) of the

Muhimbili University of Health and Allied Sciences
October, 2017

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 from Patients Care Environment at Muhimbili National Hospital, Dar es Salaam'',*** in (partial) fulfillment of the requirements for the degree of the Master of Science (Microbiology and Immunology) degree of Muhimbili University of Health and Allied Sciences.

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Co-Supervisor

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Date

DECLARATION AND COPYRIGHT

I, **Emmanuel James Nkuwi**, 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**……..

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DEDICATION

This dissertation is dedicated to my lovely family; father James Nkuwi**,** mother Tabia Nkuwi and siblings for being the source of inspiration, love, support and encouragement that has been an invaluable tool throughout the period of this academic endeavor.

ABSTRACT

Background: Environmental contamination with MRSA in routine medical care settings poses an increased risk of health care associated infections through cross- transmission. The cross-transmission is generally associated with healthcare-associated infections with increased length of stays in hospitals, healthcare costs, and mortality. Less is reported on both magnitudes and distribution of environment contamination by these pathogens in hospitals in Tanzania.

Study Objective: To determine the magnitude and distribution of MRSA contamination among various items in a patients' care surroundings at Muhimbili National Hospital (MNH).

Study Methodology: A cross sectional study was conducted where specimens from various parts of patients' care surroundings at MNH were processed for detection of MRSA using MRSA selective agar. Antimicrobial resistance pattern of the confirmed MRSA isolates was determined by Kirby Bauer disc diffusion method. Data was analyzed using SPSS software version 20.0, *p* values of < 0.05 were considered statistically significant.

Results: A total of 200 samples from hospital environment were processed**;** the prevalence of MRSA was 19.5 % with significantly higher prevalence in general wards. Patients' beds surfaces were the most contaminated among studied items (43.7%), whilst the surgical trolleys were least contaminated (7.7%). Highest proportion of isolates were resistant to Ampicillin (87.2%) where as none of the isolates were resistant to vancomycin. Ten (10) or more patients in a room and specimen source were significant predictors for MRSA contamination by bivariate logistic regression model.

Conclusion and recommendation: The reported high MRSA prevalence confirms that areas of hospital environment present underestimated important reservoir for MDR pathogens even in non outbreak settings. The findings provide the basis to emphasize on the need to formulate hygiene protocols with special consideration on high touch surfaces, Moreover larger prospective studies are recommended to assess the correlation between environmental MRSA and the acquisition of MRSA by patients or the vice versa.

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LIST OF ABREVIATIONS

ATCC	American type culture collection
CDC	Centers for Disease Control and Prevention
CFU	Colony Forming Unit
ESBL	Extended Spectrum Beta Lactamase.
GNB	Gram negative bacteria.
ICU	Intensive care unit
IDC	In dwelling catheter
IV	Intravenous
MDR	Multidrug Resistant
MIC	Minimum Inhibitory Concentration
MNH	Muhimbili National Hospital
MOI	Muhimbili Orthopedics Institute.
MRSA	Methicillin Resistant *Staphylococcus aureus*
MSSA	Methicillin Susceptible *Staphylococcus aureus*
LTC	Long term care facility
PCR	Polymerase Chain Reaction
VRE	Vancomycin Resistant Enterococci
WHO	World Health Organization

DEFINITION OF TERMS

- **Methicillin resistant *Staphylococcus aureus*-** *Staphylococcus aureus* which had growth inhibition zone of less than or equal to 21mm by 30µg Cefoxitin disc.

- **High touch surfaces-**These are surfaces that sustained more than 1 contact per interaction (as being identified by other epidemiological studies and through prior consultation with nursing staff at MNH).

- **Near patient surfaces-**These are surfaces of items which were one (1) meter from a patient's bed.

CHAPTER ONE

1.0. INTRODUCTION

1.1. Background

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a leading cause of hospital acquired infections including bloodstream infections, urinary tract infection (UTI), and surgical site infection (SSI) among others. These are strains of *S.aureus* that have acquired mechanisms for resistance to methicillin through the expression of mec gene of importance *mec* A, a gene encoding for penicillin-binding protein 2a (PBP2a) an important feature for resistance to methicillin. In most cases resistance extends to other commonly prescribed beta-lactam antibiotics, including penicillins and cephalosporins, thus narrowing the therapeutic choices with increased expenses.

MRSA is a problem in health care facilities as well as communities, but with greater concern in former settings as the transmission and effect of these pathogens is amplified due to presence of susceptible population. On the other hand hospital premises in particular are known to contain antibiotics and disinfectants residues which could exert selective pressure on contaminating organisms and contribute to the gradual appearance of resistant pathogens. Furthermore hospital localities have proven favorable in transmission of these pathogens due to existing constant interactions between health care workers, highly susceptible population (patients), visitors and various contaminated surfaces notably in cases of poor compliance to hand hygiene(1).

There has been mounting evidence that MRSA can be recovered from surfaces confined to hospital environments often with increased risk of nosocomial incidences (2). Different studies have revealed significant level of contamination by MRSA in various surfaces at hospital environment. Frequently touched (high touch) surfaces and items in the immediate vicinity of patients such as bed surfaces, floor, linen, sink hampers, door handles are reported to be more frequently and heavily contaminated. Air, especially in controlled environments such as operating theatres and tap water have also been associated with spread of Multi-drug resistant (MDR) pathogens in several hospital settings(2– 4).

The occurrence of MDR in hospital-associated pathogens has resulted in the emergence and re-emergence of difficult to treat nosocomial infections depicting the pre-antibiotic era. These infections are difficult to eradicate due to resistance to many antibiotics, thus major cause of morbidity and mortality, leading directly and indirectly to an enormous increase in cost of hospital stay for the patients and also emergence of new health hazards for the community (3,4).

Despite the anticipated important role of hospital environment in transmission of MRSA, less emphasis has been given in evaluating the occurrence of these pathogens in our hospital settings; Generally few studies have adequately assessed the relative role of the environment versus other modes of transmission of hospital acquired pathogens. Bacteriological sampling of environmental surfaces has been only indicated as part of some outbreak investigations but rarely in endemic situations. This study was undertaken to assess MRSA contamination of inanimate surfaces surrounding patients receiving care at Muhimbili National Hospital (MNH), the largest tertiary hospital in Tanzania.

1.2. Statement of the Problem

A growing body of evidence supports the contribution of contaminated inanimate surfaces in transmission of MDR pathogens to patients in healthcare settings. MRSA is of particular concern due to its ability to survive for long on dry surfaces and causation of multiple nosocomial infections(5). On the other hand, increase in incidence of nosocomial infections attributed to MRSA has been reported at our locality (6,7), commonly linked to poor outcomes and elevated healthcare related costs. The most recent studies undertaken to cite out possible reservoirs of these pathogens in health care settings were limited on assessing MRSA colonization among healthcare workers and/or patients(8). Information addressing the occurrence of these organisms on inanimate surfaces and hence their role as possible important secondary reservoirs is still scanty.

