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Research Article

Detection of Multidrug Resistant *Staphylococcus aureus* at Morogoro Regional Referral Hospital, Tanzania

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Abstract

Background: Hospital settings are associated with constant introduction of pathogens which can be transmitted among workers by patients, and visitors, resulting into potential nosocomial infections. This study compared the carriage and pattern of drug resistant *S. aureus* among patients and on equipment in hospital setting at Morogoro Regional Referral Hospital (MRRH). *Methods:* A cross sectional study was conducted by collecting samples from the anterior nares using sterile cotton swabs from patients. Furthermore samples were collected from inanimate surfaces, ward door handles; wheelchairs; and trolleys. The samples were incubated on mannitol salt agar plates aerobically at 37 ℃ for 24 hours. Antimicrobial susceptibility testing was done using; erythromycin, azithromycin, ofloxacin, gentamicin, ciprofloxacin and cefoxitin. Clindamycin inducible resistance was tested by D test as per CLSI (2019). Data analysis was carried using SPSS where Chi – square was used to compare the association of occurrence of resistance and source of isolation. *Results:* Out of 200 samples, 54 tested positive for *S. aureus* were from human while the prevalence of *S. aureus* in inanimate objects was high in beds 10 (40%). The AMR was observed more in azithromycin (26.3%) than other antimicrobials. The D-Test showed inducible clindamycin-resistant phenotype in 57.1% of the MRSA isolates. The maternity ward had the highest risk of being exposed to *S. aureus* contamination [OR = 9.9 (95% CI, 2.0-19.30), p = 0.01] and tables [OR = 4.6 (95% CI, 1.22-1.89, p= 0.03)]. The recovery wards were least likely to be contaminated with the result of four times likely to be contaminate for both patients and surfaces [OR = 5.1 (95% CI, 1.3-8.6), p= 0.04] when compared with other wards. *Conclusion:* This study presents some important findings on MRSA which is a global concern, the authors encourages more researches are done in MRSA for efficient availability in the AMR database.

Keywords

S. aureus, Antimicrobials, Multidrug Resistance, Hospital, Morogoro, Tanzania

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1. Introduction

Methicillin Resistant *Staphylococcus aureus* (MRSA) is the second leading cause of nosocomial infections and healthcare workers can often be carriers of the pathogen [1-3]. MRSA can cause a wide range of infections involving skin, soft tissue, bone, joints and infections associated with prostate devices or the indwelling catheters, which are difficult to treat due to high degree of multi-drug resistance [4]. Factors such as condition of the patients, ward setting, overcrowding, and hygienic practices play significant role in the spread of MRSA in hospital settings [2]; with negative consequences on patient management especially in hospitals where resources are limited [5-7]. According to previous studies conducted in two hospitals in Tanzania, the prevalence of MRSA in patients ranged between 8.5% and 19.5% [6, 7].

MRSA infection within hospital settings has been associated with increased morbidity and mortality as well as longer hospital stays [4, 9, 10]. Comparatively, patients with MRSA have been estimated to have 1.19-fold increase of hospital charges in comparison to patients with methicillin sensitive *S. aureus* (MSSA) [8, 9]. Vancomycin and daptomycin are recommended as the first-line treatment agents for MRSA [3]. However, there are concerns that these treatments might not be effective due to emergence of resistant strain, with linezolid, tigecycline, and quinupristin/dalfopristin suggested as alternatives [3, 5]. Unfortunately, most of these antimicrobials cannot be afforded in resource limited countries such as Tanzania, thus, emphasising the need for strict infection prevention and control (IPC) measures to limit the spread of nosocomial infections such as MRSA [3]. With limited infection control practices in the overcrowded hospitals, like the Morogoro Regional Referral Hospital (MRRH), contaminated surfaces may play an important role in the transmission of antibiotic resistant pathogens like MRSA, posing serious risks for patients, health care workers, caregivers, and visitors [3-5]. This study was conducted to determine the extent of MRSA in patients and on commonly touched surfaces by workers, patients and visitors at MRRH in Eastern Tanzania.

2. Materials and Methods

2.1. Study Design and Setting

This was a hospital based cross-sectional study conducted at the MRRH in Morogoro, Tanzania. This facility, has a bed capacity of 450 and receives patients from a large catchment area of the Morogoro and nearby regions, which include urban and rural areas. The hospital receives approximately 1000 visitors and outpatients per day and has 562 clinical and non-clinical working staff [11].

