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Research Article

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Prevalence of Nasal Carriage of Methicillin Resistant Staphylococcus Aureus Among Patients, Health Care Workers and Patients' Care Takers at Kabale Regional Referral Hospital, South Western Uganda

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Abstract

Background

Methicillin Resistant Staphylococcus aureus (MRSA) is a type of Gram-positive bacterium that is genetically different from other strains of Staphylococcus aureus by virtue its capability to overcome the actions of methicillin and many other antibiotics making it difficult to treat.

MRSA development is as the result of the triggering of the resistance gene, mecA, which react by inhibition of the β-lactams from rendering inactive transpeptidases indispensable in cell wall synthesis. MRSA is one of the known major hospital acquired bacteria that causes severe ill health and mortality world over. The global prevalence of MRSA isolated in swabs samples differs from health care facility to another in various countries, with high rates ranging from 32–52% as reported in low resource settings in the developing countries. In Kabale Regional Referral hospital(KRRH), the prevalence of MRSA stands at 54% among isolates from wound swabs on surgical ward according to studies conducted by Andrew et al., 2016. However, there is hardly any known information of the prevalence of MRSA in nasal swabs of Health care workers (HCWS), patients and patients' caretakers in KRRH Uganda. This study aimed at ascertaining to the prevalence of nasal carriage of MRSA among HCWs, patients and patients' caretakers at KRRH.

Methods

A grand total of 382 samples were collected from the several HCWs, patients and patients' caretakers in different wards of KRRH in Kabale District. The nasal swab specimens were inoculated and cultivated on Mannitol salt agar at 37°C for 24 hours and the colonies subjected to Gram staining, Catalase, Coagulase test reactions and confirmed as S. aureus bacteria on DNase testing agar. Identification for MRSA was performed using the Cefoxitin (30µg) disc on Mueller Hinton agar medium by disc diffusion technique, antibiotic sensitivity testing was conducted using the Kirby–Bauer disc diffusion method on Mueller–Hinton agar (MHA) and results were interpreted in accordance with Clinical and laboratory standards institute (CLSI) 2020 guidelines. S. aureus mecA and pvl genes were identified and subsequently detected by PCR amplification assay using gene-specific primer pairs to confirm MRSA.

Results

Out of 382 study participants, 130 participants had MRSA identified phenotypically out of which, 115 of the participants, had MRSA as confirmed by the mec A gene. Generally, the prevalence of nasal carriage of MRSA in Kabale Regional Referral Hospital was found out to be 30.1%. It was found to be 31.49% in patients, 29.7% in HCWs, and 28% among patients' caretakers. MRSA was highly sensitive/Susceptible to Ceftaroline, Clindamycin, Ciprofloxacin, Linezolid, Chloramphenicol and Tetracycline.

Conclusion
Generally, the prevalence of nasal carriage of MRSA in the study area was found to be 30.1% and 31.49% in patients, 29.7% in HCWs, and 28% among patients' caretakers. The highest nasal carriage rate of MRSA was found in patients (31.49%). MRSA was more common in OPD department, followed by medical ward, Gynecology and Obstetrics wards. MRSA strains were sensitive to Ceftaroline, Clindamycin, Ciprofloxacin, Chloramphenicol, Linezolid and Tetracycline. Most of MRSA isolates were multidrug resistant to antibiotics such as Cefoxitin, Sulfamethoxazole-Trimethoprim, and Penicillin.

