EPIDEMIOLOGICAL INVESTIGATION OF MULTI-DRUG RESISTANT *Staphylococcus aureus* FROM PATIENTS AND ENVIRONMENT AT MOROGORO REFERRAL REGIONAL HOSPITAL, TANZANIA

NANCY GWIMO

A DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN PUBLIC HEALTH AND FOOD SAFETY AT THE SOKOINE UNIVERSITY OF AGRICULTURE. MOROGORO, TANZANIA.

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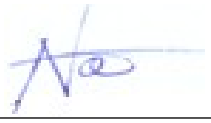
ABSTRACT

The MDR *S. aureus* is a significant nosocomial pathogen and has been associated with significant morbidity and mortality in the world. In hospital settings, pathogens, such as multidrug resistance (MDR) *Staphylococcus aureus* can be transmitted via health care workers (HCWs), patients, and visitors resulting in hospital-associated infections that are difficult to treat. This was a cross-sectional study, comparing carriage and pattern of drug resistant *S. aureus* between patients and equipment in the hospital. The study involved sampling of patients and environment at MRRH. It undertook the following: isolation of *S. aureus*, gram staining, catalase test (slide test), coagulase test, antibiotic susceptibility of isolated *S. aureus*, D Test, MRSA detection and questionnaire survey. An assessment was done on the distribution of *Staphylococcus aureus* based on age and gender and the risk factors associated with *Staphylococcus aureus* in the hospital. Also, antibiotic susceptibility test of isolated *Staphylococcus aureus* was done and from the questionnaire survey, assessment of awareness and perception among patients at the Morogoro Regional Referral Hospital (MRRH) was done. Results of this research highlighted the burden and pattern of Antimicrobial Resistance (AMR) at the hospital and can be used as a model to further collect data in Tanzania on MRSA and essentially provide an insight into the magnitude of the problem. A higher prevalence of MRSA was observed in patients compared to the environment around the hospital, making it significantly different.

DECLARATION

I, **Nancy Gwimo**, do hereby declare to the Senate of Sokoine University of Agriculture that this dissertation is my own original work done within the period of registration and that it has neither been submitted nor being concurrently submitted for a higher degree award in another institution.



07/04/2021

Nancy Gwimo — Date

(MSc Public Health and Food Safety candidate)

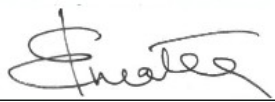
The declaration is hereby confirmed by:

Dr. A. A. S. Katakweba — Date

(Supervisor)

Prof. S. Kimera — Date

(Supervisor)



7th April 2021

Prof. M. Matee — Date

(Supervisor)

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DEDICATION

I would like to dedicate this work to my grandparents; Mrs Ntegeye Rachel, and in memory of the late Mr Sylvain Gwimo Ndimurwimo, the late Rev. Daniel Karimuribo and the late Mrs Phoebe Karimuribo. I honour and respect the sacrifices you have made in your lives and I acknowledge that I am where I as a result of that. Thank you!

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LIST OF ABBREVIATION AND ACRONYMS

AMR	Antimicrobial Resistance
CA-MRSA	Community Acquired- Methicillin-Resistant *Staphylococcus aureus*
CDC	Centers for Disease Control and Prevention.
CLSI	Clinical and Laboratory Standard Institute.
HAI	Hospital Acquired Infection
HA-MRSA	Hospital Acquired- Methicillin-Resistant *Staphylococcus aureus*
HCW	Health Care Workers
MDR	Multi-Drug Resistant
MRRH	Morogoro Regional Referral Hospital
MRSA	Methicillin-Resistant *Staphylococcus aureus*
MSSA	Methicillin-Susceptible *Staphylococcus aureus*
NIMR	National Institute for Medical Research
OTC	Over-The-Counter
PBPs	Penicillin-Binding Proteins
RCT	Randomized Controlled Trial
RHMT	Regional Health Management Team
Scene A	Staphylococcal cassette chromosome mec, (mec A regulatory gene)
URT	United Republic of Tanzania
VRSA	Vancomycin-Resistant *Staphylococcus aureus*

WHO	World Health Organization

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Staphylococcus aureus is a major human pathogen that can cause infections varying from mild to moderate skin and soft tissue infections to invasive life-threatening diseases (Lakhundi and Zhang, 2018). Due to its numerous virulence factors and ability to acquire resistance to most of the antimicrobials, it has been branded as a “superbug” (Lakhundi and Zhang, 2018). The most frequently colonised areas are nares, throat and perineum (Sollid *et al.,* 2014). When collecting specimens for isolation of *S. aureus,* nasal swabs are often preferred because the nasal vestibule is the primary site of *S. aureus* carriage, with the anterior nares of the nose being the primary carrier (Sollid *et al.,* 2014). The *S. aureus* bacterium is the second most common organism causing nosocomial infections (Al-Zoubi *et al.,* 2015). The extensive use and exposure to antimicrobials in hospital settings has resulted into multi drug resistant (MDR) *S. aureus* (Al-Zoubi *et al.,* 2015). For example, Methicillin-resistant *S. aureus* (MRSA) cause infections that are difficult to treat, thus leading to prolonged or more expensive treatments which result in longer hospital stays, lost productivity and increased mortality (WHO, 2018).

The spread of MRSA poses a serious challenge in hospital acquired infections (HAI). In hospital environmental, MRSA has been extensively reported in different areas, including in intensive care units, burn units, isolation rooms and general wards. MRSA can be recovered from 1% to 27% of surfaces in patient rooms (Yuen *et al.,* 2015). Contamination through MRSA varies among different hospital ward surfaces, as contamination is influenced by various factors such as the condition of the patients, the

ward setting, overcrowding, and hygienic practices. It has been well documented that high-touch surfaces are major reservoirs for MRSA in hospital environments (Yuen *et al.*, 2015). Higher levels of drug resistance are often in patients with a longer history of antimicrobials usage (Sighn *et al.,* 2018), and have been associated with increased mortality (Pastagia *et al.,* 2012). Kariuki *et al.* (2018) highlighted increased incidence of drug resistance in hospital acquired infections in Africa, and similar observations have been made in Tanzania (Nkuwi *et al.,* 2018). This situation has prompted the African Centers of Diseases Control and Prevention (CDC) to develop an antimicrobial resistance (AMR) framework that will address issues of surveillance of antimicrobial resistance (CDC, 2019).

1.2 Problem Statement

Antimicrobial resistance remains a huge problem globally but disproportionally affects most low-income countries, including Tanzania, with limited treatment alternatives (Blomberg *et al.,* 2004). According to a study done by WHO (2018), the problem of AMR has been steadily increasing in East Africa, including Tanzania (Wangai *et al.,* 2019). However, the data remains inconsistent due to a lack of effective and systematic routine surveillance systems (Wangai *et al.,* 2019). Lack of resources, poor hygiene as well as poor infection control practices play a huge role in the spread of community and hospital-acquired infections (Ampaire *et al.,* 2017). In overcrowded hospitals, with limited infection control measures, MDR bacteria can easily spread between health care workers, patients and visitors via contaminated surfaces (Nyambura *et al.,* 2019). Several studies have shown resistance beyond just Methicillin resistance (Wangai *et al.,* 2019). However, the MRSA bacterium is the second leading cause of nosocomial infections and has been recognized by the WHO as a priority organism in the control of AMR (WHO, 2018).

1.3 Study Justification

Studies conducted in Tanzania, with regards to *S. aureus* resistance have been limited to Dar es Salaam, Mwanza and Arusha. Morogoro being the sixth largest city in the country as reported by The United Republic Tanzanian Government, Census General Report (URT, 2012), with one of the biggest referral hospitals in the country, it was imperative to have a set of data to contribute to the AMR data frame from the region. In overcrowded hospitals, with limited infection control measures, MDR bacteria can easily spread between health care workers, patients and visitors via contaminated surfaces (Nyambura *et al.*, 2019). The comparison between patients and hospital equipment helped to identify risk factors that can help eliminate the spread of MRSA. Characterization of MRSA isolates helped distinguish the strain resistance to antimicrobials. The results showed the problem of MRSA at the Morogoro Regional Referral Hospital (MRRH), by showing the link from different sources, patients and hospital environment, and further contributed to the lack of data within the region.

1.4 Main and Specific Objectives

1.4.1 Main objective

To determine multi-drug resistant *S. aureus* between patients and inanimate surfaces at Morogoro Regional Referral Hospital.

1.4.2 Specific objectives

i. To determine antimicrobial susceptibility patterns of Methicillin-resistant *Staphylococcus aureus* and Methicillin-susceptible *Staphylococcus aureus* isolates and thus determines the prevalence of Methicillin-resistant *Staphylococcus aureus* carriage among patients and inanimate surfaces at MRRH.
ii. To determine risk factors associated with Methicillin-resistant *Staphylococcus aureus* carriage among patients and inanimate surfaces at MRRH.
iii. To assess awareness of antimicrobial usage and perceptions of the MRRH.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Methicillin-resistance and Susceptibility to *Staphylococcus aureus*

Methicillin-resistant *Staphylococcus aureus* (MRSA) can be defined as the prototype of multi-drug resistant bacterial pathogens and forms part of major causes of nosocomial infections in the world (Pantosti and Venditti, 2009). The *S. aureus* pathogen is a ubiquitous microorganism that is able to settle on human skin and anterior nares of healthy humans, with approximately 50% of the human population being persistent or intermittent carriers (Pantosti and Venditti, 2009). This gram-positive bacterium becomes a serious threat when it becomes resistant to treatment and can thus cause a wide range of infections involving the skin, soft tissue, bone, joints and infections associated with prostate devices or the indwelling catheters (Hassoun *et al.,* 2017).

Before MRSA was a problem, penicillin was used to treat *S. aureus* infections; however, overtime *S. aureus* overpowered penicillin and thus became resistant (Pantosti and Venditti, 2009). To combat that problem, methicillin was created, which was penicillin, but it worked by blocking the penicillin binding protein (PBPs), which are responsible for the construction and maintaining bacterial cell walls. This forced the *S. aureus* resistant strains to form a new protein known as PBP2a, which was not blocked by methicillin and it replaced the other PBPs, therefore allowing *S. aureus* to survive in the presence of methicillin (Pantosti and Venditti, 2009). There are two types of MRSA strains noted, hospital-acquired (HA) MRSA and community- associated (CA) MRSA (Falagas *et al.*, 2013). The epidemiology of MRSA is always changing Falagas *et al.* (2013), therefore making it harder to distinguish between HA-MRSA and CA-MRSA. Unlike MRSA,

which is resistant to methicillin, methicillin-susceptible *Staphylococcus aureus* (MSSA) also exists. The main difference between MRSA and MSSA is that MSSA cells are susceptible to β-lactam antimicrobials and are less virulent than MRSA (Rozgonyi *et al.*, 2007). The knowledge of MSSA genetic backgrounds and associated virulence factors is very important for understanding the emergence of HA-MRSA and CA-MRSA (Breurec *et al.,* 2010). MRSA are generated when MSSA acquires the me A gene, which is carried on a mobile element known as the staphylococcal cassette chromosome mec A (SCCmec A) (Moellering, 2012). Unlike MRSA, MSSA is genetically more diverse than MRSA and it is believed that MRSA originated from a limited number of MSSA epidemic lineages through the transfer of SCCmec A (Breurec *et al.*, 2010).