2.2. Patient Selection and Consent to Participate

Participants from four types of wards; recovery wards (male and female), surgical wards (male and female), maternity ward (females only) and eye clinic (male and female) were selected on the basis of being present during the time of study. Only those who provided verbal consent were enrolled. For children aged between 12-18 years their assent and permission were sought from their parents and relatives. Children below 12 years of age and patients who were using antimicrobials at the time of recruitment or within 2 weeks after their treatments were excluded in this study.

2.3. Sample Size

This was calculated using the Kish-Leslie formula [12], and was based on the MRSA prevalence of 8.5% found in Mwananyamala and Amana hospitals in Dar es Salaam, Tanzania. A total of 200 samples were collected, 100 from patients and another 100 from hospital environment surfaces [6].

2.4. Specimen Collection

Samples from patients were collected from anterior nares of 100 patients (52 males and 48 females) using sterile cotton swabs. In addition, 100 samples were collected from inanimate surfaces, including bed nets; bed rails; patient tables; ward door handles; faucets; wheelchairs; and trolleys, from the same wards. The surfaces were selected in this study because they were highly touched by patients, staff, and visitors [13]. All samples were kept in a Cary-Blair transport media at a temperature of -8 ℃ in a cool box and were processed within 2 hours of collection in the Microbiology Laboratory of the Department of Veterinary Medicine and Public Health at the Sokoine University of Agriculture (SUA).

2.5. *Staphylococcus aureus* Isolation and Identification

In the laboratory, swabs were inoculated in mannitol salt agar plates (OXOID, UK) and incubated aerobically at 37 ℃ for 24 hours. Identification of *S. aureus* was done using a combination of colony morphology, gram staining, catalase, coagulase, and DNase tests [14].

2.6. Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing was done using the Kirby-Bauer's disc diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines [14]. In brief, colonies were suspended in sterile distilled water to produce a turbidity of 0.5 McFarland standard. The suspension was inoculated on Muller Hinton agar plate (OXOID, UK) and incubated at 35 ℃ for 24 hours. The following standard antimicrobial disks (OXOID, UK) were used; Erythromycin

(15 µg), Azithromycin (15µg), Ofloxacin (15µg), Gentamicin (10 µg), Ciprofloxacin (5µg) and Cefoxitin (30 µg). As per CLSI (2019), diameters of inhibition zones were measured with a ruler and interpreted as resistant (R), intermediate (I) and susceptible (S) according to the guidelines. The detection of MRSA was done using Cefoxitin 30 µg discs (OXOID, UK) [14]. An inhibition zone of 21mm or less around Cefoxitin disc indicated MRSA. *S. aureus* ATCC 25923 was used as a positive control [14, 15].

Clindamycin inducible resistance was tested by D test as per (CLSI, 2019) guidelines [14]. Erythromycin (15 µg) disk was placed at a distance of 19 mm from Clindamycin (2 µg) disc on Mueller-Hinton agar plate. After overnight incubation, plates were examined for the formation of flattened zone of inhibition adjacent to the erythromycin disk. Formation of D-shape with Erythromycin indicated a positive clindamycin inducible resistant (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), as shown in Figure 1.

Figure 1. *Positive D test for resistance against clindamycin (CD).*

2.7. Data Analysis

The data collected was analysed using Microsoft Excel and the statistical program for social science (IBM-SPSS) version 20.0[7], Chi square test was used to compare the proportions of categorical independence and dependent variables. Univariate and multivariate analysis were performed to determine the risk factors associated between MRSA in the hospital equipment and in patients. A *p* value of <0.05 was considered a statistically significant.

3. Results

3.1. Distribution of *Staphylococcus aureus* Based on Age and Gender

The age and gender of study participants are shown in Table 1. Most of the patients were aged 24 and 64 years, accounting for 72.4% of all subjects. Of the 100 respondents, 29% tested positive to *S. aureus* of whom 20% were female and the remaining 9% were males. The isolation frequency of *S. aureus* increased with age from 12 to 64 years but decreased thereafter.

3.2. Distribution of *Staphylococcus aureus* in Hospital Environmental Surfaces

Table 2 presented the prevalence of *S. aureus* the highest isolation frequency of *S aureus* to be beds (40%), followed by tables (28%), while wheelchairs and nets had none (0%).