Key words: Nasal Carriage, Methicillin, Resistant Staphylococcus Aureus, MRSA

Abbreviations

AMR	Antimicrobial Resistance
API	Analytical profile index
ATCC	American Type Culture Collection
CA	Community acquired
CA-MRSA	Community Acquired *Methicillin Resistant Staphylococcus aureus*
CFU	Colony-Forming Units
CLSI	Clinical Laboratory Standards Institute
DST	Drug Sensitivity Test
ENT	Ear, Nose and Throat
FREC	Faculty of Medicine Research and Ethics Committee
HAI	Hospital-acquired infections
HA-MRSA	Hospital Acquired *Methicillin Resistant Staphylococcus aureus*
ICU	Intensive Care Unit
IRB	Institutional Review Board
KRRH	Kabale Regional Referral Hospital
MHA	Muller Hinton Agar
MRSA	*Methicillin-resistant Staphylococcus aureus*
MUST	Mbarara University of science and Technology
IPC	Infection prevention and Control
IPD	Inpatient department
OPD	Outpatient department
PBP	Penicillin binding protein
PCR	Polymerase Chain Reaction
PVL	Panton-Valentine Leucocidin gene
RRH	Regional Referral Hospital
SCC *mec*	Staphylococcal cassette chromosome *mec*
UNHLS	Uganda National Health Laboratories
WHO	World Health Organization

1. Introduction

Methicillin-Resistant Staphylococcus aureus (MRSA) is among the most prevalent nosocomial bacterial infections in most health care settings globally. This is because of its potential behavior to aggressively and quickly get used to antibiotics and consequently develop resistance. The health burden attributable to MRSA has significantly increased ill-health and deaths and in a similar way extended period of time in healthcare settings following infection generally. Studies have shown that MRSA species indicate elevated figures of attributable conditions such as septic shock and extended or rather indefinite care compared to species of bacteria that are susceptible to methicillin [1].

Globally the widespread presence of MRSA isolated from swabs samples differs from health care facility to another in various countries, with high rates ranging from 32–52% as reported in low resource settings in economically underdeveloped nations [2]. MRSA coming to light as a nosocomial pathogen continues to affect health care workers (HCWs), patients and patients' caretakers. Moreover, as such a high prevalence of MRSA, remain a key contributing factor in failure to manage patients effectively. The continuing rise of antibiotic resistance due to inappropriate use of antibiotics results into decreased treatment options for MRSA carriage and colonization [3].

High MRSA carriages among health care workers is known to be a pointer to the process of spread and transmission among patients in the course of administering medication, patients interaction and dispersion following the process of sneezing and coughing [4]. The widespread dispersal of MRSA strains is presumably due to extensive and irrational use of medicines and therapeutic agents in veterinary and human medicine for treating humans and animals. MRSA strains contain *mecA*, a gene that encodes for the PBP2a, which triggers a form of resistance among antibiotics that have a β-lactam ring structure methicillin inclusive, that makes them a big global threat [5].

Molecular identification, characterization and detection of *pvl* gene in community associated *S. aureus* is a marker and predictor of the existence and occurrence of pathogenic *S. aureus* in the community. Such discovery is very important and key information in regard to the existence of MRSA superbug associated infections. One of the essential virulence factors associated with *S. aureus* is Panton-Valentine leucocidin. This cytotoxin is connected with processes that result into tissue destruction and decay and also leads to misfunctioning of leucocyte membranes. The face to face interaction process between HCWs, the community and healthcare settings act as a reservoir source of MRSA spread in various health care facilities. The spread of such bacterial strains by conveyance of acquired MRSA in both the poor healthcare settings and the community environment can create negative consequences due to inadequate laboratory detection infrastructure and patient management [2].

MRSA Nasal carriage among HCWs, patients and patients' caretakers can greatly affect infection prevention and control measures in a negative way, in a sense that, quite often the affected, patients' caretakers, patients and HCWs do not have symptoms, however, such categories of people pose a risk of spreading microorganisms to vulnerable patients [2]. Considering the magnitude of infections associated with MRSA, prompt singling out of the affected HCWs, patients' caretakers and patients, together with execution of prevention and control measures that encompass intervention like isolation of affected patients, surgery management of most at risk patients by decolonization, all can lead to reducing the likelihood of MRSA infection development and subsequent spread and transmission. Knowledge and deeper understanding of occurrence and existence of MRSA prevalence and current antibiotic sensitivity pattern is essential in guiding the process of identifying and subsequently selecting the ideal antibiotics. Nonetheless, the majority of healthcare settings in Africa and Uganda as a whole, there is no clear and elaborate monitoring and tracking mechanism, and non-existence of prevention and control policy in regard to MRSA, all this triggers an ever-continual rise in MRSA nasal carriage colonization and subsequent infection [3].