2.2 MRSA AND MSSA Distribution in Tanzania and Africa

MRSA had a prevalence rate of lower than 50% in 2000, however, it has risen extensively risen over the last couple of years in many African countries, except for South Africa (Falagas *et al.,* 2013). The biggest problem with MRSA reports in Africa is that; not enough data is being collected everywhere, therefore making it difficult to pin down the exact prevalence. Over the last decade, countries like Nigeria, South Africa and countries in the basin of the Mediterranean e.g., Egypt and Algeria have more consistent data (Falagas *et al.,* 2013). Unlike MRSA, the documentation of MSSA in Africa is also poor (Breurec *et al.*, 2010). In a study conducted by Breurec *et al.* (2010), in five major towns in Cameroon, Madagascar, Morocco, Niger and Senegal; they concluded that there is a high prevalence of luk-PV genes among MSSA strains and that is a major concern. They also concluded that human mobility has been the number one factor in the spread of MSSA clones endemic to Africa.

The number of MRSA cases is said to be on the increase in Tanzania over the last couple of years (Joachim *et al.,* 2017). Previous studies conducted in Tanzania at Muhimbili National Hospital (MNH) and Bugando Medical Centre have documented an increasing prevalence of hospital-acquired MRSA by 0.4% (Mshana *et al.,* 2009), 8% (Blomberg *et al.,* 2007), 16.3% (Mshana *et al.,* 2009 IS THIS Mshana *et al*., 2009a or 2009b) and 23% (Moyo *et al.,* 2012). These studies had similar sampling approaches and used clinical specimens from hospitalized patients who presented with symptoms and/or signs of infection. However, independent studies characterising the pattern of MSSA within patients have few documented data in Tanzania. Joachim *et al.* (2018) did a comparison of MSSA and MRSA carriage among HCW in tertiary level hospitals in Tanzania and higher rates were observed for MSSA than MRSA.

2.3 Predilection Sites of MRSA in Humans

It was always believed that humans are the primary reservoirs for *S. aureus*, with asymptomatic nasal, perineum and/or nasopharyngeal carriage being the major sites from which these organisms spread from and cause infections (Moellering, 2012). However, a shift in nasopharyngeal colonization rates for CA-MRSA to other parts are lower than for MSSA. Heavy nasopharyngeal colonization is still considered a risk factor to increased infection, but a study conducted in London disputed nasal carriage of MSSA would result in MRSA formation once admitted into hospital (Moellering, 2012). This proved that MRSA does not have a selective advantage over MSSA in colonizing the nasopharynx. However, it still remains that; CA-MRSA can easily spread by direct contact and via contaminated fomites. The rate at which an infection is developed in humans as a result of MRSA colonization is estimated to be around 30% (Joachim *et al.,* 2017). The bacterium is a frequent cause of recurrent skin and soft tissue infections for example atopic

dermatitis, which is a recurrent infection as a result of MRSA, can often lead to eczema (Ong, 2014).

2.4 MRSA in Environments

The MRSA bacterium is capable of surviving for days to weeks on environmental surfaces, including hospital surfaces (Boyce, 2007). The frequency at which surfaces are touched in hospitals plays a huge role in high numbers of bacteria. General studies have further shown how HCW in particular are instrumental in the spread of the bacteria as their hands or gloves can be contaminated (Boyce, 2007). However, identifying the actual source of MRSA within the hospital environment is quite expensive therefore rapid screening methods, such as whole-genome sequencing (WGS), are useful to determine the source of the MRSA (McKew *et al.,* 2020). Unknowingly, in the process of treating patients, moving or holding equipment can transfer the bacteria to susceptible patients, other HCWs and the environment (Boyce, 2007).

2.5 How MRSA Develops in Humans

The MRSA bacterium is believed to have been first observed in 1961 and has spread ever since through different strains (Moellering, 2012). MRSA develops in humans when the mec A gene is acquired by the bacterium in the sequence type (ST) 8 which is believed to have evolved from the (ST) 250. It is believed that ST247, which is a minor variant of ST250, is one of the major MRSA strains circulating the world today (Moellering, 2012). Most MRSA bacteria are believed to have colonised the body and thus originate from such strains (Otto, 2012). Some colonization sites, such as the perineum and the throat are poorly understood and thus making it hard to eradicate carriage. Colonization of the body occurs when surface-anchored *S. aureus*-binding protein interacts with human matrix molecules (Otto, 2012).

2.6 Effect of MRSA in Humans and the Environment

Majority of MRSA infections are considered to be non-life-threatening, however the organisms are capable of producing some devastating diseases in some patients (Moellering, 2012). Moellering (2012), further lists some of these infections; necrotizing fasciitis, septic thrombophlebitis of the extremities, a ‘pelvic syndrome’ (septic arthritis of the hips, pelvic abscesses and pelvic septic thrombophlebitis), water-house-Frederickson syndrome and rapidly progressive pneumonia. It was further observed that MRSA became a risk factor among elder patients as opposed to younger ones (Pastagia *et al.,* 2012). Older patients are often immune-compromised, and a history of antimicrobial consumption increases the risk of acquiring resistance (Pastagia *et al.,* 2012).

Contaminated hospital environments are becoming responsible for multi-resistant organisms (MRO), (Fernando *et al.,* 2017). Methicillin-resistant *Staphylococcus aureus* (MRSA) can survive for long periods within the environment and thus the contamination may spread from the environment to the patients and vice versa (Kurashige *et al.,* 2016). The initial contamination on environmental surfaces is the result of the environment being touched by the hands of patients with MRSA colonization/infection (Kurashige *et al.,* 2016). Therefore, causing a continuous cycle of contamination.

2.7 Control of MRSA in Humans and the Environment

Immunotherapy has been one of mitigation at the forefront of controlling MRSA in humans in developed countries, and this has evolved over the last couple of years depending on the susceptibility of the antimicrobial agents (Moellering, 2012). More than half of staphylococcal infections were formerly treated through empiric therapy with Penicillin or Cephalosporin (Baorto *et al.,* 2019). However, with the rising level of

resistance and CA-infections, some experts have recommended combinations of therapy e.g., penicillin-resistant penicillin (flucloxacillin, dicloxacillin), clindamycin or quinolone. Others suggest clindamycin, tamethoprim-sulfamethoxazole (tmp-smx), rifampin or doxycycline. The two most remarkable resistance achieved by *S. aureus* were to the antimicrobials, methicillin and vancomycin (Hiramatsu *et al.,* 2014).

The Clinical and Laboratory Standard Institute (CLSI) (2019), recommends four groups of antimicrobial agents for testing of *S. aureus,* see Table 1. According to Table 1, the four groups of antimicrobial agents are divided into four categories; Group A is the primary testing and reporting, Group B is the optional primary test report selectively; Group C is the supplemental report selectively and the final group is Group D which is the supplement for urine test only. For this study, various antimicrobial agents were chosen across the four groups.

Vaccine strategies could be considered to be the most effective way to control human infections resulting from MRSA, however the organism still poses many challenges to the developed vaccines (Moellering, 2012). To date, no vaccines have been successfully developed. New approaches to treating MRSA infections have been proposed, such as searching for essential genes found in bacteria and not found in mammalian cells (Moellering, 2012).

This is because bacteria initiate protein synthesis by incorporating N-formyl-methionine as the initial amino acid of synthesized polypeptide chain, this results in the N-formyl-methionine cleaving off, allowing the resulting protein to function (Moellering, 2012). As Moellering (2012), further explains that this step is not found in mammals but rather in bacteria and it is ideal for antimicrobial targeting within the environment, cleaning or

disinfection of the environment is often encouraged to reduce transmission of MRSA (Boyce, 2007). However, may not always be effective. Therefore, environments such as hospitals often need improved methods. Currently, studies suggest that hydrogen peroxide vapour technology is the most effective method of decontaminating environments, especially in the healthcare settings (Boyce, 2007).

Table 1: Four groups of antimicrobials that are used to testing of *S. aureus*

Antibiotic group	Antimicrobial agent
Group A - Primary Testing and report	● Azithromycin[b]/Clarithromycin[b]/ Erythromycin[b] ● Clindamycin[b] ● Cefoxitin (surrogate test for oxacillin) ● Oxacillin ● Penicillin ● Trimethoprim- ● Sulfamethoxazole
Group B – Optional Primary Test Report Selectively	● Ceftaroline ● Daptomycin ● Linezolid ● Tedizolid ● Doxycline ● Minocycline ● Tetracycline ● Vancomycin ● Rifampin
Group C– Supplemental report Selectively	● Chloramphenicol ● Ciprofloxacin/levofloxacin ● Moxifloxacin ● Gentamicin ● Dablavancin ● Oritavancin ● Telavancin
Group D– Supplemental for urine only	● Nitrofurantoin ● Sulfisoxazole ● Trimethoprim

CHAPTER THREE

3.0 METHODOLOGY

3.1 Study Site

The study was conducted at the MRRH. The hospital is situated 6º49’38, 6” S in latitude and 37º40’18, 8”E in longitude, in Morogoro Town, in Tanzania, see Fig. 1. The MRRH is the largest referral hospital in the Morogoro Region. It is one of the two referral hospitals in the region, St. Francis Hospital being the second. The Hospital has bed capacity of 450, with about 562 working staff (URT, 2018).

3.2 Study Design and Sampling Techniques

This was a cross-sectional study involving sampling of patients and the environment at MRRH. Patients in six selected wards (male recovery ward, female recovery ward, male surgical ward, female surgical ward, maternity ward and the eye clinic) were selected, based on who the head nurse in each ward felt was fit enough to take part in the study, upon obtaining informed consent from patients. Each ward had a head nurse in charge and often these head nurses had different shifts. Upon arrival, it was expected to go to the head nurse in charge of that shift and explain the reason being done in that ward, then the nurse would indicate which patients were conscious enough to participate. Children below 12 years of age and patients who were using antimicrobials at the time of recruitment or within 2 weeks were excluded.