1.3. Study rationale

Given the increasing prevalence of surgical sites infections, wound burn infections, UTI and other forms of nosocomial infections attributed to MRSA at our settings, possible reservoirs and transmission routes of these pathogens should be explored. Findings from this study will therefore provide evidence-based information about the extent of contamination and most probable potential MRSA reservoirs among the studied surfaces. Information on significant MRSA contamination will be useful for designing, reviewing and implementing effective prevention and control measures including greater emphasis on compliance with contact precautions and more strategic cleaning and disinfection protocols hence tackling the cross transmission of these pathogens and the subsequent infections.

1.4. Conceptual framework

Rate and distribution of contamination of hospital surfaces by MRSA can be predicted by vicinity of surface to the patient receiving care and touch frequency. These surfaces are contaminated following contact by contaminated hands of patients, health care workers (HCW's) or visitors, likelihood amplified by poor hygiene and contact precautions. Figure 1, illustrate the interconnections between these independent and dependent variables.

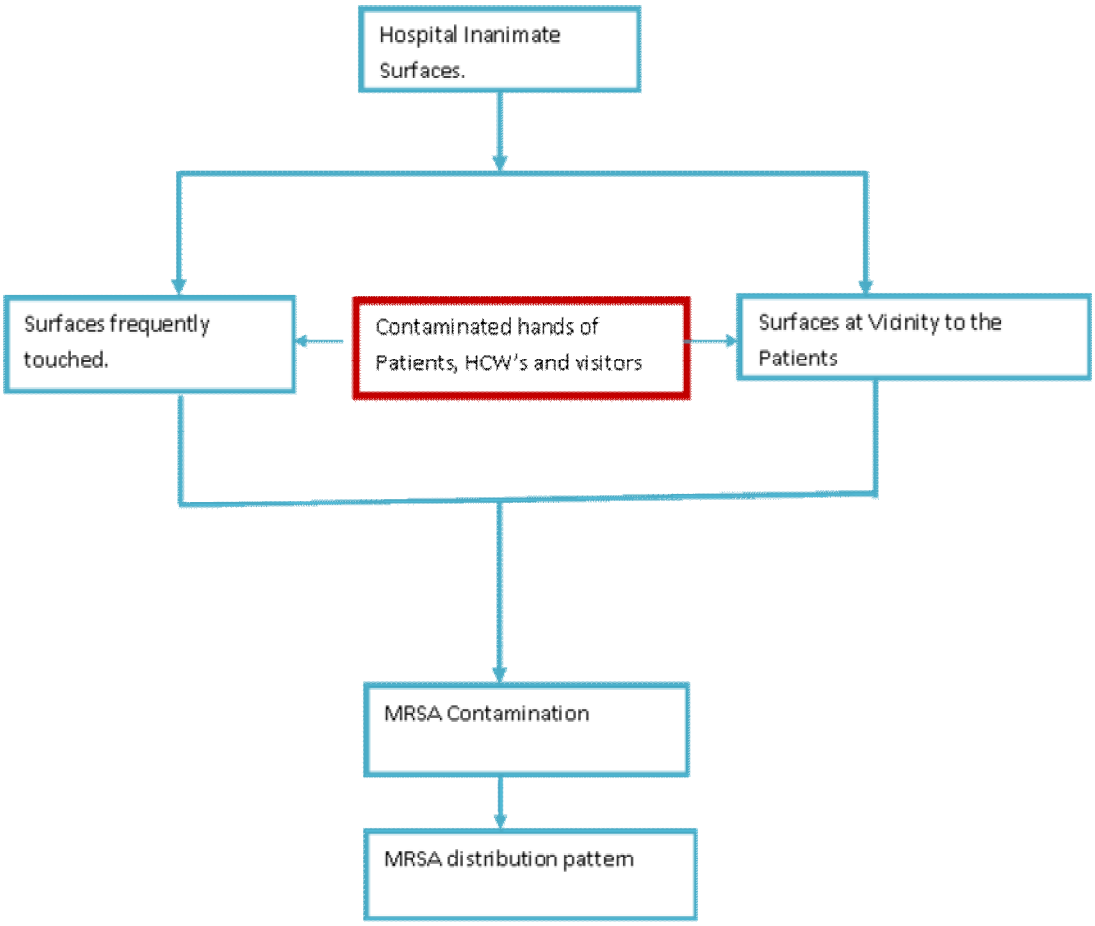


Figure 1: Conceptual framework for contamination of hospital surfaces by MRSA

Research hypothesis

Environment near patients receiving care at MNH do not present potential representing potential secondary reservoir for MRSA

1.5. Research questions

1.5.1. Is an environment near patients receiving care at MNH representing potential secondary reservoir for MRSA?

1.5.2. Do surfaces of items surrounding patients receiving care at MNH bear the same probability of being contaminated by MRSA?

1.5.3. What is antimicrobial susceptibility pattern of MRSA isolates from MNH patients care environment?

1.5.4. Do some hospital and patient factors have significant role in predicting the contamination of hospital environment by MRSA?

1.6. Study Objectives

1.6.1. Broad objective

To determine the magnitude, antimicrobial susceptibility and correlate factors for MRSA contamination in patients' care environment at MNH.

1.6.2. Specific Objectives

1. To determine the magnitude of MRSA contamination on surfaces of various matrices surrounding patients care at MNH.
2. To determine the distribution of MRSA among surfaces of various items surrounding patients receiving care at MNH.
3. To determine antimicrobial susceptibility pattern of MRSA isolated from various items and surfaces surrounding patients receiving care at MNH.
4. To determine factors correlated to MRSA contamination in hospital environment at MNH.

CHAPTER TWO

2.0. REVIEW OF LITERATURE

2.1. Prevalence of MRSA

During the past decade, the incidence of MDR has reached unprecedented levels; specifically strains of MRSA are reported to increase by fourfold worldwide(9). In most settings the increasing rates of MDR strains have generally been linked to the irrational antibiotic use, inadequate coverage of the antibiotics and inaccurate antibiotics sensitivity tests done in the laboratories. The most recent estimates of global antibiotic resistance, list MRSA as among the three agents of greatest concern associated with both hospital and community acquired infections with the resistance rates exceeding 20% in all WHO regions and even above 80 % in some regions(10).

The national nosocomial infection surveillance system data from the Centers for Disease Control and Prevention (CDC) documented great problem of nosocomial infection in intensive care units, in particular MRSA accounted for almost 60% of staphylococcal infections, (11). European surveillances reported prevalence in long term care facilities varying from one percent to 23%, (percentage of MRSA among patients), and from five percent to 54% (percentage of MRSA among *S. aureus* isolates) of which proportions of around 20% or higher are reported in UK, Northern Ireland and Belgium(10,12). Asia has the recent highest prevalence of MRSA reported in Vietnam where proportion of MRSA among hospital acquired *S.aureus* infections was 74.1% (13).

Despite of suboptimal MRSA surveillance in most African countries the general picture is one of the increasing rates. Available data report the general prevalence ranging from five percent to 45% (percentage of MRSA among patients) but higher proportions of MRSA among *S. aureus* isolates, Madagascar being a country with lowest rates reported (14,15) .In Ethiopia isolates from infected wounds revealed 76.7% of MRSA among all *S.aureus* isolates (16), where as in Libya a tertiary surgical and trauma hospital reported 31% of isolated *S.aureus* species to be MRSA (17). Moreover a high prevalence of MRSA has been reported in Kinshasa, (Democratic Republic of Congo) where 63.5% of the *S.aureus* isolated from surgical site infections in one hospital were MRSA (18).