A previous study carried out at Kabale Regional Referral hospital, found an elevated prevalence of 54% of MRSA in isolates from wound infections [6]. However, there is limited information in regard to the prevalence of nasal carriage of MRSA among Health care workers, patients' caretakers, and patients at KRRH Uganda. This study aimed to determine the occurrence and magnitude of nasal carriage of MRSA among different categories of people such as, patients' caretakers, HCWs, and patients at KRRH, Uganda.

2. Materials and Methods

2.1 Study Design

This was a cross-sectional descriptive study design that involved nasal swab specimens collection from 382 HCWs, Patients and Patients' caretakers at KRRH.

2.2 Duration and Study Population

The research study focused on HCWs, patients and patients' caretakers in KRRH and was conducted between the months of November 2021 and May 2022.

2.3 Enrollment of Study Participants

Recruitment and enrollment of study participants involved seeking prior consent and assent from them. A technique of cluster random sampling was applied and it entailed selecting study participants in a random manner on the basis of two main cluster groups that is, OPD and IPD clusters to ascertain relatively equal distribution and representation during sampling. Data and information from study participants was collected using a questionnaire through the process of interviewing to gather demographic characteristics and other variables in regard to nasal carriage of MRSA.

2.4 The Process of Sampling

The procedure for sampling involved stratification of the study groups into three main individual strata. Proportionate probability was used, whereby the average number of study participants that attend OPD and IPD each day at Kabale Regional Referral hospital was calculated. This was ascertained using a process of dividing the number of study participants in each individual study group of the three groups who had attended OPD and IPD at KRRH daily in the previous one week before commencement of the study by the total number that visited OPD and IPD at KRRH in that very week and multiplied the number by the sample size of 382. The recruitment process of the study participants involved the use of consecutive sampling technique for a period of three weeks at KRRH.

2.5 The Inclusion Criteria

HCWs, patients' caretakers and patients on OPD and IPD who consented or assented and were aged 15 years and above. HCWs, Patients and Patients' caretakers who had never tested for MRSA and were working or requiring healthcare services at IPD or the OPD of KRRH. The same study participants who had provided consent or assent to get involved in the study. Written informed assent. For the study participants that fell in the category of children aged 15 to 17 years, written informed assent was provided by their parents or guardians who also provided informed consent.

2.6 Exclusion Criteria

HCWs, patients and patients' caretakers, who qualified to be in the study, but failed to give or provide the sample.

3. Data Collection Tools

Data collection was done using interviewer questionnaire on HCW, Patients' caretakers and patient's data more especially on socio demographics, reasons for seeking health care services or admission, Present diagnosis, antibiotic drugs used, time and duration of admission, workplace or occupation. This questionnaire was availed to study participants by principal investigator or the research assistant in the ward or on OPD. Laboratory Request form was used to capture nasal swab culture and drug susceptibility results.

3.1 Sample Collection and Transportation

The collection of nasal swab specimens from study participants was done by use of sterile cotton swabs, whereby each study participant was subjected to the process of collecting the specimen by rotating the sterile swab inside the nose of consenting patients, HCWs and patients' caretakers early in the morning at the time of admission or duty rotation for the case of HCWs. Transportation of the nasal swabs to the laboratory for testing, then involved dipping the collected nasal swabs into a tube containing Brain heart infusion broth (BHI) media, and subsequent labeling with the participants' study number, date and time of sample collection, before transportation to the Microbiology section in the main laboratory of KRRH for culturing.

