

Methicillin Resistant *Staphylococcus aureus* nasal carriage and associated factors in a rural tertiary hospital in Eastern Uganda: A prospective cross-sectional study

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Abstract

Background: Asymptomatic carriage of Methicillin-Resistant *Staphylococcus aureus* (MRSA) can predispose the host to a wide array of infections that can be difficult to treat due to antibiotic resistance. To inform public health strategies, the study sought to describe MRSA nasal carriage frequencies and the associated factors concerning nasal carriage among patients attending Mbale Regional Referral Hospital (MRRH). **Methods:** Nasal swabs were obtained from consented (aged >15years) participants presenting to the hospital for medical care between January and April 2018 [L1]. Direct Culture of swabs was performed on blood agar and then incubated at 37°C for 24 hours. Identification of *S. aureus* was done using conventional biochemical tests. Phenotypic screening and confirmation of MRSA was done using cefoxitin disc (30µg) test and MICs on the Phoenix M50 instrument respectively. Patient demographic characteristics and the MRSA nasal carriage risk factors were collected using a pre-tested questionnaire. **Results:** Overall, majority of the participants were in-patients (138, 63.3%) with the proportions of both females and males among the participants being 154/218 (70.6%) and 64/218 (29.3%) respectively. Mean age for both female and male participants was 40.16 (SD± 17.04) years respectively. *S. aureus* nasal carriage rate among the participants was 22.9% (50/218), with 57.9% (29/50) of the harboured strains phenotypically expressing methicillin resistance (*mecA* mediated). Phenotypic co-expression with inducible clindamycin resistance and vancomycin resistance was displayed in 45.5% (23/50) and 2% (1/50) of the studied isolates respectively. Colonisation with MRSA did not show any significant relationship with all the studied factors. **Conclusion:** There was a moderate *S. aureus* nasal carriage among the participants in Mbale Regional Referral Hospital with a highly noted phenotypic expression of methicillin resistance among the isolated *S. aureus* strains. The studied factors were not significantly associated with the rate of MRSA nasal carriage. For surveillance purposes to combat future outbreaks, there is a need to do a larger study to better draw generalizable conclusions of carriage in the population.

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Background

S. aureus among the most significant human bacterial pathogens worldwide (1), with MRSA currently being the most common antibiotic-resistant strain in most of the hospital and community settings (2)(3). *S. aureus* is also the second leading cause of nosocomial bacteremia (4) and has been highlighted as a priority organism of interest by the WHO (5). Asymptomatic nasal carriage of MRSA can predispose the host to a wide array of infections that would pose a challenge in the management of the cases in the current era of antibiotic resistance (6)(7).

The magnitude of MRSA remains greatly unknown in developing countries yet surveillance systems to guide interventions require expertise and resources, which are inadequate(8). Studies conducted elsewhere by Conceição *et al.*, and Aiken *et al.*, documented MRSA prevalence rates of 26.9% and 7.0% respectively (9)(10). However, in resource-limited settings, not much has been done to explore on MRSA nasal carriage as reported by Bebell *et al.*, 2016 and yet MRSA is currently recognized as the leading

cause of hospital-acquired infections (11). Empirical and inappropriate use of antibiotics, multiple-pathology and use of invasive devices have attributed to a high rate of MRSA hence extended hospital duration and high treatment costs among patients (12). Documented reports about Methicillin resistance in WHO regions an exceeding 20% proportion with the highest recorded being 80% in some regions (13). In Africa, heterogeneity has been reported in the MRSA prevalence rates both intra-country and inter-country (14). Data collected from nine African countries indicates that MRSA prevalence rates range between 12% and 80% (15)(16). In East Africa and Uganda in particular has documented high proportions of between 31.5 to 42% within patients and healthcare professionals (17)(18).

Identifying the source, reservoirs and vectors for the spread of antibiotic-resistant bacteria poses significant challenges in tracking antimicrobial resistance. The patient's carried endogenous microflora may all play a vital role in the cause of nosocomial infections (19)(20). In a study conducted on surgical wound infections in the hospital indicated that 65.9% of the cases were caused by MRSA strains (21). There are currently no documented reports on nasal colonization and carriage rates of MRSA among patients and associated carriage factors in Mbale Regional Referral Hospital. With most patients coming from different settings, our study aimed to determine the prevalence of *S. aureus* and MRSA nasal colonization, and further identify the risk factors associated with asymptomatic carriage of MRSA.

Methods

Study design

This was a descriptive cross-sectional study carried out between January and April 2018 at Mbale Regional Referral Hospital on both in and Outpatients.