The highest proportions of MRSA among *S.aureus* isolates in East Africa were recently reported in Kenya where a study done on skin and soft tissue infections in Nairobi reported 84.1% of MRSA among all *S. aureus* isolates (19).

In Uganda proportions of MRSA have been reported to be within a range of 20% to 30%. However a study done on prevalence of MRSA among isolates from surgical site infections in Mulago hospital reported as high as 31.5% of MRSA among isolated *S. aureus* (20) .

Tanzania hasn't been spared for the burden as there have also been reports on alarming increase in prevalence of MRSA among *S. aureus* isolates. The dramatic increase in MRSA rates is evidenced by different studies conducted in the same settings at different times. In a study conducted on bacteria isolated from bloodstream infections at a tertiary hospital in Dar es Salaam Seven years ago 23.3% MRSA was reported, where as 0.4% MRSA were reported in the same settings 15 years ago (7). However even higher proportions of MRSA among *S. aureus* isolated have been reported in recent studies. A study done on bacteria pathogens causing surgical site infections in MNH three years ago, MRSA was reported to comprise 44% of *S.aureus* isolates (6).

2.2. Clinical Significance of MRSA

MRSA causes significant proportion of hospital-associated infections, commonly causing bacteremia, pneumonia, and wound infections (21). Hospital acquired infections caused by MRSA affect patient care by increasing morbidity, mortality, and costs derived from increased durations of hospitalization and use of more-expensive antimicrobial agents. In particular, patients with bacteremia due to MRSA have significantly longer durations of hospitalization and higher hospital charges than do patients with bacteremia caused by methicillin-susceptible *S. aureus* (MSSA). In one meta-analysis of comparing mortality in patients with bacteremia caused by MRSA and MSSA found bacteremia due to MRSA associated with significantly higher mortality rate (22).

Elsewhere a study for excess costs and utilization associated with MRSA infections reported higher costs incurred and more co-morbidities compared to when infections are due to MSSA (23). All of these adverse outcomes provide strong incentive to control the emergence and dissemination of MRSA strains.

2.3. Distribution of MRSA in Patient Care Environment

Recently, there has been increasing body of evidence demonstrating the presence of MRSA in the patient care environment, often at the level of contamination exceeding the number of pathogens necessary for the transmission (5,23). In most cases therefore contamination has been linked to transmission of several key nosocomial-associated pathogens. Contamination of hospital surfaces with MRSA has been linked with viability of organisms shed by previous occupants, asymptomatic carriers, horizontal transmission from healthcare workers, visitors or as well as migration of the organisms through airflow or other means (25).

The study in Japan to investigate the existence of airborne MRSA in a hospital environment found 20% of airborne *S.aureus* being MRSA (26). Another study done to evaluate the presence of MRSA in surfaces with close proximity to patients at intensive care unit (ICU) in Brazil reported 60% of *S.aureus* isolates being MRSA (27).

In Africa studies have also shown significant proportions of MRSA among *S.aures* isolated from patients' surroundings. A study conducted in Nigeria reported 25% of MRSA among *S.aureus* isolates from various hospital surfaces (28).

In Uganda, (East Africa) a study in the surgical units of Mulago hospital reported 38% MRSA among *S.aureus* isolated from surfaces (29). There is paucity of information regarding the occurrence of MRSA in patients care surroundings in Tanzania; however one study conducted at a referral hospital to identify risks of white clinical coats towards spreading nosocomial infection, though not specific for MRSA found *S. aureus* accounting for 91.67% of all contaminants reported (30).

The useful insights about role of MRSA contaminated surfaces on spreading of certain nosocomial infections have been in place through molecular typing, where genetic relatedness between environmental and patients isolates have been important evidence. One study assessing environmental reservoirs of MRSA in isolation rooms in U.S recovered genetically related strains from the patient and their environment in 70% of typed isolates, strongly suggesting possible contribution of environmental contamination to endemic MRSA(4). Investigation of relatedness between bacterial isolates from the hospital

environment and patients' surgical wounds revealed a significant plasmid matching between the isolates (31).

The distribution and survival MRSA on various surfaces is generally influenced by temperature, humidity and decontamination activities while factors for their transmission involve ability of pathogens to remain viable on a surface, the rate at which contaminated surfaces are touched by patients and healthcare workers, the context in which the patient is exposed, and the levels of contamination that result in transmission to patients (5) .

Multiple studies have reported MRSA contamination more confined to air and dry surfaces such as bedrails, linens, door handles and floor of which surfaces at more vicinity to patients are far frequently contaminated followed by other high touch sites. A higher incidences of MRSA on surfaces at ICU in Brazil were reported on the side rails and bed cranks, tables, buttons on the infusion pumps and aprons at increasing order (27).

Studies in Africa have also shown the distribution of MRSA on hospital surfaces almost similar with example of study in Nigeria reported higher MRSA detection rates from bedrails, over bed table tops and door handles at decreasing order (28).

A concern about a potential role of environmental contamination as a reservoir for MRSA and other resistant species have contributed to the current great emphasis on the importance of cleaning and disinfecting "high-touch surfaces" and monitoring these activities to maintain a sanitary environment in the hospital through different healthcare infection control guidelines.

2.4. Antimicrobial resistance pattern of Environmental MRSA

Multidrug resistance among isolates from hospital environment is quite an anticipated phenomenon as these pathogens are subjected to selective pressure for resistance due to persistent antibiotics and disinfectant use in the settings. Regularly the observed resistance pattern has been similar to that of isolates from patients near studied environment, suggesting further the cross transmission. A study for hospital airborne MRSA transmission in Japan reported similarity in resistance pattern between isolates from environment and those from patients, with highest percent of isolates being resistant to Ampicillin and least to Vancomycin among drugs tested (32).

In the region study for environmental MRSA from trauma ICU in Egypt reported large percent of resistant isolates towards Ampicillin and Tetracycline where as lowest resistance among isolates was reported towards Vancomycin and Linezoid (33). Moreover a study in Uganda reported higher rates of multidrug resistance among MRSA isolated from the surgical units, notably resistance to b-lactams, Sulphamethoxazole- trimethoprim and Tetracycline while resistance rates to Erythromycin, Gentamicin and Chloramphenicol were very low (29).

2.5. Factors predicting environmental contamination by MRSA

Compliance to hygiene, touch frequency, patient load, frequency /number of MRSA colonized or infected patients, length of hospital stay, and procedures like catheterization can have significant association with the detection rates in hospital environment. Frequency of surface touch have been associated with rate at which one can get contaminated by MRSA. Hospital surfaces with more contacts per interaction are more likely to be contaminated compared to those touched less frequently. In the light of these findings surfaces like bedrails, door handles are more frequently contaminated than curtains, light switches and other rarely reached objects (34). Meanwhile the frequency of touch to hospital surfaces is largely attributed to number of patients, HCW and visitors.