The limitations were due to the discouragement of prescription antibiotics to children as it poses a risk to increased chances of obesity (McCarthy *et al.,* 2018). Structured

questionnaire was used to collect social demographic information including age, sex, level of education and residence. Risk factors associated with MRSA including current and previous medical history and use of antimicrobial in the past 3 months were excluded. Swabs were collected from patients and from points of contact: bed nets, bed rails, patient tables, ward door handles, faucets, wheelchairs and trolleys.

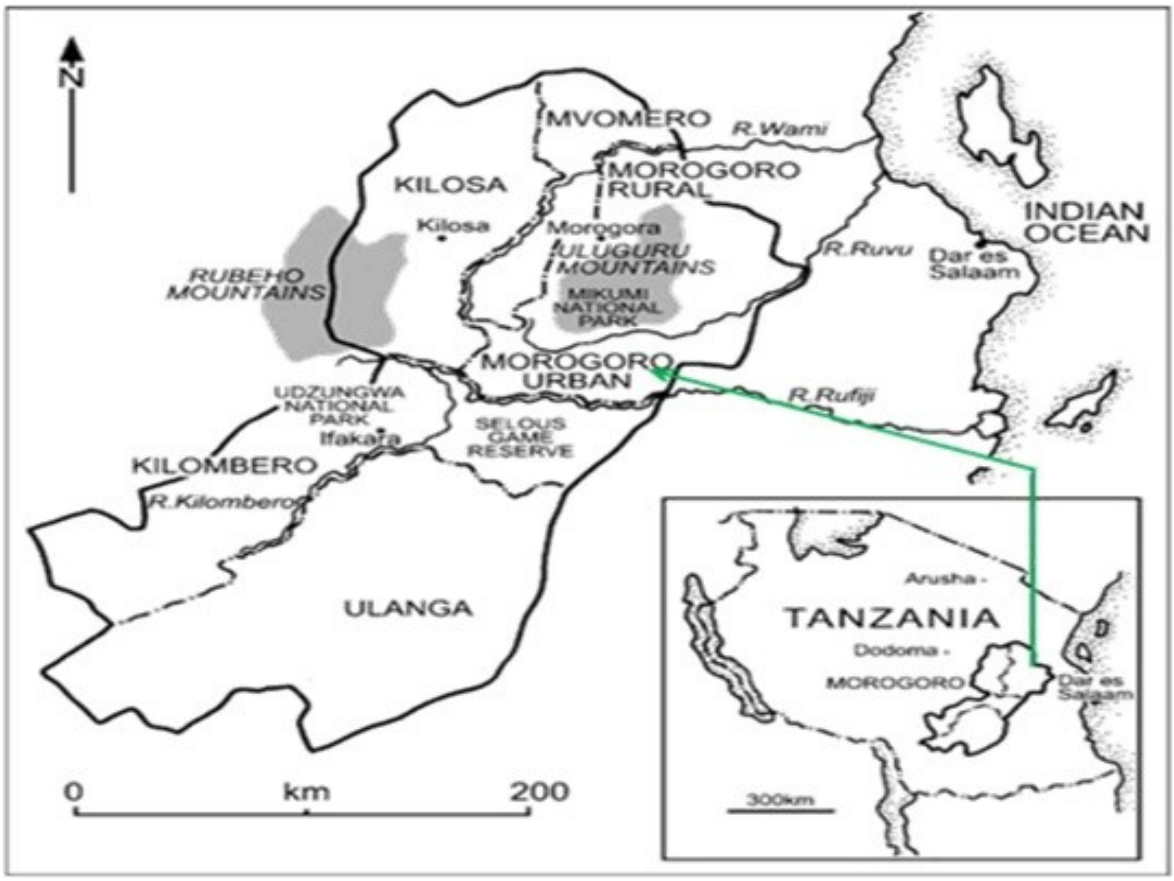

Figure 1: Map of Morogoro region Tanzania showing Morogoro urban , where MRRH is located

Source: Sokoine University of Agriculture GIS Department

3.2.1 Sample sources and size determination

The approach to determine the sample size was done using the prevalence of MRSA in Dar es Salaam hospitals of 8.5% as determined by Joachim *et al.* (2017) by using the

formula by Gebremedhn *et al.* (2016), $n= Z^2\alpha/2\ P\ (1-P)/e^2$, which gave a total sample size of 120. Therefore, a total of one hundred patients were randomly selected from six different wards. Then a further one hundred environmental surface samples were collected from the six different wards. A total of 200 samples were enrolled in this study, 100 of the samples came from patients and another 100 came from the hospital environment.

The samples taken from 100 patients were from six different wards; male recovery ward (20%), female recovery ward (16%), male surgical ward (15%), female surgical ward (8%), maternity ward (20%) and the eye clinic (21%). From the total sample, 52% were male patients and 48% female patients. Similarly, for the environmental surfaces, samples were taken from 100 points of contact: bed nets (14%); bed rails (29%); patient tables (15%); ward door handles (14%); faucets (14%); wheelchairs (8%); and trolleys (6%). The environmental samples were collected from the six different wards; male recovery ward (39%), female recovery ward (33%), male surgical ward (5%), female surgical ward (9%), maternity ward (11%) and eye clinic (3%). The environmental samples distribution based on suggested hospital samples for 100 hospital admissions by Kurashige *et al.* (2016).

3.2.2 Isolation and identification of *Staphylococcus aureus*

Once the swabs were collected, they were placed into bijou bottles containing sterile Cary-Blair transport media. All samples were stored in a cool box with ice packs before they were transported to the microbiology laboratory, within 2 hours of collection. Samples were cultured in blood agar and mannitol salt agar (Oxoid, Basingstoke, UK). The bacterium culturing was done by streaking each swab on labelled media then incubated at 37°C and examined for growth after 24 hours and thereafter, readings were taken and recorded (Geofrey *et al.*, 2015).

3.2.2.1 *Gram staining*

Specimen was placed on a slide, then flooded with crystal violet and this was left for 60 seconds. Then the slide was washed with water for 5 seconds until the specimen appeared blue-violet to the naked eye. The slide was then flooded with iodine solution and let to stand for about a minute. Then the slide was rinsed with water for 5 seconds. Then ethanol drops were added to the slide until the violet colour was removed from the specimen and washed with water for 5 seconds. Finally, the slide was flooded with dye again and was left to stand for about 1 minute. Then the slide was rinsed again for 5 seconds to remove excess dye and gram-positive bacteria were identified by the formation of purple cocci in grape-like clusters (Smith and Hussey, 2005).

3.2.2.2 *Catalase test* (slide test)

A sterile wooden stick was used to transfer a small amount of colony growth in the surface of a clean, dry glass slide. The drop of 3% H2O2 was placed on the glass slide and the evolution of oxygen bubbles was formed (Hansen and Steward, 1978).

3.2.2.3 *Coagulase test*

Plasma of 1 in 10 physiological saline (mix of 0.2 ml of plasma with 1.8 ml of saline) was diluted. Then 18-24hr broth culture was placed into a sterile test tube, followed by 0.5 ml of diluted rabbit plasma pipetted in the test tube. The 5 drops of (0.1 ml) were added and mixed. The test tube was incubated at 35 degrees Celsius and after 1 hour clotting was observed (Thirunavukkarasau and Rathish, 2014).

3.2.3 Antibiotic susceptibility of isolated *Staphylococcus aureus*

Isolates were tested for antimicrobial susceptibility using antibiotic disk from Oxoids

(Onanuga and Temedie, 2011) that contained six antimicrobials; erythromycin (15µg), azithromycin (15µg), ofloxacin (15µg), gentamicin (10 µg), ciprofloxacin (5µg) and cefoxitin (30 µg). These were chosen due to their wide availability and usage in Tanzania (Onanuga and Temedie, 2011). A standard inoculum was prepared by direct colony suspension in saline and compared with 0.5 McFarland standard turbidity and inoculated on the Muller-Hinton agar plate (Oxoid, Basingstoke, UK). The plates were incubated at 37°C for 16–18 hours. The results were interpreted according to the CLSI guidelines (2019).

3.2.4 D test

Clindamycin-inducible resistance was also tested by the D test as per CLSI guidelines (2018). Erythromycin (15 µg) disk was placed at a distance of 17-26 mm from Clindamycin (2µg) disc on Mueller-Hinton agar plate. After overnight incubation, plates were examined for the formation of a flattened zone of inhibition adjacent to the Erythromycin disk. Formation of D-shape with Erythromycin indicated a positive Clindamycin inducible resistance (iMLSB). Resistance to both clindamycin and erythromycin was recorded as constitutive resistance (cMLSB) and isolates that were resistant to erythromycin only were recorded as Methicillin Sensitive (MS). Diameters of inhibition zones were measured with a ruler and interpreted as resistant, intermediate and susceptible (CLSI, 2019).

3.2.5 MRSA detection

The detection of MRSA was done using cefoxitin discs (OXOID, UK) according to the CLSI (2019), guidelines. All isolates resistant to cefoxitin were considered as MRSA. An inhibition zone of 21mm or less around cefoxitin disc indicated MRSA. For quality

control, *S. aureus* ATCC 25923 was used (Joachim *et al.*, 2017).

3.2.6 Questionnaire survey

The selection of respondents was done by identifying patients who were given the approval by the head doctor to participate within the selected six wards (recovery male, recovery female, surgical male, surgical female, maternity ward and the eye clinic). The inclusion criteria for respondents were patients who had been in the hospital for longer than 24 hours; children above the age of 12 years. The exclusion criteria were people who have been on antimicrobials for up to two weeks, children under the age of 12 years and those who were not willing to participate in the study. A total of 80 questionnaires were conducted in order to understand the patient perception on antimicrobials, HAI and public hospitals. The questionnaire contained open and close-ended questions, both open and close-ended questions were employed to ensure that respondents have an opportunity to express themselves and give answers to ensure necessary information was obtained (Appendix 4). The information collected included; education levels, age, gender and origin of patients. Furthermore, detailed questions, on antimicrobial usage and knowledge; perception of public or government hospitals; and education on HAI were asked (Appendix 4).

3.3 Ethical Issues

Ethical clearance was obtained from the National Institute for Medical Research (NIMR/ HQ/R.8a/Vol.IX/3261 - 20th November 2019). Permission to carry out this study was also sought from the Regional Health Management Team (RHMT) (DC.65/245/99 - 24th February 2020), the Morogoro Region District Office and management of the MRRH. All patients were informed about the objectives of the study, including potential benefits and

harms. Participation in the study was purely voluntary and participants had the right to refuse, without any consequences in their treatment regime. All information related to this study was kept confidential. Written and verbal consent was obtained from each participant. For children aged between 12-18 years their assent (affirmative agreement to participate in research) was sought and the permission requested from their parents. Each child was given the opportunity to ask questions and the investigator took the opportunity to explain anything that was not clear. This gave a child the opportunity to decide if they want to take part, and to decline if they were not interested. Patients were referred to attending physicians for further management.