3.3. Risk Factors Associated with *S. aureus* in the Hospital

Risk factors of exposure to *S. aureus* were assessed using a logistic regression analysis as seen in Table 3. The maternity ward had the highest risk of being exposed to *S. aureus* contamination for both patients and surfaces with an Odd ratio (OR) of 9.9 [OR = 9.9 (95% CI, 2.0-19.30), p= 0.01], while tables had an OR of 4.6 of being contaminated due to their regular exposure on touch by patients, hospital workers and surfaces [OR = 4.6 (95% CI, 1.22-1.89, p= 0.03)] compared to other items, and the recovery wards (male and female) had an OR of 5.1 to be contaminated [OR = 5.1 (95% CI, 1.3-8.6), p= 0.04] when compared with other wards.

3.4. Antimicrobial Susceptibility Test of Isolated *Staphylococcus aureus*

MRSA resistant to cefoxitin, erythromycin and azithromycin was 26.3% for each, while resistance to ciprofloxacin, gentamicin and ofloxacin was 10.5, 7 and 3.5%, respectively (Table 4). For MSSA resistance was highest against erythromycin (44.1%), followed by azithromycin (41.2%), cefoxitin (5.9%), and ofloxacin (5.9%). There was neither resistance against gentamicin nor ciprofloxacin. Compared with MSSA, MRSA isolates had significantly lower resistance against erythromycin, but higher resistance against gentamicin and cefoxitin (Table 4). No differences were observed for ciprofloxacin, azithromycin.

3.5. Prevalence of MRSA in the Samples and the Wards

The overall isolation frequency of MRSA was 8.5% being 13% among patients and 4% in samples from surfaces. Isolation frequencies varied by patients and environment as shown in Table 5. Table 6 shows the distribution of MRSA where the occurrence was relatively high in the recovery and maternity was (23.1%) than in other wards.

3.6. D Test

As seen on Table 7, 3.5% (7/200) of isolates tested positive for the D-Test (iMLSB), 6% (12/200) had reaction on both clindamycin and erythromycin (cMLSB) and 13% (26/200) were negative for the D-Test (MS).

Table 1. *Isolation frequency of Staphylococcus aureus by age and gender.*

Characteristic	***S. aureus* Positive N = 29 (%)**	***S. aureus* Negative N = 71 (%)**
Age		
12-25	3 (10.3)	11 (15.5)
26-44	11 (37.9)	36 (50.6)
45-64	10 (34.5)	18 (25.4)
65+	5 (17.2)	6 (8.5)
Total	29 (100)	71 (100)
Gender		
Male	9 (31.0)	46 (64.8)
Female	20 (69.0)	25 (35.2)
Total	29 (100)	71 (100)

Table 2. *Frequency distribution of Staphylococcus aureus in different environment surfaces.*

Source	***S. aureus* positive**	
	N = 25	**(%)**
Environment		
Nets	0	(0)
Beds	10	(40)
Tables	7	(28)
Faucets	4	(16)
Wheelchairs	0	(0)
Doors	4	(16)
Trolleys	0	(0)
Total	25	(100)

Table 3. *Logistic regression analysis of factors associated with S. aureus exposure.*

Risk factor	***P-value***	**OR**	**95% CI**	
			Upper	**Lower**
Gender				
Male	0.88	1.05	0.51	2.18
Female	0.01*	0.08	0.01	0.42

Risk factor	*P-value*	OR	95% CI	
			Upper	Lower
Sample type				
Bed	0.01*	0.08	0.01	0.42
Door	0.53	0.62	0.12	2.68
Faucet	0.90	0.92	0.21	3.69
Net	0.99	0.00	0.04	0.06
Patient	0.70	1.20	0.47	3.22
Table	0.03*	4.57	1.22	1.89
Trolley	0.88	0.024	0.00	0.037
Wheelchair	0.99	0.001	NA	0.028
Source				
Human	0.01*	0.08	0.01	0.42
Object	NA	NA	NA	NA
Ward				
Eye	0.01*	0.08	0.01	0.42
Maternity	0.01*	9.92	2.0	19.3
Recovery	0.04*	5.09	1.29	8.60
Surgical	0.10	3.82	0.89	6.75

*Statistically significant factors ($p< 0.05$)

Table 4. *Antimicrobial resistance patterns of MRSA and MSSA isolates against the tested antibiotics.*