Colonization or /infection of patients by MRSA in a particular hospital unit has been implicated significantly in near patients surface contamination in many surveillance studies. Assessment of environmental contamination due to MRSA found that inanimate surfaces near affected patients commonly become contaminated with MRSA and that the frequency of contamination is affected by the body site at which patients are colonized or infected (35). Furthermore the underlying medical conditions/diseases of patients in a particular hospital unit would significantly predict near surface contamination. MRSA were recovered from 58.8% of surfaces in the rooms of patients with diarrhea compared with 23.3% of surfaces in rooms of patients without diarrhea, the difference was statistically significant with $P < .0001$ (36). Presence of invasive devices such as indwelling catheters has further been shown to be an important predictor for near surface contamination as significant association was shown to exist between presence of indwelling catheters on patients and environmental contamination (OR 6.12, 95% CI 1.23–30.37) (37).

Patient's load in a hospital unit has been mentioned to significantly predict rate at which items in hospital areas are contaminated. It is with greater number of patients when certain items in the hospital are increasingly accessed hence recontamination and flow of personnel including visitors is less managed. A study has reported significant decrease in Hospital acquired infections associated with single–patient rooms design compared to open wards design (38).

Degree of facility sharing plays significant role in contamination, as evidenced by increased contamination rates of the frequently touched surfaces or objects in comparison to those less accessed, in a randomized cross-over study, recontamination of high-contact surfaces in ICU occurred after 4 hours from standard cleaning measures (39).

2.6. Detection of MRSA

Molecular techniques and culture-based methods are principal approaches for the detection of MRSA in microbiology samples. MRSA detection in both methods is preceded by isolation of *S.aureus* from samples through culturing and species identification. Molecular methods for the detection of MRSA rely on the detection of *mec* gene by polymerase chain reaction (PCR). Molecular approach enables the rapid, detection of target genes with sensitivities ranging from 82% to 100% and specificities ranging from 64 to 99% (40).

However the use of molecular assay is largely restricted to reference centers and they are not currently utilized by most routine diagnostic laboratories with possible lesser value in low- MRSA prevalence settings.

Conventional culture-based methods are grouped into two common types based on the principle applied in each system.

i. **Disc diffusion methods: (**Kirby Bauer and Stokes method); a colony of *S.aureus* isolate is inoculated on a non selective media impregnated with antibiotics discs of the drugs used to screen for MRSA and incubated. Oxacillin or Cefoxitin are common drugs used for MRSA detection, the later being the best recommended as it is the better inducer for *mec* A gene. An isolate is considered MRSA or MSSA depending on the diameter of inhibition zone as interpreted by referring to standard guideline with inclusion of control strains for quality control (41).

ii. **Dilution methods:** Involves dilution of antimicrobial used in screening for MRSA (Oxacillin or Cefoxitin) at minimum inhibitory concentration in either agar or broth media to which *S.aureus* isolates will be inoculated. Following incubation and if controls are satisfactory, any growth would be indicative of MRSA. For example blood agar with 4mg/l Cefoxitin or mannitol salt agar with oxacillin can be used for early detection of MRSA with appreciable sensitivity and specificity (42).

Development of selective media with chromogenic enzyme substrates to enhance one step detection of MRSA is a recent recommended approach. Growth of non-staphylococcal strains is repressed by high salt concentration and enzymic substrate is used to achieve a specific colour reaction to detect *S. aureus* where as growth of meticillin-susceptible *S. aureus* strains is repressed by the addition of various combinations of antibiotics. Use of Chromogenic media is featured by improved sensitivity and specificity with decreased isolation days and cost saving compared to molecular methods (43). Other methods for detection MRSA includes detection of Penicillin binding protein 2a (PBP2a) through latex agglutination and use of automated systems including Phoenix (Becton Dickinson) Vitek/Vitek2(bioMe´rieux), and Microscan (Dade Behring).

CHAPTER THREE

3.0. STUDY METHODOLOGY

3.1. Study area

The study was carried out in General wards, ICU's and operating rooms at MNH, the largest tertiary health care facility in Tanzania. The hospital serves as a teaching and referral hospital to the population of Dar es Salaam and the whole country with 1,500 bed facility. The hospital admits up to 1,200 inpatients per week with nearly 3000 employees, also receiving a minimum of 2,000 patient's visitors per day. Being a tertiary hospital, this complexity and big size of population within patient's care units creates an environment for possible contamination of "high touch" surfaces.

Samples were taken to and processed at MUHAS Microbiology laboratory, a university Laboratory offering teaching and research services to the university and general community.

3.2. Study Design

Across-sectional study was undertaken between May and June 2017 aiming at detecting MRSA on surfaces of pre-selected items surrounding patients care at MNH.

3.3. Study Subjects

Door handles, sinks, bed side surfaces, and surgical trolleys were considered frequently touched items and comprised the study subjects. Items with surfaces that were obviously dirty soiled were excluded.

3.4. Sample size estimation

The sample size was estimated using the formula for calculation of sample size as,

$n = \frac{z^2 p (100 - p)}{\varepsilon^2}$; Where n = sample size; z = standard normal deviate equal to 1.96 for 95% confidence level; p = expected proportion with characteristic of interest; ε = margin of error taken at six percent (6%). The proportion of MRSA contaminated surfaces was derived from the cross-sectional study conducted in the surgical units of Mulago hospital in Kampala, Uganda where prevalence of MRSA from the sampled inanimate surfaces was 18.9% (29).

The estimated sample was then;

$$n= \frac{1.96^2\ 18.9(100 - 18.9)}{6^2} = 164$$

The sample size will be adjusted for any error during sample collection and processing by using the following formula; n=n*(100%/100%-f %)

=164*100/100-10=182.2

The minimum required sample size was 182.

3.5. Sampling technique

Items for sampling were conveniently obtained in 50 patients' care rooms from different hospital units, for the purpose of this study, four (4) items were sampled from each of the selected room. The numbers of patients' rooms were distributed in such that to maximize the number of ICU's and Operating rooms, given that majority of rooms in these settings were general wards. Samples were obtained from bedside surface, surgical trolleys, door handles and hand washing sinks. Items to be studied were selected on the basis of their high accessibility and therefore the anticipated high touch frequency. The high touch frequency of these particular items and their increased possibility of transmitting important pathogens have been emphasized in studies undertaken for quantitative approach to define "high-touch" Surfaces in Hospitals (44) and with prior consultation with MNH nursing staff.

3.6. Specimens and Data collection Procedures

3.6.1. Data collection

Data extraction forms were used to collect information regarding unit identity, number of patients, patient's gender, and cleaning & disinfection protocols. This information was obtained from nurses in charge of units involved in sampling.

3.6.2. Specimens collection

The specimens were collected in the weekdays, within hour after daily cleaning and disinfection had taken place. Surfaces were sampled based on recommendations of the CDC environmental cleaning toolkit (45) whereby specimens were collected using sterile cotton swabs by gently rubbing the swab moistened with sterile physiological saline on the surfaces and rotating the swab round to 360 degrees. A standardized surface area (of not

greater than 10cm^2) was swabbed for each selected item. All swabs were pooled in tubes containing Amies transport media, tubes were labeled for specimen sources, date and time of collection, the hospital unit and immediately delivered to MUHAS Microbiology laboratory for processing.