3.2 MRSA, Isolation, Identification and Phenotypic Detection

Culture of collected nasal swab specimens was performed on Mannitol salt agar at a temperature 37°C for 18-24 hours. Bacterial growth on the culture media shown by the presence of colonies was ascertained and colonies then subjected to testing by carrying out tests such as Gram staining, Catalase, and Coagulase. Confirmation of *S. aureus* bacteria was done by identification of colonies on DNase testing agar. Cefoxitin (30µg) antibiotic disc was used to detect and screen for phenotypic MRSA on Mueller Hinton agar medium by disk diffusion technique [6]. Interpretation of results was done using guidance from the Clinical and laboratory standard institute (CLSI) 2020 guidelines on the basis of *S. aureus* Cefoxitin sensitivity or resistance whereby a zonal area of inhibition of ≥ 22 mm and ≤ 21 mm diameters respectively. Quality control was performed using *S. aureus* ATCC 25923 strains for methicillin sensitive *S. aureus* (MSSA) and ATCC 43300 for methicillin resistant *S. aureus* (MRSA) as described in the CLSI, 2020 guidelines.

3.3 Storage and Preservation of the Isolates

This was performed by aseptically picking pure growth colonies from Mannitol salt agar purity plates using a sterile wire loop and emulsified them in 1ml of 15% glycerol broth and stored in a freezer at −80°C until required for subsequent phenotyping and genotyping testing for MRSA.

3.4 Antimicrobial Sensitivity Pattern testing

Testing for antimicrobial sensitivity pattern was achieved by use of the Kirby–Bauer disc diffusion technique in the laboratory by sub culturing bacterial isolates on Mueller–Hinton agar (MHA) in accordance with Clinical and Laboratory Standards Institute (CLSI) 2020 guidelines. The process entailed emulsification of *S. aureus* colonies into 5 ml of 0.85% saline, and compared the turbidity by adjusting it to match 0.5 McFarland standard (1.5 ×108 cfu·ml−1). Testing was then achieved by spreading uniformly the inoculum from the tube on MHA using sterile cotton swabs. Determination of the MRSA bacterial isolate sensitivities to antibiotics was achieved using the Kirby-Bauer disc diffusion technique. The areas that showed zones of inhibition were ascertained by measuring using a meter ruler in millimeters(mm). Control organisms of *S. aureus* ATCC 25923 and ATCC 29213 were applied during the process of testing as control strains. Antibiotics used included Ceftaroline(30µg), Ciprofloxacin(5µg), Erythromycin (15µg), Levofloxacin(5µg), Gentamycin(10µg), Chloramphenicol(30µg), Tetracycline(10µg), Linezolid(30µg), Cefoxitin(30µg), Clindamycin(2µg), Penicillin(10U), Azithromycin(15µg), and Trimethoprim–sulfamethoxazole (1.25/23.75µg), as recommended by CLSI 2020 guidelines.

3.5 Genomic DNA Extraction from Bacterial Isolates by Boiling Method.

Genomic DNA extraction from bacterial isolates was done at DC Laboratories in Kamukuzi, Mbarara City, Uganda. Mannitol Salt agar was used by determine growth of bacterial isolates at a temperature of 37°C for a period of 18-24 hours. A Pea sized amount of the colonies were scrapped off the plate and placed in Labelled 1.5ml tubes with sample identification numbers of bacterial growth culture. A volume of one hundred microlitres (100 µl) of PCR water (RNAase free water/TE buffer) was pipetted and added to the tubes. A loopful of bacterial colonies from bacterial growth culture was transferred and emulsified in PCR water in respective labelled tubes and vortexed.

The tubes were then boiled at 95^0c for 1 hour and 30 minutes in a dry heat block (dry bathe), and allowed the tubes to cool after boiling. The tubes were then Centrifuged at 15000 Revolutions per minute (RPM) for 3 minutes (Maximum speed). A Supernatant solution volume of eighty microlitres(80µl) was put in labelled test tubes by pipetting procedure. DNA quantification was conducted using a Nanodrop spectrophotometer method equipment that quantified DNA in the samples at a wavelength of 260nm and 280nm. A volume of two microlitres(2µl) of the DNA extract was pipetted and gently put on the Nanodrop to quantify DNA in each of the samples. The lysate was stored at -20^0c until use for PCR Amplification.