3.4 Data Analysis

Data obtained were analysed using Microsoft Excel and the Statistical Program for Social Sciences (SPSS) version 20.0 (Joachim *et al.,* 2017). Categorical variables were summarized using proportions. Chi-square tests or Fisher's exact tests were used to compare differences between proportions. Further descriptive statistics such as: means, standard deviations, and frequencies were generated.

CHAPTER FOUR

4.0 RESULTS

4.1 Macro-morphology

Appearance of *S. aureus* on mannitol salt agar and blood agar were used for preliminary identification of *S. aureus*. Mannitol salt agar was preferred due to its high salt content that fermented mannitol resulting in the formation of yellow colonies, which is a useful tool for identifying *S. aureus* (Shittu *et al.,* 2006). Blood agar is a nonselective media that is recommended when identifying *S. aureus* as a way to decrease time and expenses and it is great enrichment for fastidious bacteria (Sharp and Searcy, 2006). On mannitol salt agar turned the agar yellow and formed yellow colonies as observed on Fig. 2.

Figure 2: Appearance of *S. aureus* colonies on manual salt agar

In blood agar white colonies were formed to indicate that it was *S. aureus* as seen in Fig. 3 below.

Figure 3: Appearance of *S. aureus* colonies on agar

4.2 Micromorphology

After isolating *S. aureus* on media, gram staining, catalase and coagulase tests were conducted in order to further distinguish *S. aureus* as seen on Fig. 4 and 5.

4.2.1 Gram-staining

The sample was identified as positive, once it was observed that under the microscope grape-like cocci were formed. The grape-like cocci were purple in colour, with the background remaining yellow as seen on Fig. 4.

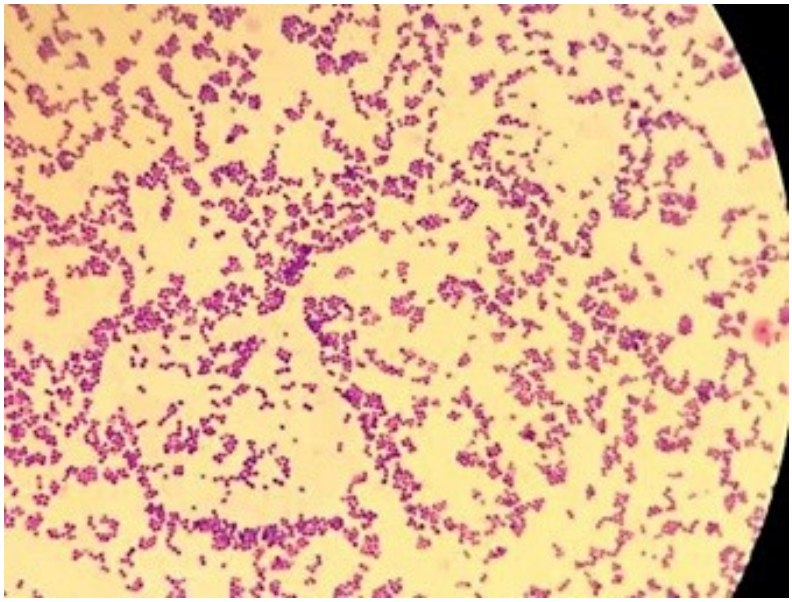

Figure 4: Gram-positive cocci in clusters

4.2.2 Catalase Test (Slide test)

The sample was identified as positive for *S. aureus* once cloud formations occurred on the slide. A gram-negative bacteria sample was tested and the sampled remained clear with no cloud formation as seen on Fig. 5.

Figure 5: Positive catalase for *S. aureus* test for *E. coli*

4.2.3 Bio-Channel Test (Coagulase)

The sample was identified as positive *S. aureus* and formed cloudy lumps on the slide as seen on Fig. 6.

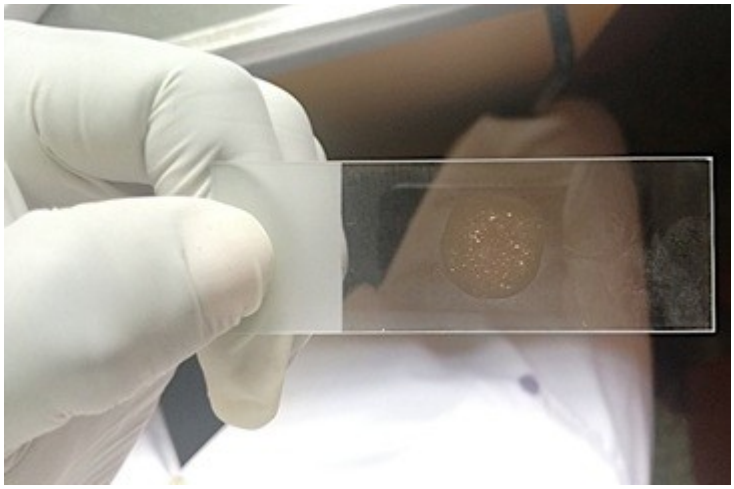

Figure 6: Positive coagulase test for *S. aureus*

4.3 Distribution of *Staphylococcus aureus* Based on Age and Gender

The patients who took part in the data collection were characterised by their age and gender see Table 2. The respondents were divided into four groups, the youngest being sixteen years and the oldest above sixty-five. The frequencies in the groups are about the same for the (26-44) years and (45-64) years. The frequency is almost double for the youngest (16-25) to the oldest (above 65 years). Also, from Table 2, the frequency for the females is almost twice that of males. The results in Table 2 indicate that; out of 100 respondents, 29% tested positive to *S. aureus*. Then out of the total tested positive, 20% were female and the remaining 9% were males, the rate *S. aureus* positive respondents in each age group indicate that it increases with age.

Table 2: Frequency of *Staphylococcus aureus* isolates in respondents of different age and gender

Characteristic	*S. aureus* Positive n =29 (%)	*S. aureus* Negative n =29 (%)	Positive *S. aureus* in each group (%)
Age			
16-25	3 (10.3)	11 (15.5)	21.4
26-44	11 (37.9)	36 (50.6)	23.4
45-64	10 (34.5)	18 (25.4)	35.7
65+	5 (17.2)	6 (8.5)	45.5
Total	29 (100)	71 (100)	
Gender			
Male	9 (31.0)	46 (64.8)	16.4
Female	20 (69.0)	25 (35.2)	44.4
Total	29 (100)	71 (100)	

4.4 Distribution of *Staphylococcus aureus* in Hospital Environmental Surfaces

Table 3 shows the prevalence of *S. aureus* in the hospital environment. The highest frequency is from beds (29%) and lowest is trolleys (6%). A total of twenty six percent (26%) of the environmental surfaces were found to have *S. aureus* present. From the results, the specific area that is leading in is tables (46, 7%). The nets, trolleys and wheelchairs also tested negative for *S. aureus.*

Table 3: Frequency of *Staphylococcus aureus* in different environment surfaces

Source	*S. aureus* Positive n=26 (%)	*S. aureus* Negative n=74 (%)	Positive *S. aureus* in each group (%)
Environment			
Nets	0 (0)	14 (100)	0
Beds	10 (38.4)	19 (25.6)	34.48
Tables	7 (26.9)	8 (10.5)	46.7
Faucets	4 (15.4)	10 (13.2)	28.6
Wheelchairs	0 (0)	8 (10.5)	0
Doors	4 (15.4)	10 (13.2)	28.6
Trolleys	0 (0)	6 (7.9)	0
Overall	**26 (100)**	**74 (100)**	

4.5 Comparison of Sampling Wards *Staphylococcus aureus* Recovered between Humans and Environment

The finding of *S. aureus* positive for each different ward has been presented in Fig. 7. The recovery male ward had the highest number of bacteria identified (57.7%) followed by the recovery female ward (26.9%), then the surgical male ward (7.7%). The surgical female ward, maternity wards and the eye clinic all had zero *S. aureus* identified within its environment.

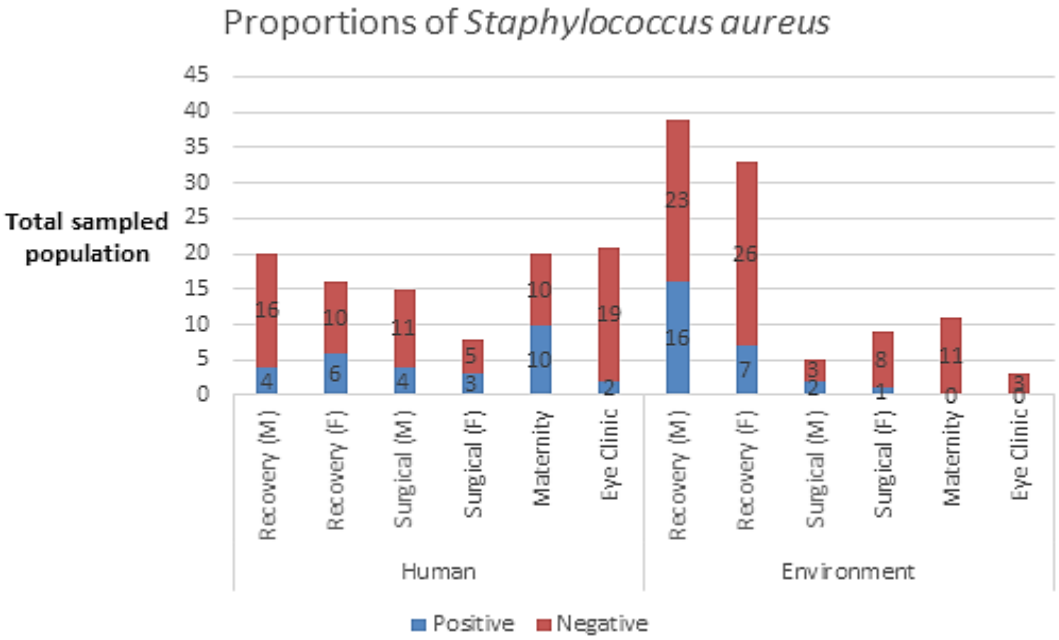

Figure 7: Graph showing the number of for each ward for human and environment samples

4.6 Risk Factors Associated with *Staphylococcus aureus* in the Hospital

Risk factors of exposure to the *S. aureus* were assessed using and a logistic regression analysis as seen in Table 4. Maternity had the risk of being ten times more exposed to *S. aureus* contamination from patients [OR = 9.9 (95% CI, 2.0-19.30), p= 0.01] followed by recovery wards (male and female) [OR = 5.1 (95% CI, 1.3-8.6), p= 0.04] compared with other wards. The tables were five times at risk of being exposed to *S. aureus* contamination from patients [OR = 4.6 (95% CI, 1.22-1.89, p= 0.03)] compared to other

items.