3.7. Laboratory Procedures

3.7.1. MRSA screening Agar preparation

The medium was prepared in accordance with the manufacturer's instructions (Liofilchem^{TM} Italy); 37mg of Chromogenic Agar powder was mixed with 495ml distilled water and autoclaved for 15minutes at 121°C then cooled to 50°C. Reconstitution of agar supplement was done by mixing five milligrams of sterile distilled water to a five grams Vial of supplement powder.

The supplement was added to the media solution then poured to Petri dishes and solidified under room temperature followed by media drying at 50°C for 20minutes.Media performance was tested by inoculating the standard Methicillin resistant *Staphylococcus aureus (*MRSA ATCC 29213) and incubated aerobically for 24hours at 35°C while sterility tests was done by incubating one uninoculated media plate (for each 495ml preparation) under similar conditions.

3.7.2. Isolation and detection of MRSA

Swabs specimens were immediately cultured on plates containing MRSA screening agar (Liofilchem^{TM} Italy) and incubated aerobically at 33°-35°C for 24 hours or 24 hours more if no growth, after which any growth with colony color ranging from mauve-red-pink was indicative for MRSA.

3.7.3. Identification and characterization of isolates

3.7.3.1. Colonial appearance

Preliminary identification of isolates growing on the MRSA screening agar had based on mauve-pink coloration and medium sized appearance of the colonies(fig 1 below); This identification criteria was as defined by the manufacturer (Liofilchem^{TM} Italy).

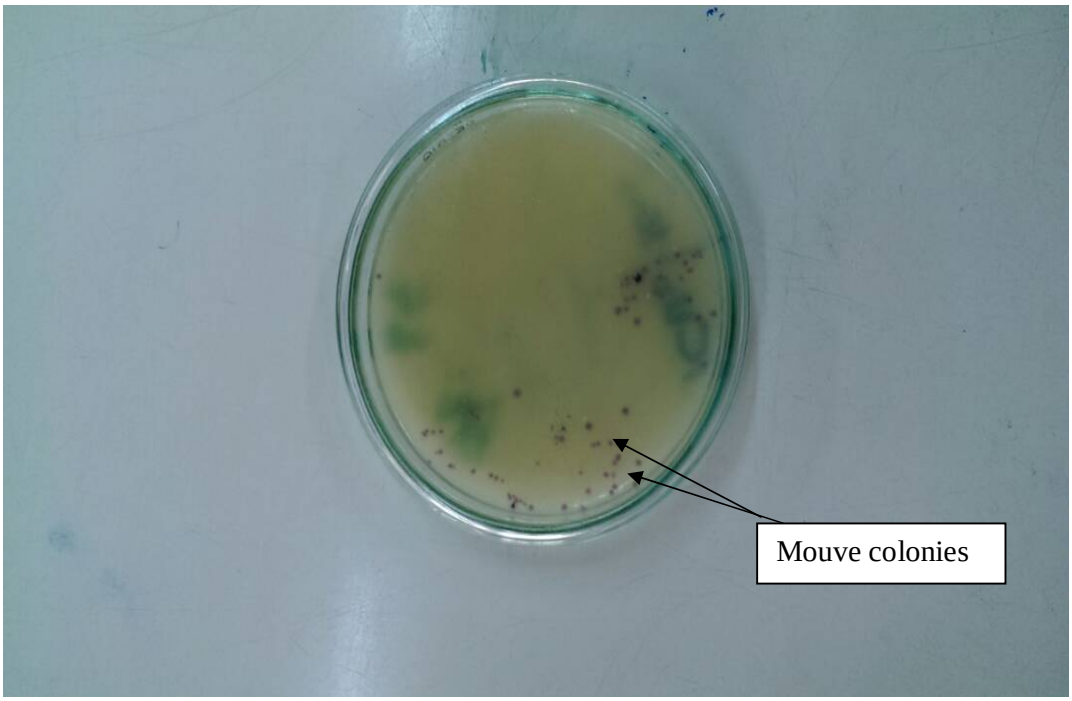


Figure 2: MRSA colonies grown on screening agar (characteristic mouve-pink clour)

3.7.3.2. Gram staining

Gram stain was used to identify isolates on the basis of their cellular morphologies and Gram reactions. One drop of sterile normal saline was placed on a labeled glass slide. A small portion of colony was picked from the culture media using a sterile applicator stick and mixed gently to emulsify on the slide. The slide was left to air dry and then fixed by passing the slide over a flame three times. The slide was left to cool before staining. The slide was stained by flooding the fixed smear with crystal violet stain for 30 seconds.

The stained slide was then washed gently with running water and blotted with paper towel before Lugol's iodine was added and left on the slide for 30 seconds. The iodine was then rinsed off gently with flowing tap water.

Decolourization was done by use of acetone and the slide then rinsed off with water. Counter staining was done by flooding the slide with dilute Carbonfuchsin and allowed to remain on the slide for two minutes. The Carbonfuchsin was then washed off with tap water. The slide was left to air dry. The slide was examined on microscope for the Gram stain reaction.

3.7.3.3. Catalase test

Thirty percent hydrogen peroxide was used as the reagent to test for catalase production.
A colony of the bacteria was picked from the media by using a sterile applicator stick and placed on a clean glass slide. One drop of the reagent was placed on the colony on the slide. Effervescence was observed indicating presence of *S.aureus.*

3.7.3.4. Coagulase test

Zero point five milliliters of 1:10 diluted plasma were put into a test tube and the test tube was inoculated with one colony of the test organism growing from the media. Incubation of the tube was done at 37˚C for four hours and observed hourly for clot formation and also 24 hours for the colonies that form weak agglutination in four hours. Positive test was a complete clot formation or any degree of clot formation while the negative was lack of clot formation.

3.7.3.5. MRSA confirmation and Antimicrobial susceptibility testing

Cefoxitin disk 30 µg was used for MRSA confirmation where by isolates giving a growth inhibition zone diameter of 21mm or less, were reported as oxacillin resistant and considered MRSA (46).

Kirby Bauer disk diffusion was a method for both confirmation of MRSA and antimicrobial susceptibility testing according to the guidelines of the Clinical and Laboratory Standards Institute (46). Five colonies of the organism were emulsified in five milligrams of sterile normal saline and mixed well; the turbidity was compared to 0.5 Mac Farland standards. A sterile cotton swab was used to inoculate the sample to Mueller-Hinton agar plates and allowed to dry followed by addition of antibiotic disks and aerobic incubation at 35^0C for 24 hours. The following antibiotics were used; 20/10 µg Amoxicillin/clavulanic acid,10 µg Gentamycin, 30µg Vancomycin, 10 µg Ampicillin, 30 µg tetracycline, 1.25/23.75 µg Trimethoprim-sulfamethoxazole and 30µg Chloramphenicol. The choice of the antibiotics was done according to Clinical and laboratory standards Institute (CLSI) guideline of 2013.

Susceptibility for Vancomycin was further tested using an in-house prepared Vancomycin Muller-Hinton Agar (VMHA); 16 µg/mL. Isolates with inhibition zone diameter of 15mm or less by Vancomycin disk were considered candidates for inoculation to VMHA and incubated aerobically at 35^0C overnight. Isolates that were to grow on this media were to be

considered VRSA. Algorithim for Vancomycin susceptibility testing was adopted from CDC guideline 2010 (47).