3.6 Detection and PCR Amplification of Genetic Markers

PCR amplification and detection of S. aureus genetic markers mecA and pvl genes was achieved using gene-specific primer pairs as shown in Table 1. The PCR amplification protocol was done using a 25µl PCR tube. A total volume of 5.5µl PCR buffer RNAase free water was pipetted and added in the tube, this was followed by addition of the Master Mix 12.5µL and 2.0µl dNTPs-200mM each); 1.0µl of each primer (primer F and primer R) (10µM); and 5.0µl of template DNA.

A volume of twenty microlitres(20µl) of the Master mix (prepared master mix) was pipetted and dispensed in each PCR tube (132 tubes). A volume of five microlitres(5.0µl) of the DNA templates was added in tubes 1-130, followed by addition of three microlitres(3.0µl) of positive control into a labelled tube and five microlitres(5.0µl) of negative control into another labelled tube. The samples were vortexed and centrifuged in a mini spin centrifuge. The samples were then Loaded and run for 2 hours and three minutes on the conventional PCR Thermocycler (CLASSIC K960 Thermocycler). ATCC 25923, ATCC 33591, and ATCC 49775 *S. aureus* positive control strains were set ensure validity of the test results.

4. Gel Electrophoresis.

A 1% Agarose Gel electrophoresis was prepared by weighing 1g

of Agarose powder using an analytical weighing scale(balance) and dissolved in 100mls of TRIS Borate EDTA(TBE).

The mixture was then boiled in the Microwave for 2-3 minutes until clear as glass and allowed the Gel to cool to approximately 50^0c. A volume of 5.0µl of DNA staining dye (Safe view Classic TM-Cat #G108) {5µL per 100mls} was added and the gel poured into the gel casting mold, followed by application of the comb and allowed it to set before gently removing the comb.

The samples and the DNA ladder/marker 100bp (NEB-Biolabs #N3231L), were loaded into the wells in Electrophoresis Gel. This involved adding 2.0µl of DNA loading dye (Thermo Scientific #R0611) to each PCR product tube. A volume of 20µl of the product was then loaded into the well/gel and subsequently loaded the gel into the electrophoresis tank. The electrophoresis apparatus was properly set by connecting red to red and black to black electric poles. The electrophoresis parameter (voltage, current and time that is, voltage:200 volts, Time:1 hour(60minutes), Current:80 Mille Ampere) were set and adjusted accordingly before starting the process of electrophoresis by pressing the start button. The process of visualization of PCR amplicons was done in agarose gel containing SafeViewTM DNA stain/visualization dye (5ul/100ml) using a Dark Reader Transilluminator (Gene-Flash Trans-illuminator) equipment and the photograph captured for use. DNA fragments of 147 bp and 433bp, were used to correspond to *mecA and pvl genes* as shown in table 1a [7].

S. aureus genetic markers mecA and pvl, primer sequence.

Earmarked gene	Primer Direction	Primer sequence	Size of the Amplicon in bp	References
mecA	**F**	GTGAAGATATACCAAGTGATT	147	(Karmakar et al., 2018).
	R	ATGCGCTATAGATTGAAAGGAT		
pvl	**F**	ATCATTAGGTAAAATGTCTGGACATGATCCA	433	(Karmakar et al., 2018).
	R	GCATCAAGTGTATTGGATAGCAAAAGC		

Table 1a: S. Aureus Genetic Markers mecA and pvl, Genes Primer Sequence

4.1 Quality Assurance and Control.

Assurance of quality was achieved by strictly following standard Operating procedures, ensuring sterility by using sterile non-powdered latex gloves, monitoring of storage conditions of reagents in the refrigerator at 2-8°C, and administering questionnaires as a form of pilot testing before the commencement of the study. N95 face masks were used during sample collection and culturing of samples to avoid contamination. All samples collected were cultured immediately and standard *S. aureus* reference strains, including the Methicillin sensitive strains *S. aureus* (MSSA) ATCC 25923 and ATCC 29213, and a Methicillin resistant strain *S. aureus* (MRSA) ATCC 43300 were used. The controls were procured from UNHLS.