Study area

The study was conducted at both the Outpatient and In-patient departments (wards) of Mbale Regional Referral Hospital located in Mbale municipality. The hospital serves as both a regional referral and a tertiary teaching hospital for the medical school of Busitema University and School of clinical officers. It has a bed capacity of 400 and serves a population of approximately 4 million people.

Target population

The study targeted all patients seeking medical care at Mbale RRH who are asymptomatic carriers of MRSA. These included those from the inpatient (medical, surgical and maternity wards) and outpatient departments.

Inclusion Criteria

We included Patients aged 15 years and above, seeking medical care at either the In-patient or the Outpatient department at MRRH and had consented to take part in the study. Written informed consent for children aged 16 and 15 years was sought from their parents or guardians.

Sample size and recruitment of study participants

The sample size estimation was calculated using Kish Leslie formula. Consent was sought prior to recruiting of the participants. A cluster random sampling technique was used whereby participants were selected randomly with respect to the two broad clusters i.e. OPD and in-patients to ensure equal representative sampling. A questionnaire was administered through interviewing of participants to capture both demographic factors and predictor factors for MRSA nasal carriage.

Sample collection and transportation

From each participant, specimens for *S. aureus* culture were collected from both the anterior nares using sterile broth moistened dry swabs. The swabs were immediately placed into a tube with Cary-Blair transport media (BBL™, BD bioscience), labelled with the participants' study number, initials, date and time of sample collection. Samples were immediately delivered to the Clinical Microbiology Laboratory, Mbale RRH for processing.

Isolation and identification of *S. aureus*

The swabs were first inoculated on Blood Agar and incubated at 37°C aerobically for 24 hours after which plate reading was done. Gram staining was performed on discrete colonies displaying the culture characteristics of *S. aureus* to confirm for Gram-positive cocci in clusters. A series of conventional biochemical tests such as catalase test using 3% hydrogen peroxide, bound and free coagulase using reconstituted rabbit lyophilized plasma (Remel, Europe, Ltd. Dartford, Kent DA2 6PT, UK) were performed to identify the *S. aureus*. Further confirmation was done by sub-culturing of the isolated colonies of *S. aureus* onto Mannitol Salt Agar (MSA, Oxoid, CM0085. Thermo-scientific, US) and incubated at 37°C for 24 hours aerobically. After 24 hours, the plates were examined for growth with interest in mannitol fermenting colonies that appeared as yellow colonies, measuring 1-2 mm in diameter and slightly raised.

Preservation of the Isolates

By use of a sterile pre-flamed wire loop, pure growing colonies were scraped off the Blood agar purity plates and suspended in 1ml of 15% glycerol broth the frozen at -80°C till needed for MRSA phenotypic testing.

Phenotypic detection for MRSA

Agar disk diffusion (Kirby Bauer) technique on 4% sodium chloride Mueller Hinton Agar (BBL™, BD) was employed. The inoculum was prepared by picking distinct colonies from a fresh pure loon culture on Blood agar and suspended into 5mls of a 0.85% saline water to make a bacterial suspension. The suspension was vortexed for 15 seconds and the turbidity adjusted visually by adding sufficient saline water to achieve a 0.5 McFarland standard. A sterile cotton swab was dipped into the suspension, rotated several times and the excess fluid removed by pressing on the sidewalls of the tube. The dried surface of the agar was evenly streaked with the sterile swab aseptically rotating the plate at 60° to ensure uniform

distribution. A ceftioxin disc (30 µg, HIMedia, Mumbai, India) was placed on the surface of the inoculated plate using sterile forceps. The setup was incubated at 37°C for 24 hours aerobically. The plates were read after 24 hours of incubation and the zone diameters of inhibition were measured using a Vernier calliper. The measured zones of inhibition were recorded and interpretations were done following the interpretive cut-offs (MRSA_ ≤ 21 mm and MSSA _ ≥ 22 mm) as per the Clinical Laboratory Standard Institute (CLSI) guidelines (22). The positively screened isolates were subjected to ceftioxin MICs (>8mg/mL) to confirm for MRSA carriage on the Phoenix M50 instrument using PMIC panels (BD) for confirmation.

Data Analysis

Data analysis was done using Stata Corp. version 13. Descriptive statistics including proportions, means were used to describe the participants and to determine prevalence. We considered a univariate analysis at a level of significance of 0.2 to describe the categorical variables by sex and obtain baseline characteristics. The *P-value* was adjusted to allow for multiple logistic regression. Associations were generated using Odds ratio at 95% CI and a *P-value* of ≤0.05. We performed a logistic regression to determine any association between the factors collected as predictors and MRSA nasal carriage as the outcome.