Zones of inhibition were determined by measuring the size of clear zones with a graduated ruler. The measurements were done in millimeters, compared with the CLSI standards for interpretation (CLSI, 2013) and reported by indicating Resistant or Sensitive.

3.7.4. Quality control

Aseptic techniques were observed in all steps of specimens' collection and laboratory processing to minimize contamination. Standard *S.aureus* (ATCC 25923) was used for quality control during gram staining, catalase test, coagulase tests and assurance of disks viability in antimicrobial susceptibility testing. Standard MRSA (ATCC 29213) was used for quality control during MRSA Agar media preparations. The control strains were subjected to the same conditions as the test organisms.

3.8. Data management and analysis

Data obtained from laboratory experiments and data extraction forms were crosschecked and coded before entry into computer software. All data were cleaned and analyzed using Statistical Package for Social Sciences (SPSS) version 20.0. Descriptive statistics; frequencies and cross tabulation were used where as Chi-square test and p values were used as a measure of association and significance.

Binary logistic regression was used to obtain the crude odds ratio for the significant predictors .A *p* value of less than 0.05 was considered to be statistically significant. Data were presented in form of tables and figures.

The independent variables were surfaces of selected items in patients' care areas, cleaning and disinfection frequency, gender and number of patients in a ward/patient's care unit. Primary outcome of interest was MRSA contamination status on studied items.

3.9. Ethical considerations

Ethical clearance was obtained from the Senate Research and Publications Committee of the Muhimbili University of Health and Allied Sciences. Clearance for undertaking the study was obtained from the MNH Administration. Information was given to patients whom near surfaces were sampled. Nature and reasons for undertaking the study were also explained to in charges of the units involved.

3.10. Study findings dissemination plan

Findings from this study will be shared through presentations at department of Microbiology and Immunology, a copy written report will also be submitted to MNH management for possible actions based on the findings. Furthermore a manuscript draft will be prepared for a possible publication.

CHAPTER FOUR

4.0. RESULTS

4.1. Distribution and characteristics of the Patients care units and Studied Objects

Fifty (50) patients' care rooms at MNH, comprising of Thirty six (36) General wards, Seven ICU's and Seven Operating rooms were involved in sample taking (Table1). Of the patients' rooms studied, 15 were Males rooms while 19 were for female patients, and 16 were used for both sexes (ICU's and operating rooms). Thirty one rooms had equal or more than ten patients while 12 rooms had less than ten patients. The hospital cleanness and disinfection protocol in these facilities involved the use of diluted commercial disinfectant, liquid soaps and mops to clean various items surrounding patients receiving care. Cleanness and disinfection was done twice a day at five hours intervals, no special protocol was in place for objects considered highly touched.

Table 1: Distribution of sampled Patient's facilities

		No. of rooms	(%)
Hospital Service	General wards	36	(72)
	ICU's	7	(14)
	Operating theatres	7	(14)
Gender of occupants	Male Wards	15	(30)
	Female Wards	19	(38)
	Mixed gender	16	(32)

4.1.2. Prevalence and Distribution of Environmental MRSA pathogens

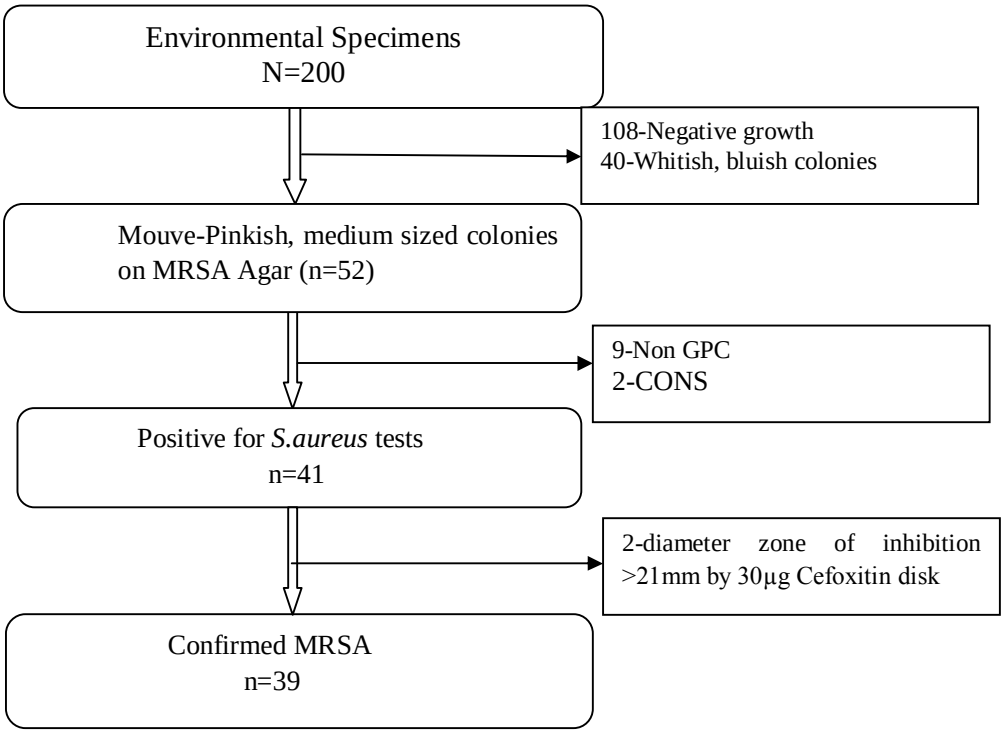
A total of 200 environmental samples were collected during the study, of which108 samples gave no growth on MRSA selective agar; 40 specimens had growth features not distinctive for MRSA; 11 isolates were negative for *S.aureus* identification tests and 41 samples grew *S.aureus.* Among *S.aureus* isolates, 39 were confirmed to be MRSA making a prevalence of 19.5 %(39/200) (Figure 3 below).

Amongst the hospital service areas units, higher prevalence was observed from general wards (20.1%) with no statistical significance difference between medical and surgical

wards (Table 2). Items in operating theatres presented with the lowest MRSA prevalence (14.3%) while those from ICU's had MRSA prevalence of 17.9%.

Prevalence of MRSA on surfaces of items found in areas occupied by female patients was higher(28%) than that of items found in males patients' areas (12.5%), and the difference was found to be statistically significant (p=0.043) (Table2).

As for studied items the highest prevalence was seen in bed surfaces (34%), while surgical trolleys were contaminated least (6%) (Table2).