Table 4: Logistic regression analysis of factors associated with exposure

	OR	***P-value***	**95% CI**	
Risk factor			**Upper**	**Lower**
Gender				
Male	1.05	0.88	0.51	2.18
Female	0.08	0.01	0.01	0.42
Sample type				
Bed	0.08	0.01	0.01	0.42
Door	0.62	0.53	0.12	2.68
Faucet	0.92	0.90	0.21	3.69
Net	0.00	0.99	4.1-E265	6.4+E20
Patient	1.20	0.70	0.47	3.22
Table	4.57	0.03*	1.22	1.89
Trolley	2.4-E08	0.88	0.00	3.7+E30
Wheelchair	1.0-E08	0.99	NA	2.8+E55
Source				
Human	0.08	0.01	0.01	0.42
Object	NA	NA	NA	NA
Ward				
Eye	0.08	0.01	0.01	0.42
Maternity	9.92	0.01*	2.0	19.3
Recovery	5.09	0.04*	1.29	8.60
Surgical	3.82	0.10	0.89	6.75

*Statistically significant factors ($p< 0.05$)

4.7 MRSA and MSSA Detection

Cefoxitin was used to identify positive MRSA isolates by measuring the inhibition zones of 21mm or less as seen in Fig. 8. Then when the inhibition zone was more than 21mm it was assumed to be MSSA.

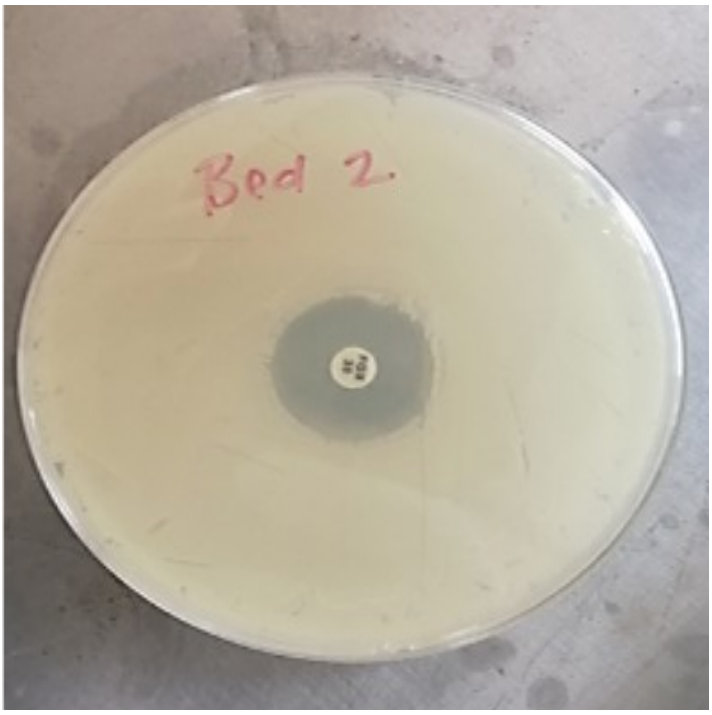

Figure 8: A zone of inhibition in MRSA detection

From all the samples tested for MRSA, it was found that the total prevalence of MRSA was 8.5% in all the samples. Within patients the prevalence of MRSA was 13% and within the hospital environment it was 4%. The overall highest MRSA prevalence was found in objects within the female recovery ward (50%), this was also the highest in environmental surfaces in each ward as observed in Table 5.

Table 5: MRSA prevalence in each ward

WARDS	Patients N=13 n (%)	Environment N=4 n (%)
Recovery (M)	1 (7.7)	1 (25)
Recovery (F)	3 (23.1)	2 (50)
Surgical (M)	2 (15.4)	0 (0)
Surgical (F)	2 (15.4)	1 (25)
Maternity	3 (23.1)	0 (0)
Eye Clinic	2 (15.4)	0 (0)

4.8 Antibiotic Susceptibility Test of Isolated *Staphylococcus aureus*

After measuring inhibition zones as seen in Fig. 9, representing erythromycin (E15), azithromycin (AZM15), ofloxicin (OF15), cefoxitin (FOX30), ciprofloxacin (CIP30) and gentamicin (GEN10) that tested positive.

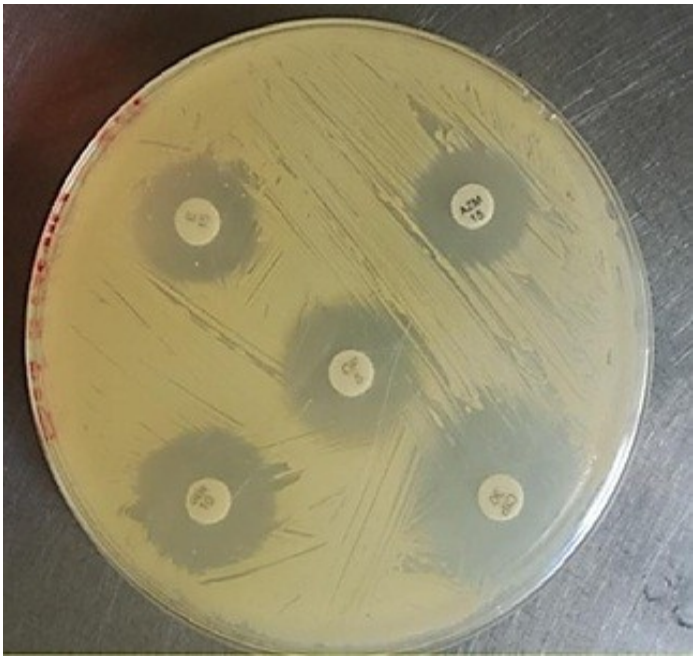

Figure 9: Inhibition zones for around different antimicrobials

Most of the MRSA identified samples were resistant to: cefoxitin (26.3%), erythromycin (26.3%) and azithromycin (26.3%). Resistance to ciprofloxacin, gentamicin and ofloxicin were 10.5, 7 and 3.5% respectively as observed in Table 6. Similarly, MSSA identified samples had the highest resistance to erythromycin (44.1%), followed by azithromycin cerofoxin (28.2%), ofloxicin, and gentamicin at 41.2, 5.9, 5.9, 2.9% respectively and zero resistance to ciprofloxacin. In Table 6, it was assumed equal variance in patterns between MRSA and MSSA with a significance p value of less than 0.005 for all antimicrobials. The equal variance was accepted for erythromycin, ofloxicin, azithromycin, gentamicin and cefoxitin. However, the trend was not observed for ciprofloxacin, azithromycin.

Table 6: Antimicrobial resistance patterns among MRSA and MSSA isolates

Drugs	***MRSA*** ***N=57 n (%)***	***MSSA*** ***N=34 n (%)***	***P=value***
Erythromycin (E 15)	15 (26.3)	15 (44.1)	0.0
Azithromycin (AZM 15)	15(26.3)	14 (41.2)	0.088
Ofloxicin (OF 15)	2 (3.5)	2 (5.9)	0.474
Gentamicin (GEN 10)	4 (7.0)	0 (0)	0.051
Ciprofloxacin (CIP 30)	6 (10.5)	1 (2.9)	0.054
Cefoxitin (FOX 30)	15 (26.3)	2 (5.9)	0.000
	57 (100)	**34 (100)**	

4.9 D Test

The D test, which was used to study the macrolide lincosamide streptogramin resistance (MLSB), both constitutive and inducible in *S. aureus*. The results based on the zone of inhibition (D-pattern) are given on Fig. 10.

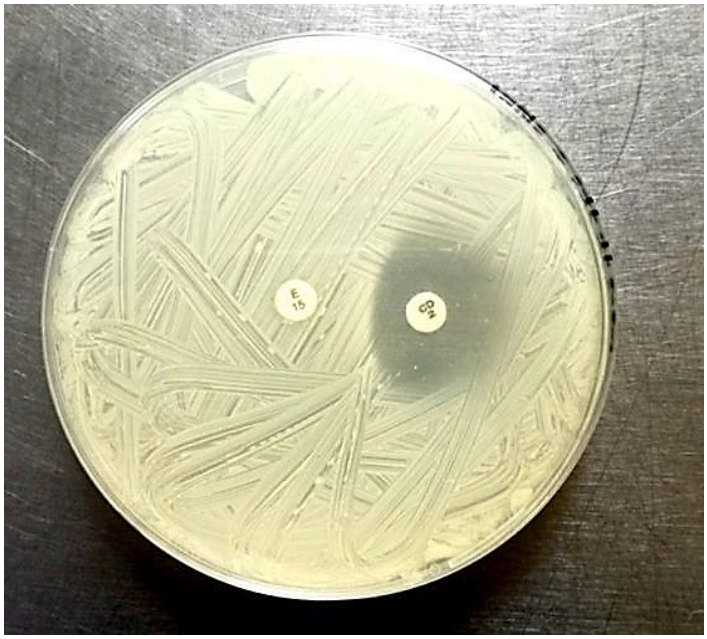

Figure 10: Positive D test for resistance against clindamycin (CD)

A total of 23% (46/200) of the total samples, made up from 100 patient samples and 100 environmental samples, that were tested for antimicrobial resistance were used for the D test. There was a statistically significant association between MRSA and multiple drugs resistance (MDR) among *S. aureus* isolates (p = 0.000) as seen on Table 7 below. These results show that: 3.5% (7/200) tested positive for the D-Test **(**iMLSB), 6% (12/200) had reaction where both clindamycin and erythromycin (cMLSB) tested positive and 13% (26/200) tested negative for the D-Test (MS).

Table 7: Table: Prevalence of different antimicrobials resistance types among MRSA and MSSA isolates

Resistance type	Overall N=200 n (%)	MRSA N=40 n (%)	MSSA N=50 n (%)	P value
iMLSB	7 (3.5)	4 (57.1)	3 (42.9)	0.053
cMLSB	12 (6)	8 (66.7)	4 (33.3)	0.028
MS	26 (13)	8 (30.8)	18 (69.2)	0.000
MDR	45 (22.5)	20 (44.4)	25 (55.6)	0.000

4.10 Assessment of Awareness and Perception among Patients at MRRH

Characteristics of respondents

Out of the 100 patients, only 80 respondents had valid questionnaire responses; 13 (16.25%) of the respondents were those who tested positive for MRSA while 67 (83.75%) were negative. The number of respondents who took part in the questionnaire was analysed based on their age, gender, level of education and place of residence against positive MRSA and negative MRSA tests as seen on Fig. 8.