Drugs	*MRSA* (N=57) *N (%)*	*MSSA* (N=34) *N (%)*	*P=value*
E 15 µg	15 (26.3)	15 (44.1)	0.00
AZM 15 µg	15 (26.3)	14 (41.2)	0.09
OF 15 µg	2 (3.5)	2 (5.9)	0.47
GEN 10 µg	4 (7.0)	0 (0)	0.05
CIP 30 µg	6 (10.5)	1 (2.9)	0.05
FOX 30 µg	15 (26.3)	2 (5.9)	0.00
Total	57 (100)	34 (100)	

E: Erythromycin, AZM: Azithromycin, OF: Ofloxacin, GEN: Gentamicin, CIP: Ciprofloxacin, FOX: Cefoxitin

Table 5. *Prevalence of different antimicrobial resistance type among MRSA and MSSA isolates.*

Resistance Type	Erythromycin	Clindamycin	D-Test	*S. aureus* N (%)	MRSA N (%)	MSSA N (%)	P value
$iMLS_B$	R	S	D+	7 (7.8)	4 (10)	3 (6)	*0.053*

Resistance Type	Erythromycin	Clindamycin	D-Test	*S. aureus* N (%)	MRSA N (%)	MSSA N (%)	P value
$cMLS_B$	R	R	-	12 (13.3)	8 (20)	4 (8)	*0.028*
MS_B	R	S	D-	26 (28.9)	8 (20)	18 (36)	*0.000*
MDR	S	S	-	45 (50)	20 (50)	25 (50)	*0.000*
Total no. (%)	N/A	N/A	N/A	90 (100)	40 (100)	50 (100)	

$iMLS_B$: Inducuble Macrolide-lincosamide-streptogramin B phenotype, $cMLS_B$: Constitutive Macrolide-lincosamide-streptogramin B phenotype, MS_B: MS_B phenotype, N/A: Not applicable

Table 6. *MRSA prevalence in each ward by patients and environment.*

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)

Table 7. *Prevalence of different antimicrobial resistance type among MRSA and MSSA isolates.*

Resistance type	Overall N=200 N (%)	MRSA N=40 N (%)	MSSA N=50 N (%)	P value
$iMLS_B$	7 (3.5)	4 (57.1)	3 (42.9)	*0.053*
$cMLS_B$	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. Discussion

This study reports isolation *S. aureus* to increase with age from 21.4% among patients 16- to 25-years to 45.5% among those aged 65 years, the isolation of the bacteria has been reported to increase with age. This is in accordance with a study conducted in Germany [15]. Where the older the patience was, they were likely to acquire *S. aureus* due to several factors such as immunocompromised that comes with age and longer hospital stays. In the hospital environment, the beds and tables had the highest frequencies of *S. aureus* that were respectively 38.4% and 26.9%, followed by faucets and door handles which were equally at 15.4%. We did not isolate *S. aureus* on nets, wheelchairs, and trolleys. Our findings differ with those observed in a community hospital in Nigeria, which found doors to have the lowest isolation frequency of *S. aureus*, while bed linens (nets) had a much significant rate of *S. aureus* [16]. However, this trend made sense as the hospital beds and tables always had patients, HCW and visitors on them as opposed to nets that were never used, wheelchairs and trolleys that were often sanitised and rarely used.

The overall prevalence of MRSA for patients and the hospital surface at the MRRH was 8.5%. The MRSA prevalence findings were consistent with previous studies conducted in Dar es Salaam that looked at two regional hospitals (Mwananyamala and Amana) in the city [6]. The studies documented an increase prevalence hospital-acquired MRSA within the two regional hospitals: Muhimbili National Hospital (MNH) and Bugando Medical Centre. The overall prevalence of MRSA nasal carriage at the two hospitals was 8.5%

for patients admitted. In the study, clinical specimens were used from hospitalized patients who presented symptoms to track the number hospital-acquired *S. aureus* infections [6, 21].

Furthermore, it was found at the MRSA prevalence at the MRRH was higher among patients (13%) than hospital surfaces (4%). The prevalence was higher for patients as opposed to the hospital surfaces because of regular cleaning of hospital environment with disinfectants [2]. However, the prevalence for among nasal carriage patients was slightly higher at 13% than what was previously observed in Dar es Salaam (8.5%) [6]. The MRSA prevalence differed within the different wads sampled, the female recovery ward and the maternity ward had the highest prevalence of 23.1%. This was physically observed by the overcrowding of these two wards in comparison to the rest of the wards. The surgical male ward, surgical female ward, and the eye clinic each had the prevalence rate of 15.4%. The surgical wards are often assumed to have lower MRSA prevalence in comparison to other hospital wards because of the vigorous disinfection surgical wards tend to undergo prior surgeries [17]. The 15.4% rate observed at the MRRH was slightly higher than observed in Malaysia when comparing MRSA prevalence in different wards [17].