CONS=Coagulase negative Staphylococcus, GPC=Gram positive cocci

Figure 3: Flow diagram for laboratory MRSA identification

Table 2: Prevalence and Distribution of MRSA in various units ans studied surfaces

	Total Specimens	Positive (%)	*p* Value
Overall prevalence	**200**	**39(19.5)**	
General wards			
Medical	44	9(20.5)	0.899
Surgical	100	21(21.0)	
Special units			
ICU	28	5(17.9)	0.812
OT	28	4(14.3)	
Gender of occupants**			
Female's units	76	22(28.9)	0.026*
Male's units	60	7(11.7)	
Number of Patients***			
≥10 patients	76	23(30.3)	0.006*
<10 patients	96	12(12.5)	
Surfaces			
Bed Surfaces	50	17(34)	0.010*
Door handles	50	13(26)	
Sinks	50	6(12)	
Surgical trolleys	50	3(6)	

Key; *=statistically significant, OT=Operating theater, ICU=Intensive care unit; ** =Data from OT's, ICU's and pediatric wards omitted during analysis; * =Data from OT's omitted during analysis,**

4.1.3. Antibiotic resistance pattern of MRSA isolates

Antibiotic susceptibility testing (AST) was performed to all MRSA isolates giving resistance pattern as summarized in Table 3 below. Highest proportion of isolates was resistant to Ampicillin (87.2%) followed by trimethoprim/sulfamethoxazole (co-trimoxazole) (61.5%). Slightly above fifty percent (51.3%) of isolates had resistance to Tetracycline, while similar proportion of isolates (30.8%) had resistance to

Chloramphenicol and Amoxicillin/clavulanic. Least percent of isolates were resistant to Gentamicin (28.2%) where as none was resistant to Vancomycin.

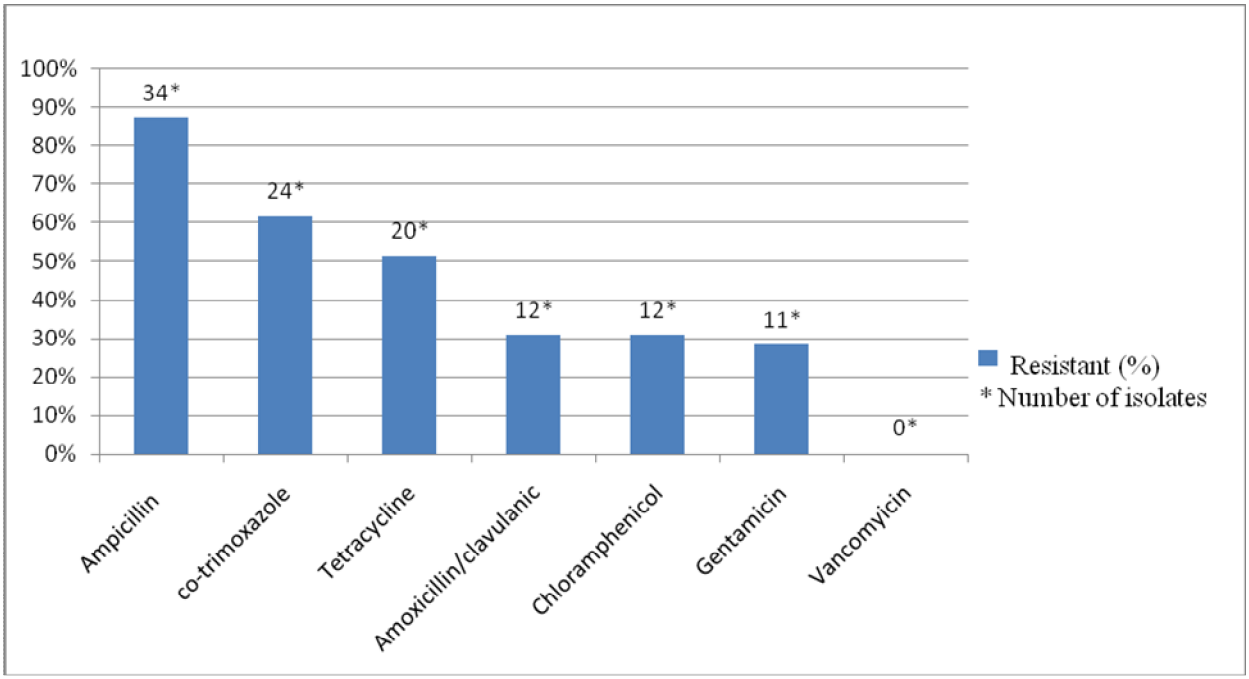


Figure 4.Antimicrobial resistance pattern of 39 Environmental MRSA isolates

4.1.4. Correlate factors for environmental MRSA contamination

Variables that showed significant association by chi square independence tests; patients' number, sex of room occupants and specimen source were further analyzed for binary logistic regression model to determine their odds ratios. There was statistically significant difference in number of MRSA isolates between rooms with ten or more patients in a room compared to those with less than ten patients (p = 0.004), MRSA were four times more likely to be detected in samples from rooms with ten or more patients (Odds ratio [OR] 4.75 [95% confidence interval (CI): 1.624-13.895];Also MRSA were significantly isolated from bed surfaces and doorknobs compared to surgical trolleys (p =0.014 and 0.036 respectively), Isolates were six times more likely to be detected from bed surfaces compared to surgical trolleys (OR 6.26 [95% CI: 1.443-27.153]) while it was five times more likely that door knobs were contaminated by MRSA compared to surgical trolleys (OR 5.21 [95% CI:1.321-25.426]) .

There was no statistical significant difference in number of isolates from sinks compared to those from surgical trolleys *(p* = 0.691). There was also no statistically significant differences in MRSA isolates between the patients room occupied by the different sexes (OR 1.650 [95% CI: 0.139-19.571]; *p* = 0.691). (Table 4)

Table 3: Correlates of MRSA contamination by bivariate logistic analysis

Variables(no. of specimens)	**MRSA (%)**	**OR(95%CI)**	***p*- value**
Gender of occupants**			
Female (76)	22(28.9)	1.650(0.139-19.571)	0.691
Males(60)	7(11.7)	1	
Number of patients***			
≥10 patients(76)	23(30.3)	4.75(1.624-13.895)	0.004*
<10patients(96)	12(12.5)	1	
Specimen source			
Bed surfaces(50)	17(34)	6.26(1.443-27.153)	0.014*
Door handles(50)	13(26)	5.21(1.321-25.426)	0.036*
Sinks(50)	6(12)	0.906(0.296-2.771)	0.863
Surgical trolleys(50)	3(6)	1	

Key; *=statistically significant; ** =Data from OT's, ICU's and pediatric wards omitted during analysis; * =Data from OT's omitted during analysis; Reference values were set to variables known to be of less likelihood for positively predicting the outcome**

CHAPTER FIVE

5.0. DISCUSSION

To the best of our knowledge, this is the first study in our settings undertaken to elucidate the role of frequently accessed items in patient's care environment as secondary reservoir of medically important pathogens. On the other hand, compared to previous studies elsewhere this is among few studies that involved diverse hospital units and studied items, hence more inclusive findings.

The overall prevalence of MRSA on different surfaces from this study (19.5%) is similar to the findings from some studies undertaken in the region. Studies in Uganda and Egypt, respectively documented prevalence of MRSA on hospital inanimate surfaces as 19 % and 21.8% (29,33). The findings from Uganda might have shared even closer figures with the current study as the two study areas are of similar settings (both studies undertaken at referral hospitals) with similar study designs. On other hand the current findings show a higher prevalence of MRSA on inanimate surfaces compared to findings from a study in Nigeria and Ethiopia where the reported prevalence was 5% and 1.7%, respectively (3,28). Although details on the compared studies aren't much provided, variations in environmental MRSA prevalence can be expected, especially if factors like geographical location (prevalence of MRSA colonization in local population), hospital's characteristics (size, patient load), hospital's cleaning/disinfection protocols, study design (surfaces sampled, sampling technique used), and surface's characteristics (type of material and texture) are taken into consideration.