Quality Control

Standard Operating procedures were followed, aseptic transfer techniques were performed, reagents were kept at 2-8°C, and the questionnaire was pretested before the commencement of the study. *S. aureus* ATCC 25923 was used as the positive control strain for the identification of biochemical tests and susceptibility tests on 4% sodium chloride Muller Hinton Agar. Commercially available control strain of *S. aureus* ATCC 700699 was used as a live positive control for MRSA on the PMIC panels.

Results

Demographic characteristics of patients

The study recruited 218 participants aged 15 years and above presenting to Mbale Regional Referral Hospital for medical care. Majority of the participants were in-patients 138/218 (63.3%) with the proportions of both females and males among the participants being 154/218 (70.6%) and 64/218 (29.3%) respectively. Mean age for both female and male participants was 40.16 (SD± 17.04) years respectively as shown in table 1 below.

The proportion of patients with *S. aureus* and MRSA nasal carriage attending Mbale Regional Referral Hospital

The prevalence rate for *S. aureus* nasal carriage among the participants was 22.9% (50/218) as indicated in table 2 below. Proportions for MRSA nasal carriage among the participants were found to be 13.3% (29/218) and 9.63% (21/218) of whom were colonized with MSSA (table 3).

Distribution of Methicillin resistance among *S. aureus* Isolates.

Of the 50-studied *S. aureus* isolates, 57.9% (29/50) of the isolates were found to be phenotypically expressing methicillin resistance (*mecA* mediated) while 42.1% (21/50) were methicillin sensitive as seen in figure 1. Co-expression of MRSA with inducible clindamycin resistance and vancomycin resistance was observed among 45.5% (23/50) and 2% (1/50) respectively.

Multivariate analysis of studied factors for association with Methicillin Resistant *S. aureus* nasal colonization.

Colonization with Methicillin Resistant *S. aureus* was not significantly associated with age (OR=0.9825889; 95% CI=0.9612351-1.004417, *P*-value=0.117), history of hospitalization (OR=1.156944; 95% CI=0.5689258-2.352712; *P*-value=0.687), contact with animals (OR=1.141084 ; 95% CI=0.8069104-1.613653; *P*-value=0.455), antibiotic use (OR=0.7516896; 95% CI=0.3804775-1.485974; *P*-value=0.411) and HIV status (OR=0.7215959; 95% CI=0.2871594-2.107922; *P*-value=0.551).

Discussion

Prevalence of *S. aureus* nasal carriage

Results from this study indicated that the nasal carriage rates for *S. aureus* among the participants was 22.9%, a rate significantly higher than that reported from studies from Thika Hospital, Kenya (8.9%), Nigeria (18.3%), and China (2.4%) (23)(24)(25). Studies conducted elsewhere by Bebell *et al*, Ouedraogo *et al*, Moniri *et al* and Ateba *et al* revealed slightly higher rates of 28.5%, 29.1%, 32.9%, 38% and 29% respectively compared to the finding in our study (11)(26)(27)(28). However, our finding was similar to reports from North Germany by Mehraj *et al*, Northwest Ethiopia and Italy, which documented *S. aureus* nasal carriage rates of 21.9% and 23% respectively (29)(30). The difference in the various findings would have been attributed to the sample size used giving the different studies a better power to detect the *S. aureus* nasal carriage. In addition, the studies were conducted in different geographical regions and the regions might have differing antibiotic usage practices hence differences in the carriage bio-films.

MRSA carriage Prevalence and associated carriage risk factors

In our study, we determined the prevalence of MRSA and MSSA among randomly selected participants who presented to the hospital for medical care. Our study documented an overall prevalence rate of 13.3% (29/218) for MRSA among the participants who were included in the study. However, we noted a high 57.9% (29/50) methicillin resistance expression among the carried *S. aureus* strains. The finding for the overall carriage rate (13.3%) among the participants was lower than the reported prevalence of 46% from Mulago hospital, Kampala, Uganda (18). However, our finding was higher than a prevalence rate of

2.8% reported by Bebell *et al* from Mbarara Hospital, Uganda (11). Our observed high (57.9%) methicillin resistance expression among the isolated *S. aureus* strains was contrary to reports from three regional hospitals in Tanzania that is Muhimbili National Hospital (10.5%), Mwananyamala and Amana regional hospitals (24.7%) (31)(32). An attribution to the high MRSA carriage could have been due to the high numbers of in-patients who were included in the study and duration of hospital stay could have had an effect of acquisition. In addition, the choice of the participants included could have affected the prevalence since our study did not consider the children age group. Among the MRSA strains identified, 45.5% (23/50) and 2% (1/50) phenotypically co-expressed inducible clindamycin resistance (MLS_B resistance marker) and vancomycin resistance respectively. A study by Mwanbi *et al* conducted in Uganda on clinical isolates reported a closely similar prevalence rate (36%) of clindamycin resistance (33). Resistance to clindamycin would have been due to an increased use of the drug for patient management in the region.