The lowest prevalence in patients was among the male recovery ward (7.7%), which was physically observed by the lack of patients and visitors.

The rate of MRSA according to different ages was most surprising as patients between the ages of 18 to 30 had the highest rates for MRSA at 50%. It was assumed that MRSA prevalence increases with age, due to age decreasing the host's ability to resist exposure [18]. However, the biggest factors in acquiring MRSA remained to be the environment. The 18-30 age group had the highest number of people in the hospital and hence a larger prevalence was absorbed. Those between the ages of 31 to 60 had a prevalence of 31.8%, then followed those between the ages of 7 to 17 at 13.6% and those older than 60 had the lowest rates of MRSA with 4.5%. We found the highest MRSA prevalence in the environment was in the recovery female ward (50%) followed by the recovery male ward (25%) and the surgical female ward (25%) respectably. A similar trend was observed at the Muhimbili National Hospital, female wards had higher rates of MRSA [7]. The prevalence was expected to be higher in men as opposed to women, this is due to the behaviour theory observed, which indicates that women were more likely to practice personal hygiene in comparison to men [19]. The rates were higher for female patients due to females having more people sampled (69%) while only 31% of the samples were male.

The antimicrobial susceptibility test of isolated *S. aureus* found that most of the MRSA were highly resistant to cefoxitin, erythromycin and azithromycin, followed by moderate resistance to ciprofloxacin, gentamicin and ofloxacin. The data for MSSA prevalence in Tanzanian hospitals is rather inconsistent [20]. MRSA can be recovered from 1% to 27% of surfaces in patient rooms [2]. There 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 [20]. 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 [16]. The D test results found in this study were significantly lower than those previously reported, where the prevalence of inducible Clindamycin resistance (21.3%), constitutive Clindamycin resistance (3.4%), MS phenotype (resistance to Erythromycin alone (12.4%)), and multidrug resistance was found to be 16.9% [6]. This could be due to no usage of Clindamycin within the population sampled or correct usage of Clindamycin unlike what was discovered in Dar es Salaam Joachim *et al.* (2017) [6].

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. The trend showed higher resistance to commonly used antimicrobials as observed in MSSA samples in comparison to MRSA [5, 21]. 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 where higher prevalence was found in patients within ICU (intensive care unit) who had been to hospital more than three times [21].

5. Study Limitations

Due to the study being conducted at the peak of the SARS-CoV-2, Corona virus (COVID-19) outbreak in Tanzania, several limitations were put in place including the decreased number of people allowed in the hospital. In future, it would be advisable to include what possible factors visitors might have in contributing to the increased numbers of *S. aureus* bought into the hospital.

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 as previously observed longer stay in hospital can be factor that can contribute to patients testing positive for MRSA [7]. However, the MRRH data is an added information to guide any other regional hospital outside Dar es Salaam Tanzania's economic capital. Furthermore, we were not able to perform molecular genotyping due to lack of resources and time bound factors.

6. Conclusion

This study 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. The presence of *S. aureus* was observed more in older patients and hospital surfaces that were commonly used. The authors encourage more studies on MRSA so as to establish the true prevalence of such a bacterial strain in Tanzania.

Abbreviations

AMR: Antimicrobial Resistance
HAI: Hospital Acquired Infection
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
RHMT: Regional Health Management Team
WHO: World Health Organization

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Ethical Approval and Consent to Participate

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 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.

Consent for Publication

The authors and participants agreed not to publish any individual person's data than the bacterial isolates. Therefore the consent to publish is not applicable in this submission.

Author's Contributions

NG, AK, SK and MM, conceiving, designed and executed the study; NG, PBM, AK, SK and MM, did the sample analysis; NG, AK, SK and MM, analysed the data; NG, AK, SK and MM, participated in literature search; NG wrote the initial draft which was reviewed critically by all authors while PBM read the final version of the manuscript. All authors have read and approved the final version of the manuscript.

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Conflicts of Interest

The authors declare no conflicts of interest.

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