Females had the highest number of respondents who tested positive within the questionnaire with 9/80 (11.25%). Respondents who were from Morogoro had the second highest number of MRSA cases with 8/80 (10%). However, it was observed that respondents who had higher levels of education had less cases. Those with form six level had zero case, undergraduate 1/80 (1.25%) and those with postgraduate also had zero case

as seen in Fig. 11.

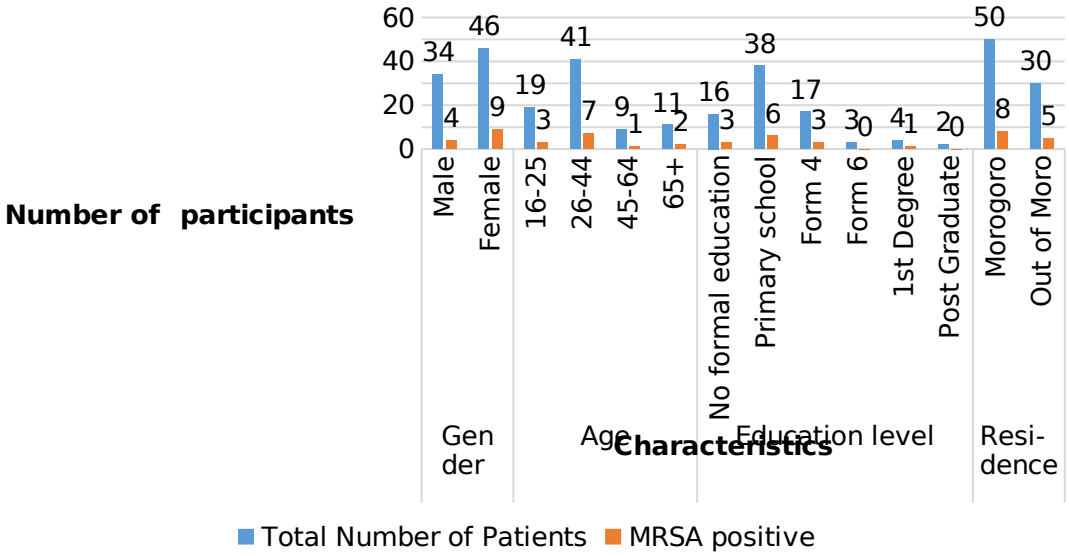

Figure 11: Characteristics of respondents involved in questionnaire

4.11 General Perception of Respondents

4.11.1 General awareness on antimicrobials

Awareness of antimicrobials, regular usage of antimicrobials and methods of antibiotic prescriptions were considered as potential factor for MRSA existence and/or emergence. Table 8 shows the results of a questionnaire survey. The respondents were required to respond to the following questions: Awareness of antimicrobials, usage of antimicrobials and doctor's prescription. The results are given in numbers and then proportional percentage of the respondents who tested positive to MRSA.

Majority of respondents said yes to antimicrobials awareness and usage of antimicrobials as prescribed by the doctor, as well as no use of antibiotics. After the MRSA test was done the proportionality percentage shows that there was a low percentage of positive MRSA respondents who said no to awareness of antimicrobials, likewise there was a low

proportional percentage of positive MRSA for respondents who use antibiotics after doctor's prescription.

Table 8: General awareness and usage of antimicrobials among respondents

Question Response	**Patients N=80**	**MRSA positive, n (%)**
Antimicrobials awareness	Yes(n=38) No(n=33)	8 (21.1)
	Unsure(n=9)	3 (9.1)
		2 (22.2)
Usage of antimicrobials	Yes (n=51)	11 (21.6)
	No(n=29)	2 (6.9)
Number of antibiotic usages	None (n=49) 1-	11 (22.4)
per annum	5(n=21)	1 (4.8)
	>5(n=13)	1 (7.7)
Doctor's prescription	Yes (n=45)	7 (15.6)
	No(n=35)	6(17.1)

4.11.2 Perception of the hospital

How respondents perceived the government hospital was considered a factor in a number of MRSA cases. Many people seek treatment only as a last resort or don't at all, due to lack of trust in government hospitals. Table 9 summarises the hospital perception of all questionnaire respondents. The results show that the majority of respondents go to hospital immediately when they feel sick, despite lack of trust in hospital equipment. Fewer respondents said that; a patient can be symptomatic, after the MRSA test was done, a high proportionality percentage of positive MRSA of those who indicated symptomatic.

Likewise, a high percentage of positive MRSA was noted for respondents who had no trust in hospital equipment. Equally high proportionality percentage of positive MRSA was noted for respondents who were at the hospital for the first time.

Table 9: Perception of the MRRH among respondents

Overall perception on the use of hospitals	**Patients N=80**	**MRSA positive, n (%)**
Going to hospital when sick	Immediately(n=50) Only when it's an emergency (n=25) Never go to the hospital(n=5)	8 (16) 4 (16) 1(20)
Perception of government hospitals	Trust(n=67) No trust(n=13)	11 (16.4) 2 (15.4)
Trust of hospital equipment used	No(n=49) Trust(n=31)	4 (8.2) 9 (29.0)
A person can be sick and not seem	Asymptomatic(n=49) Symptomatic (n=31)	3 (6.1) 10 (32.3)

4.11.3 Awareness of HAI among respondents

Education levels were relatively low among respondents thus it was important to analyse the level of awareness with regard to HAI among respondents. The respondents answered questions about; treatment, the responsibility to stop and education to HA infections. Table 10 shows that; the majority of respondents indicated that antimicrobials could cure, while acknowledging the need of education regarding HA infection. After the MRSA test was done. The highest proportional percentage was found in the respondents who said that they were unsure of the HA infection treatment. Followed by the respondents who said that there was no need for HA infection education. Those who believed washing with

water was sufficient to treat HAI tested negative to MRSA.

Table 10: Perception on awareness with regards to infections

Overall perception on education regarding infections	**Patients N=80**	**MRSA positive, n (%)**
Treatment of HA infections	No treatment (n=13) Rubbing	3 (23.1)
	Cream (n=5) Washing with	0 (0)
	water (n=5) Antimicrobials	0 (0)
	(n=29)	3 (10.3)
	Prescription from a doctor(n=26)	6 (23)
	Unsure (n=2)	1 (50)
Responsible for stopping HA infections	Doctors (n=49)	11 (22.4)
	Cleaners (n=2)	0 (0)
	Patients (n=2)	0 (0)
	Guests (n=27)	2 (7.4)
Education regarding HA	Not need (n=12)	3 (25)
	Needed (n=68)	10 (14.7)

CHAPTER FIVE

5.0 DISCUSSION

This study took place in a hospital setting at the Morogoro Regional Referral Hospital (MRRH). It was an epidemiological investigation of MDR S aureus. The study compared carriage and pattern of drug resistant *S. aureus* between patients and equipment in the hospital. Through comparing isolates in different carriage routes, results of this study highlighted the burden and pattern of AMR in MRRH and provided an insight into transmission pattern. MRRH can be used as a model for other hospitals in Tanzania on MRSA.

The WHO is concerned of the AMR problem that is steadily increasing in East Africa, where MRSA is becoming a “superbug”, and *S. aureus* is becoming resistant to other antimicrobials hence the relevance of this study. At the time of this study, no previous study had been conducted on MRSA screening among patients at the time of admission to hospitals in Tanzania. Most studies investigating drug resistance among *S. aureus* isolates have been limited to MRSA.

However, the data on MRSA inconsistent due to lack of effective and systematic routine surveillance systems. One study conducted in Dar es Salaam, observed a statistically significant association between MRSA and multiple drugs resistance among *S. aureus* isolates, however, none of the investigated risk factors were found statistically significant associated with MRSA.

Other previous studies conducted in Tanzania at Muhimbili National Hospital (MNH) and Bugando Medical Centre. Used clinical specimens from hospitalized patients who presented with symptoms and/or signs of infection. They documented an increasing prevalence of hospital-acquired MRSA. Other studies on the prevalence of MRSA were conducted at two regional hospitals. (Mwananyamala and Amana) in Dar es Salaam. Also there has been nasal MRSA colonization in HCW in Tanzania. MRRH is located in Morogoro a region less populated and economically developed than Dar es Salaam.

This was a cross-sectional study, involving sampling of patients and environment at MRRH. It undertook the following: Isolation of *S. aureus*, Gram staining, Catalase test (slide test), Coagulase test, and antibiotic susceptibility of isolated *S. aureus*, D Test, MRSA Detection and Questionnaire Survey. Macro-morphology was a preliminary identification process by appearance of *S. aureus* on mannitol salt agar and blood agar. After isolating *S. aureus* on media gram staining, catalase and coagulase tests were conducted in order to further distinguish *S. aureus* in micromorphology process. Following is a summary of the results of the tests:

The distribution of *S. aureus* based on age and gender revealed that; male and female were equally affected, in other words there were more women in the survey and so a big number of women tested positive. Meaning anybody could be equally affected. Also results showed that the respondents above the age of forty-five (45) were more vulnerable to *S, aureus*. The frequency of Staphylococcus aureus in different wards and different

environmental surfaces showed that nets, wheelchairs and trolleys were negative to *S. aureus*. This is most likely because the staff take good care of those equipment for their work. Faucets and doors were equally affected obviously because the regular handling by different people. But higher positive *S. aureus* was noted on tables and beds. This is understandable, because these are places, where patients spend longer time sleeping, sitting, keeping stuff or having meals. Still the staff's good work was evident in the surgical male and female wards, maternity wards and the eye clinic which had minimum, or zero *S. aureus* identified within its environment. Otherwise, the highest positive *S. aureus* was noted in the male ward followed by female one.

The risk factors associated with *S. aureus* in the hospital were assessed; the maternity ward was the highest, followed by male and female recovery wards, Tables' risk factor was also higher than other items. The MRSA and MSSA were detected, the highest MRSA prevalence was recorded in the female recovery ward at fifty percent (50%). Then the antibiotic susceptibility test of isolated *S. aureus* was done it was found that; Most of the MRSA identified samples were highly resistant to: cefoxitin, erythromycin and azithromycin, followed by moderate resistance to ciprofloxacin, gentamicin and ofloxacin. Likewise, MSSA identified samples had the highest resistance to erythromycin, followed by azithromycin, cerofoxin, ofloxicin, and gentamicin. Ciprofloxacin tested negative. The D-test, which was used to study the macrolide lincosamide streptogramin resistance (MLSB), both constitutive and inducible in *S. aureus*, results showed that; there was a statistically significant association between MRSA and multiple drugs resistance (MDR) among *S. aureus* isolates.