In our study, colonization by MRSA did not indicate any significant statistical association with all the tested variables. However, we observed different levels of consistency with the findings of previous studies. Our study indicated that antibiotic usage did not increase the likelihood of colonization by MRSA (OR=0.7516896; 95% CI=0.3804775-1.485974; *P*-value=0.411), while HIV status was found to have no effect on the nasal carriage rate even with low immunity (OR=0.7215959; 95% CI=0.2871594-2.107922; *P*-value=0.551). The statistical association findings by Bhattacharya *et al* that reported no association with immune status or recent antibiotic use (34), were similar to the findings in our study. Nonetheless, the no association with all the variable factors might be attributed to the difference in the characteristics and varied geographical location for the participants. The study had a limitation with the small sample size, which could have inferred a less power and generalization of results for MRSA carriage in the population.

Conclusion

Our study revealed that there was a moderate *S. aureus* nasal carriage among the participants but with high methicillin resistance expressed (57.9 %) among the isolated strains. Assessment of the factors with MRSA nasal carriage rates revealed that none of the factors significantly influenced carriage. The study has been the first in Mbale, Uganda to provide baseline data on *S. aureus* and MRSA nasal carriage. Therefore, there is need to do a larger extensional surveillance to understand the drivers and transmission patterns for *S. aureus* and MRSA within the hospital and community. However, the study had some limitations and in particular, there is still need to further phenotypically or genotypically assess the isolates using *PBP2a* agglutination assay or by PCR respectively to better understand the carriage. There is also need to evaluate a link between hospital acquired infection, carriage and possibility of zoonotic transmission.

Abbreviations

MCRI: Mbale Clinical Research Institute

MRSA: Methicillin-Resistant *Staphylococcus aureus*

MRRH: Mbale Regional Referral Hospital

WHO: World Health Organization

OPD: Out Patient Department

CI: Confidence Interval

CLSI: Clinical Laboratory Standard Institute

ATCC: America Type Culture Control

SD: Standard Deviation

HIV: Human Immune Virus

MSSA: Methicillin Susceptible *Staphylococcus aureus*

OR: Odds ratios

MRRH-REC: Mbale Regional Referral Hospital Research Ethics Committee

CSV: Comma Separated Version Files

CoNS: Coagulase Negative *Staphylococcus*

N/A: Not Applicable.

Declarations

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Ethics approval and consent to participate

Ethical approval was obtained from the Faculty of Medicine Research Committee at Mbarara University of Science and Technology (Ref: DMS/6) and Mbale Regional Referral Hospital Ethics Review Committee (Ref: MRRH-REC-IN-COM 110/2017). Written informed consent was obtained from the patients who had satisfied the study eligibility criteria for inclusion. The consenting process was done in a private room and all the participants were allocated identification numbers to ensure the confidentiality of the participants.

Consent to publish

Not applicable

Availability of data and materials

The data sets in comma separated version files (CSV) are available and have been attached as supplementary information files.

Competing interests.

The authors have no competing interests

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The authors pooled funds to support this project from design, data collection, and data analysis through manuscript writing.

Author contributions

This work was conducted in collaboration between all authors. Authors NT, GK, RN, BA and EK designed the study, wrote the protocol, participated in the data and statistical analysis process, literature searches and wrote the first draft of the manuscript. Author GM did the original conceptualization, supervised the fieldwork laboratory analysis and participated in the write-up of the manuscript. Authors POO and RM participated in the manuscript writing and proof reading. Author LA did the overall supervision of the work right from the development of the protocol, statistical analysis and manuscript conceptualization. All authors read and approved the final manuscript.

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Tables

Table 1: Demographic characteristics of patients attending Mbale RRH.