Nevertheless, as in this study samples were taken shortly after daily cleaning, our findings on the prevalence rates provide an alarming indication on ineffectiveness of the process. Moreover these findings show that MRSA is capable of frequently contaminating hospital contact surfaces even in the absence of any reported or known outbreak.

Findings from this study have shown that highest prevalence was from general wards (20.1%) compared to ICU's and Operating rooms. These findings are in line with those in some earlier reports. A study undertaken in Egypt to assess MRSA prevalence on items from ICU reported as low prevalence as four point six (4.6%) while at different time in

Nigeria a study undertaken in general wards settings reported as higher as 25% prevalence (28,33).

Although the difference between medical and surgical wards wasn't significant in our case, the higher prevalence of MRSA on these general wards may be attributed to diverse clinical conditions of patients attended in, of which a proportion might be colonized or infected by MRSA. It is also in general wards where personnel and items flow management is less strict as compared to ICU or operating rooms, hence the differences. Despite the low number of detected pathogens from ICU's and Operating rooms, their presence should be importantly considered as possibility of transmission and effects to patients receiving care in these areas increases owing to their immuno compromised states.

Higher prevalence of MRSA in facilities with female patients' documented in this study portrays the contrary nature of association between contamination and patients' gender as the findings from elsewhere reported lesser contamination in female wards (37). The role of gender in defining the contamination rates in hospital settings can be connected to difference in hygiene practices or the different rate of MRSA colonization/infection between male and females in the hospital, both of which were not measured in this study.

MRSA were more prevalent from bed surfaces (34%) and least from surgical trays (6%). Higher prevalence of MRSA from bed surfaces has similarly been reported elsewhere in Nigeria and UK where in both studies bed surfaces took the lead on the contaminated objects (28,36). The consistently higher prevalence of MRSA on bed surfaces suggest the fact that sites closer to patients are likely to be contaminated than those farther. The beds surfaces are considered patient contact surfaces and therefore the detected pathogens might have been shed by the infected/colonized patients occupying the particular beds. Though patient's status for colonization and/infection wasn't determined in this study, it has been reported elsewhere that MRSA colonization especially at the groin area correlates strongly with colonization of the body and environment, predominantly bed surfaces (4).The 26% prevalence of MRSA on door handles is higher than one earlier reported in Nigeria (28). The disparity in findings might be explained by the nature of selected door handles between these studies, not specified in earlier report but as for the current study mostly targeted were toilet door handles hence expected prevalence in view of poor toilet hygiene among patients. Generally however, door handles are considered both patients and public contact

surfaces hence increased chance for their contamination, with lesser subjection to thorough disinfection on regular basis as they are usually not visibly soiled.

The 12% MRSA prevalence on hospital sinks is lower compared to the one found in UK where it was 33% (25). Timing for specimens' collection however was different between these two studies where as in an earlier report specimens were taken before cleaning and disinfections, probably contributing to the reported higher prevalence. In several occasions contamination of hand washing sinks can be defined by bed-sink ratio with lower ratios affecting the trends towards decreasing contamination rates, however no attempt was made in establishing the bed-sink ratio in patients rooms included in the present study.

The comparably least prevalence of MRSA from surgical trolleys (6%) corresponds to the findings in Nigeria where surgical trolleys and medical tables comprised least prevalence of the studied objects (48). Similar reports on low contamination rates of surgical trolleys corroborate the idea that items whose exposure is limited to healthcare workers only (HCW contact items) have lesser chances for contamination as this is a group of individuals likely to adhere to aseptic and contact precaution techniques including hand washing.

Patients' load was significantly associated with MRSA status on the studied surfaces as evidenced by higher prevalence of MRSA in wards with ten or more patients compared to those with less than ten patients (p =0.01).These findings support earlier observation elsewhere in which single- patient bedrooms design had significantly reduced hospital acquired infections (HAI's) compared to semi private or open wards design(38). The lower prevalence of MRSA in rooms with fewer patients may be in part related to other changes to the physical environment associated with fewer patients rooms including improved sink to bed ratios, semi-private toilets, and more frequent room cleaning, particularly terminal room cleaning that is done when the room is vacated after patients discharge. Moreover fewer patients might be associated with reduced opportunities for direct contact with contaminated surfaces and can potentially prevent indirect contact by limiting HCWs from moving from patient to patient without performing hand hygiene. It was also observed during this study that the flow of personnel (patients, visitors and HCW's) was lesser manageable in those units that had larger number of patients compared to units with fewer patients such as ICU's and operating theatres.

The antibiotic resistance of MRSA isolates from this study shares the pattern with isolates from similar studies in the region. High rates of resistance to Ampicillin and Trimethoprim-Sulfamethoxazole with least resistance to vancomycin and Amoxicillin/clavulanic were also reported from other studies on environmental MRSA in the region (29,49). Furthermore the general picture of susceptibility pattern from this study also correlates with the susceptibility pattern of MRSA isolated from patients with hospital acquired infections in the region, suggesting further the possibility of cross transmission from environment to patient and vice versa.

MRSA strains are particularly resistant to all ß-lactam agents, including cephalosporins and carbapenems. The observed extended resistance to other commonly used antimicrobial agents can be explained by persistent selective pressure by antibiotics and disinfectants within healthcare settings. Like findings from other studies, it can also be derived from this study that in an event of MRSA infections resulting from cross transmission from hospital environment, the therapeutic choice will be narrowed where as vancomycin might be a drug of choice.

5.1. Study limitation

1. This study was carried out at a tertiary hospital in urban settings thus generalization of the prevalence to smaller or peripheral facilities should be made with caution.
2. Despite the protocol for cleaning and disinfection being commonly used across the hospital units, individual variability on implementation was not assessed, and this could be an important confounder on the contamination pattern.
3. Quality of disinfectants used was not assessed, quality of disinfectants would determine rate at which the organisms are detected from cleaned surfaces.
4. Patient’s MRSA colonization and/infection status was also not known, during the course of sample taking, this could also be an important variable affecting MRSA contamination of items in the hospital.

CHAPTER SIX

6.0. CONCLUSION AND RECOMMENDATIONS

6.1. Conclusion

There was high prevalence of environmental MRSA from this study with bed surfaces being mostly contaminated; number and gender of occupants in a hospital unit were significant predictors. It is therefore evident that areas of hospital environment present underestimated important reservoir for HAI's associated pathogens even in non outbreak settings unlike earlier reported.

6.2. Recommendation

In line with the findings of this research i recommend the following.

i. Establishing stricter guidelines on managing the flow of personnel and equipments between hospital units and emphasis on single patients' facilities.
ii. Conducting routine surveillance of hospital surface contamination i.e. even in non outbreak situations.
iii. Larger prospective studies are needed to assess the correlation between environmental MRSA and the acquisition of MRSA by patients or the vice versa.

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