From the questionnaire survey, assessment of awareness and perception among patients at MRRH was done. As well as the analysis against positive or negative MRSA based on age, gender, level of education and place of residence. These factors were thought to be necessary for a follow up and awareness mobilisation. Overall doctor's prescription on usage of microbial were adhered to. On the perception of hospital, respondents who had no trust in hospital and those who had visited for the first time many of them tested positive to MRSA. This implies that the hospital is doing a good job by attracting patients. Related to HA infections, the highest MRSA rates were observed within patients who believed that other patients around them could be carriers of HAI and exhibit zero symptoms. It was noted in terms of how regularly patients went to the hospital, the group that said it was their first time in the hospital had higher MRSA prevalence than those who regularly went or those who only went for emergencies.

Bearing in mind Joachim's assertion that; the data for MSSA in Tanzania is however rather inconsistent (Joachim *et al*., 2018). This study also looked into other previous related studies. Following are some of recorded results: The prevalence of MRSA at MRHH, in patients and environment (among 100 patients and 100 environment surfaces). The findings are consistent with previous studies by Joachim et al. (2017) that looked at two regional hospitals (Mwananyamala and Amana) in Dar es Salaam. According to Yuen *et al.* (2015), MRSA can be recovered from 1% to 27% of surfaces in patient rooms. The MRSA prevalence rate at the MRRH is within the expected range.

While, in the environment, it showed that more common bacteria were resistant to MRSA as opposed to MSSA (Joachim *et al.,* 2018). It was further found that the prevalence of MSSA in the MRRH environment was 28.9%% that was slightly higher than 24.4% for MRSA and thus expected (Zabienlinski *et al.*, 2019). The age was a huge factor as often patients are exposed to antimicrobials for longer and hence resistance is built. The finding in this study concedes with Okamo *et al.* (2016) whereby higher MRSA prevalence was observed in older participants of a study done in Tanzanian university students. In Iran, a study done by Ahmadi *et al.* (2019) showed that the most susceptible group for acquiring MRSA was the group above fifty (50).

The D test results were significantly lower than those previously obtained by Joachim *et al.* (2017), where the prevalence of inducible Clindamycin resistance, constitutive Clindamycin resistance, MS phenotype (resistance to Erythromycin alone), and multidrug resistance was found to be 21.3%, 3.4%, 12.4%, and 16.9%, respectively. This could be result of no usage of Clindamycin within the population sampled or correct usage of Clindamycin unlike what was discovered by Joachim *et al.* (2017).

In this study, high prevalence for resistance to both Clindamycin and Erythromycin was observed as opposed to just Clindamycin. Most probably this is the result of overexposure to those two antimicrobials in the hospital. Higher prevalence was observed in MRSA positive samples in comparison MSSA for iMLSB, cMLSB and MS. This could be a result incompetent usage of Clindamycin.

According to Vivoni *et al.* (2005) the trend showed higher resistance to commonly used antimicrobials as observed in MSSA samples in comparison to MRSA (Vivoni *et al.*, 2005). The higher prevalence of MRSA in patients who often used antimicrobials without a doctor's prescription was observed as often these patients might not finish the dose, take too much or take the wrong antimicrobials. This was slightly different to the study conducted Dar es Salaam, Tanzania by Godfrey *et al.* (2015), where higher prevalence was found in patients within ICU (intensive care unit) who had been to hospital more than three times. This could also be attributed by the source of nosocomial infections. Though there was a trust in using government hospital it was observed that group of respondents had a slightly higher percentage on MRSA.

The only study conducted in Morogoro region that observed individual's perception on antimicrobials was by Katakweba *et al.* (2012), the study however looked at the awareness of antibiotic usage in small scale farming not in frequency in antibiotic consumption. The study found that around 30% of its respondents were not aware of antimicrobials in comparison to this study's results of 41.3%.

In this study, an overall total of 24.4% were found to be MRSA while 28.9% were MSSA in patient samples. Most of the MRSA identified samples were resistant to cerofoxitin (28.3%). A total of 27.5% (55/200) of the samples were colonized by *S. aureus* with 29% of patients and 26% of environmental surfaces. This is roughly the median range of *S. aureus* positive samples found in similar studies in Tanzania (Nkuwi *et al.*, 2018).

The resistant epidemiological investigation of multi-drug Staphylococcus aureus study found that; The difference in prevalence between respondents from Morogoro and those

from outside was rather small as many factors such as the type of area (urban/ rural) where the respondents had come from were not considered. The highest MRSA prevalence was observed in the group of respondents with undergraduate degrees at 25% in comparison to any other groups within the characteristics overall. However, this rate of positive MRSA is close to the rate the respondents who were the majority who have primary school education level.

The general lack of awareness on antimicrobial could start from the level at which patients have received treatment in the past, that could have resulted in the incorrect usage of antimicrobials and hence higher prevalence numbers. A study conducted by Viberg *et al.* (2010) found that many people in Tanzania used over-the-counter (OTC) drugs from drug sellers who are often not qualified pharmacists (Katakweba *et al.,* 2012). This is seen more prominent in situations where people have mild symptoms to an illness as opposed to serious conditions. The most common symptoms that these drug sellers seem to provide antimicrobials for were stomach-ache, cough, genital complains, flu and diarrhoea (Viberg *et al.,* 2010). The highest MRSA prevalence was observed in those who took no antimicrobials within the last year, while the lowest was observed in those who used antimicrobials 1 to 5 times within the last year. This could be as a result of bacteria remaining untreated in the body or using the wrong form of treatments for infections as a result of misdiagnosis.

As explained by Moellering (2012), MRSA can appear asymptomatic and cause no harm to majority of the population so most people within this group could simply have been asymptomatic and thus required no further treatment. General lack of awareness with regards to antimicrobials was slightly higher and unsure of antimicrobials was the highest with the group. Those who were aware of antimicrobials had the second highest

prevalence.

The questionnaire survey conducted helped to determine factors from patients that were associated with MRSA carriage among patients and inanimate surfaces at the hospital, such as; lack of awareness on antibiotic usage, cleanliness, overcrowding, and lack of education and poor perception of public hospitals.

This similar trend was observed in those who had trust with the hospital equipment used, whereby higher MRSA prevalence was noted. The highest MRSA prevalence was observed in the group of respondents who believed the hospital did not need to educate the patients, staff or visitors more on HAI infections. This could be due to the fact that, most people were more worried with major illnesses, they were in hospital to treat, and also COVID-19 prevention posters were everywhere, warning about the pandemic. Overall, when it came to HAI treatment the highest rate of MRSA was observed in the group of people who believed that HAI required no treatment and should be left alone. When it came to who was most responsible for stopping the spread of MRSA, those who believed HCW had a higher MRSA followed by those who believed guests visiting had the second highest rates. It was observed that those who thought themselves as patients and hospital cleaning staff were responsible over the spread of HAI had zero cases of MRSA.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

This dissertation sought to investigate how pathogens, such as multidrug resistance (MDR) *Staphylococcus aureus* can be transmitted through patients and the hospital environment, resulting in hospital associated infections that can be difficult to treat. As commonly known MDR *S. aureus* is a significant nosocomial pathogen which is high associated with morbidity and mortality.

This study was conducted in the peak of the SARS-CoV-2, Corona virus (COVID-19) outbreak in Tanzania. Due to this pandemic problem, extra measures were taken within the hospital, where more hand-washing stations were put in place, regular usage of masks was enforced, and social distance was practiced with more regular cleaning schedules for each ward. Specific time was given to visitors and the number of visitors was limited to each patient as opposed to how it was done prior the outbreak. Social distancing was encouraged, masks were regularly worn, and social distancing was encouraged. This

scenario played a part in the results observed as many changes regarding hygiene were made within the hospital.

Credit is due to the MRRH staff for keeping some risk factor at minimum notably: nets, wheelchairs and trolleys, Faucets doors, eye clinic, maternity surgical male and female wards over and above attracting first timer patients. More attention is still needed in male and female recovery wards, tables and beds.

The patients screened at MRRH had already been in hospital for a minimum of at least 24 hours and had undergone treatment in the hospital. This could have been a contributing factor to the observed cases recorded. However, the MRRH data is an added information to guide any other regional hospital outside Dar es Salaam Tanzania's economic capital.

General lack of knowledge with regards to antimicrobials was slightly higher and unsure of antimicrobials was the highest with the group. Those who were aware of antimicrobials had the second highest prevalence. This could be the result at how the patients have received treatment in the past, which could have resulted in the incorrect usage of antimicrobials and hence higher prevalence numbers.

Age was found to be a critical factor as often patients are exposed to antimicrobials for longer and hence resistance is built. Overall lack of education played a major role in determining education levels, often lower education levels resulted in higher MRSA prevalence.

The higher prevalence of MRSA in patients who often used antimicrobials without a doctor's prescription was observed. In this study, high prevalence for resistance to both

Clindamycin and Erythromycin was observed as opposed to just Clindamycin.

6.1 Recommendations

Group discussions were not possible as there were rules in place against large gatherings of more than three people, as a result of the COVID-19 and many patients were bed bound, so no one on one discussion were done. These findings have reported minimum prevalence of MRSA and furthers studies should be conducted after the COVID-19 stringent measures have been waved away.

Female wards were often found to be overcrowded; it was not a surprise that women respondents had a higher MRSA prevalence rate. Limiting visitors or expanding the wards could solve the problem.

Further molecular characterisation might be helpful in determining the MSSA and MRSA genotypes. It was important to note that further education needed to be provided on what HAI is as well as education with regards to CAI (community acquired infections) was necessary.

Overexposure to both Clindamycin and Erythromycin in the hospital resulted in prevalence for resistance. Higher prevalence observed in MRSA positive samples in comparison with MSSA for iMLSB, cMLSB and MS could be a result incompetent usage of Clindamycin.

The study recommends that:

i. Further screening and control of visitor overcrowding at the hospital is needed in order to limit the exposure to MRSA and MSSA.

ii. More awareness on HAI and CAI is needed within the Morogoro region as lack of awareness plays a significant role in the number of cases.

iii. Stricter laws should be implemented by the government to limit the OTC sell of antimicrobials in Tanzania.

iv. Consider doing this study in wide range of regional hospitals in Tanzania after the pandemic of COVID-19.

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APPENDICES

Appendix 1: Patient consent form (Kiswahili)

RUHUSA YA KUFANYA UTAFITI

MAADA : UCHUNGUZI WA MAAMBUKIZI YATOKANOYA NA MATUMIZI YA MADAWA KATIKA HOSPITALI YA RUFAA MKOA MOROGORO, TANZANIA)

MTAFITI : NANCY GWIMO

Nimefahamishwa kuhusu utafiti wenye maada hii: UCHUNGUZI WA MAAMBUKIZI YATOKANOYA NA MATUMIZI YA MADAWA KATIKA HOSPITALI YA RUFAA MKOA MOROGORO, TANZANIA.