Demographic factors	Characteristics	Males (n=64)	Females (n=154)
Age	Mean age (40.16; SD± 17.04) in years	45	38
Animal contact	Yes	42	87
	No	22	67
Residence	Resident in town	18	62
	Resident in village	46	92
History of hospitalization	Has ever been hospitalized	21	52
	Never been hospitalized	42	102
Antibiotic usage	Use antibiotics	34	81
	Does not use antibiotics	30	73
HIV sero-status	Negative	45	107
	Positive	5	21
Isolated organism	Unknown	14	26
	CoNS	44	72
	No growth	9	43
Methicillin sensitivity	<i>S. aureus</i>	11	39
	MRSA	11	25
	MSSA	0	14
	N/A	53	114

Key: MRSA- Methicillin Resistant *S. aureus*, MSSA- Methicillin Susceptible *S. aureus*, CoNS- Coagulase Negative *Staphylococcus*, n- proportions per gender

Table 2: Proportions of patients with *S. aureus* nasal carriage.

Isolated Organism	Frequency (n)	Percentage (%)
<i>S. aureus</i>	50	22.9
CoNS	116	53.2
Not Applicable	52	23.8
Total	218	100

Key: n-number of participants, %-prevalence rate

Table 3: Proportions of MRSA and MSSA among patients at Mbale RRH.

Key: MRSA-Methicillin Resistant *S. aureus*, MSSA-Methicillin Susceptible *S. aureus*

Methicillin Sensitivity	Frequency	Percentage	Cumulative frequency
MRSA	29	13.30	13.30
MSSA	21	9.63	22.93
Not Applicable	168	77.07	100.00
Total	218	100.00	

Table 4: The multivariate analysis of studied factors for association with Methicillin Resistant *S. aureus*

Methicillin Sensitivity	OR	P-value	95% [Confidence Interval]	
Age	0.9825889	0.117	0.9612351	1.004417
Residence	0.7119681	0.347	0.3505714	1.445921
History of Hospitalization	1.156944	0.687	0.5689258	2.352712
Contact with animals	1.141084	0.455	0.8069104	1.613653
Antibiotic use	0.7516896	0.411	0.3804775	1.485974
HIV status (Positive)	0.7215959	0.551	0.2470208	2.107922
(Unknown)	0.71886	0.481	0.2871594	1.799557

nasal *colonization*.

Key: OR-odds ratios

Table 4: MRSA distribution by disc diffusion and MIC testing .

Methicillin Sensitivity	Cefoxitin disc screening (≤ 21 mm), (% [n])	MIC confirmatory (% [n]), Cut-off (>8 mg/mL)
MRSA	72 (36)	57.9 (29)
MSSA	28 (14)	42.1 (21)
Total	100 (50)	100 (50)

Key: MRSA-Methicillin Resistant *S. aureus*, MSSA-Methicillin Susceptible *S. aureus*

Figures

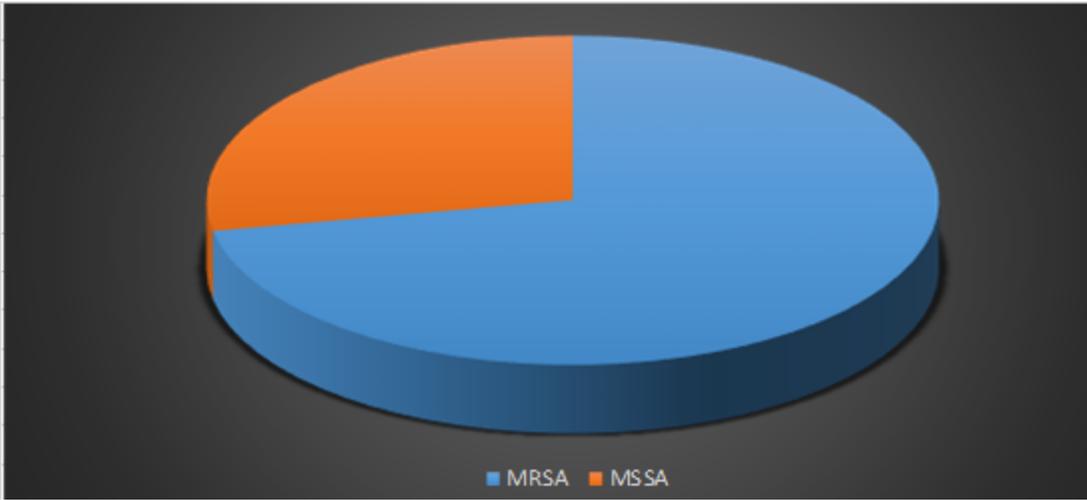


Figure 1

Distribution of Methicillin Sensitivity among carriers of *S. aureus* Key: MSSA-Methicillin Susceptible *Staphylococcus aureus*, MRSA-Methicillin Resistant *Staphylococcus aureus*