Nimezungumza na mtafiti, Nancy Gwimo kuhusiana na utafiti anaoufanya kama sehemu ya masomo ili ku kamilisha shahada ya uzamili katika Afya ya jamii na usalama wa

chakula katika Chuo Kikuu cha Kilimo Morogoro ambayo ina simaniwa na:

Dkt. A.A.S KATAKWEBA (SUA), Profesa. S.I. KIMERA (SUA) and Profesa. M.I. MATEE (MUHAS).

Nimeelezwa madhara yanayoweza kutokea na uzito wa utafiti huu. Pia nilipata nafasi ya kumuuliza, Nancy Gwimo swali lolote kuhusiana na utafiti huu ikiwa ni pamoja na kushiriki kwangu.

Ninaelewa ninashiriki kwa hiari yangu, pia niko huru kutoshiriki na kwamba nina weza kusimama kushiriki muda wowote. Kama kuna lolote jingine nalohitaji kufahamu kuhusiana na utafiti huu nitawasiliana kwa simu na: Nancy Gwimo (0621607652), au Msimamizi, Prof.Kimera (07875696840).

Kwa saini yangu hapa chini nina kubali kwa hiari (tia alama):

□Sampuli toka kwenye Ngozi yangu zichuliwe

□Sampuli toka kwenye vifaa vinavyonizunguka zichukuliwe.

Ninaelewa kuwa takwimu zitakazo tokana na kushiriki kwangu zita tumika kwa ajili ya kuandika taarifa ya shahada ya uzamili pamoja na makala mbali mbali ya kiutaaluma. Sina kipingamizi kwa matumizi hayo.

Sahihi…….Tarehe……

(KWA WALIO NA UMRI CHINI YA MIAKA 18, MLEZI/MZAZI WASAINI)

……

(JINA ANDIKA KWA HERUFI KUBWA).

Appendix 2: Patient consent form (English)

CONSENT FORM

RESEARCH TITLE: EPIDEMIOLOGICAL INVESTIGATION OF MULTI-DRUG RESISTANT *Staphylococcus aureus* AT MOROGORO REGIONAL REFERRAL HOSPITAL, TANZANIA

RESEARCHER: NANCY GWIMO

I have been given information about the research titled, EPIDEMIOLOGICAL INVESTIGATION OF MULTI-DRUG RESISTANT *Staphylococcus aureus* AT MOROGORO REGIONAL REFERRAL HOSPITAL, TANZANIA and discussed the research project with Nancy Gwimo who is conducting this research as part of a degree in Master of Science (MSc.) Public Health and Food Safety supervised by DR. A.A.S KATAKWEBA (SUA), PROF. S.I. KIMERA (SUA) and PROF. M.I. MATEE (MUHAS) at Sokoine University of Agriculture.

I have been advised of potential risks and burdens associated with this study and I have had an opportunity to ask Nancy Gwimo any questions I may have about the research and

my participation.

I understand my participation in the research is voluntary, I am free to refuse to participate and I am free to withdraw from the research at any time. If I have to enquire about the research, I can contact Nancy Gwimo (0621607652) and supervisor, Prof.Kimera (07875696840).

By signing below, I am indicating my consent (please tick):

□ Have my swab samples taken?

□ Have swabs taken on objects around me?

□ I understand that the data collected from my participation will be used for purpose of master dissertation and journal publication, and I consent for it to be used in that manner.

Signed (if under 18 guardian signature is required) Date

.....…...../......../.....

Name (please print) .....

Appendix 3: Questionnaire (Kiswahili)

MASWALI YA UTAFITI

Ndugu Mshiriki

Maswali ya fuatayo yametungwa ili kupata taarifa za mshiriki pia kujua ni kiasi gani mshiriki anafahamu kuhusu matumizi na maambukizi yatokanayo na matumizi ya madawa yakujikinga (antibiotics).

Tafadhali JAZA au CHAGUA jibu sahihi kwa kila swali

(Taarifa zote zitokanazo na maswali haya yata tunzwa kwa siri)

SEHEMU YA 1 – MASWALI YA AWALI

Tarehe…….(siku/mwezi/mwakaka)Jinsia Mme/Mke…….....

Wilaya…….....Kabila……

Umri: □16-25 □26-44 □45-64 □65+

Unahusikaje? □Mgonjwa □Mfanyakazi wa afya

□Vinginevyo (Tafadhali eleza).....

Elimuuliyonayo *(weka alama)*

1. Sikwenda shule	
2. Shule ya msingi	
3. Kidato cha nne	
4. Kidato cha sita	
5. Shahada ya kwanza	
6. Shahada ya pili nakuendelea	

SEHEMU YA 2 –UFAHAMU WA MADAWA YA KUJIKINGA (ANTI BIOTICS)

Je unafahamu madawa ya kujikinga (anti biotics)?

□Hapana/ Sina Uhakika

□Ndiyo

Je ulishawahi kutumia madawa ya kujikinga (antibiotics)?

□Hapana

□Ndiyo *(kama ndiyo, tafadhali taja jina lake)*

.....

.....

Ni mara ngapi kwa mwaka unatumia madawa yakujikinga (antibiotics)?

□Hapana

Mara 1 - 5

Zaidi ya mara 5

Je ulishawahi kutumia madawa bila maelekezo ya daktari?

□Hapana

□Ndiyo

SEHEMU YA 3 –MTAZAMO KATIKA HOSPITALI

Je ni mara ngapi una kwenda hospitali ukiwa mgonjwa?*(weka alama kwenye jibu sahihi)*

1. Mara nijisikiapo mgonjwa	
2. Wakati nikikosa nafuu baada ya tiba nyingine	
3. Siendi hospitali	

Je unayo imani na watu wanaokupa huduma hospitalini?

□Ndiyo

□Hapana *(kama hapana, Tafadhali toa maelezo)*

.....

.....

Je una imini kuwa vifaa vya hospitalini kuwa visafi na havina maambukizi?

□Hapana

□Ndiyo (,*kama ndiyo, tafadhali toa maelezo ni mambo gani yanayo changia mgonjwa kuzidiwa akiwa hospitalini)*

.....

.....

.....

Je unafikiri mtu anayeonekana kuwa na afya nzuri kuwa anaweza kuwa na ugonjwa huku

hajisikii mgonjwa?

□Hapana

□Ndiyo *(kama ndiyo tafadhali toa maelezo kwa nini unafikiri inawezekana)*.....

.....

.....

SEHEMU YA 4 –UFAHAMU WA MAAMBUKIZI HOSPITALINI

Je una dhani ni njia gani inayofaa zaidi ili kutibu maambukizi *(weka alama majibu yote yanayo husika)*

□Asitibiwe

□Matibabu kwa dawa zaku paka

□Matibabu kwa kuosha

□Matibabu kwa madawa ya kuzuia magonjwa(antibiotics)

□Matibabu kutoka kwa daktari

□Maambukizi hayatibiki

□Sina uhakika

Je unafikiri ni jukumu la nani anayeweza kuzuia kuenea kwa maambukizi hospitalini na kwa vipi? *(weka alama majibu yote yanayo husika)*

□Madaktari (ki vipi?)

.....

□Wahudumu wa usafi (ki vipi?)

.....

□Wagonjwa (ki vipi?)

.....

□Wageni (ki vipi?)

.....

Je unafikiri watu wengi wanahitajika kuelimishwa kuwa wanaweza kupata maambukizi hospitalini?

□Hapana

□Ndiyo*(kama ndiyo, Tafadhali toa maelezounavyo fikiri itakavyokuwa)*

.....

.....

.....

MWISHO

Tuna kushukuru sana kwa kujibu haya maswali. Majibu yako yatasaidia kuelewa ufaham uuliopo kutokana na maabukizi.

Asante

Nancy Gwimo, (MSc MPH Candidate, Sokoine University of Agriculture)

Appendix 4: Questionnaire (English)

QUESTIONNAIRE

Dear Respondent

This questionnaire is designed to know general information of the respondent and understand how much the respondent understands on consumption of antibiotics and hospital acquired infections.

Please COMPLETE or CHOOSE the correct response for ALL questions

(All the information in this questionnaire will be treated confidentially)

SECTISECTION 1 – PRE-INTERVIEW QUESTIONS

Date:.....(DD/MM/YYY)…..Gender: □Male □Female

District:.....Tribe: .....

Age:□16-25 □26-44□45-64□65+

Are you? a □Patient □Health Care Worker □Other *(please specify)*.....

Highest level of education *(tick where applicable)*

1. No formal schooling	
2. Primary school	
3. Form Four	
4. Form Six	
5. Undergraduate Degree	
6. Postgraduate Degree	

SECTION 2 –KNOWLEDGE ON ANTIBIOTIC

Do you know what is antibiotics?

□No/ Not Sure

□Yes

Have you ever taken antibiotics?

□No

□Yes (if yes, please name the antibiotic you have taken)

.....

.....

.....

How often per year do you take antibiotics?

□Never

□1 to 5 times

□ More than 5 times

Have you ever taken antibiotics without a doctor's prescription?

□No

□Yes

SECTION 3 –PERCEPTION OF HOSPITALS

6. How often do you go to the hospital when you are sick? *(Tick where applicable)*

1. As soon as I feel sick	
2. Only when other forms of treatments have failed	
3. I don't go to the hospital	

7. Do you trust the people providing health care to you in hospitals?

□Yes

□No *(if no, please explain why not?*

.....

.....

.....

8. Do you trust hospital facilities to be clean and free of contamination?

□No

□Yes

9. Do you believe a person can get sicker from going to the hospital?

□No

□Yes *(if yes, please explain what factors could contribute to a person getting sicker in hospitals)*

.....

.....

.....

.....

10. Do you think a healthy-looking person can have an illness and not feel it

□No

□Yes *(if yes, please explain how you think this could be possible)*

.....

.....

SECTION 4 – EDUCATION ON HOSPITAL ACQUIRED

□Cleaning Staff (How?) .....

□Nurses

(How?).....

□Patients (How?).....

□Visitors

(How?).....

11. Do you think more people need to be aware that they can acquire infections in hospitals?

□No

□Yes *(if yes, please explain how you think this could be possible)*

.....

.....

THE END

Thank you very much for completing this questionnaire. Your answers will help me understand the general views on hospital acquired infections.

Regards

Nancy Gwimo, (MSc MPH Candidate, Sokoine University of Agriculture).