4.2 Data Analysis and Results Interpretation

Data collected was entered into excel spread sheet, analysis and results interpretation was then performed using Stata Corp. version 13. Results were summarized in form of percentages, frequencies, and proportions to describe the characteristics of study participants and to ascertain the prevalence.

4.3 Ensuring and Adherence to Ethics

The research process started with approval of research proposal by the department of medical Microbiology, FREC and IRB. The researcher then sought for permission from KRRH Hospital Director to conduct the study in the hospital. The study participants voluntarily provided written informed consent and informed assent after being sensitized and subsequently recruited and enrolled in the study. Confidentiality was ensured by the use of only anonymous codes and Laboratory numbers to identify participants. Prior health education sensitization talk on the study was conducted that targeted the study participants, that is HCWs, patients' caretakers and patients.

Study participants who lost interest in the study, their right to withdraw or not to participate was respected and guaranteed.

There was minimal risk associated with the process of sampling and taking specimens for culture and antimicrobial susceptibility testing to Microbiology laboratory. The use of N95 face masks, face shields, sterile latex gloves, laboratory coats, waterproof apron and sterile swabs ensured prevention of infection. The researcher handled all Study participants data was kept confidentially and only used for the study purpose. Bacterial isolates collected as a result of a process of culturing were stored safely in KRRH Microbiology laboratory freezer under lock and key. Testing protocols of handling patient data were meticulously followed. No data was collected until the supervisor and the department of Medical Microbiology together with the FREC and IRB approved the study.

5. Results

5.1 Study Participants Socio-Economic and Demographic Characteristics

A total of 382 samples were collected from the various study participants that included, HCWs, patients and patients' caretakers in different wards of KRRH in Kabale District. 181 samples were collected from patients, 100 samples from patients' caretakers, and 101 samples were collected from health care workers working on OPD and IPD as shown in table 1

Characteristic	Frequency	Percentage (%)
Sex		
Females	237	62.04%
Males	145	37.95%
Department/Ward		
Dental	7	1.83%
ENT	2	0.52%
Eye	2	0.52%
Gynecology &Obstetrics	50	13.08%
MCH	30	7.85%
Maternity	40	10.47%
Medical ward	47	12.30%
OPD	125	32.72%
Occupational therapy	2	0.52%
Orthopedics Unit	5	1.30%
Pediatric ward	10	2.61%
Physiotherapy	3	0.78%
Private Wing	10	2.61%
Psychiatric ward	5	1.30%
Surgical ward	40	10.47%
TB ward	4	1.04%
Participant Category		
Heath care workers	101	26.4%
Patients	181	47.38%
Patients' Caretakers	100	26.18%
District		
Kabale	275	71.98%
Kanungu	8	2.09%
Kisoro	3	0.78%
Mbarara	1	0.26%
Ntungamo	18	4.71%
Rubanda	51	13.35%
Rukiga	25	6.54%
Rukungiri	1	0.26%
Marital status		
Married	320	83.76%
Single	39	10.21%
Divorced	23	6.02%
Residence of participants		
Home	374	97.9%
Barracks	7	1.83%
School	1	0.26%
Occupation of the participants		
Health care work	104	27.22%
Trader	25	6.54%
Farmer	160	41.88%
Others	93	24.34%

Table 1: Study participants socio-economic and demographic variables.

Majority of the people under study in KRRH were females (62.04%). The highest percentage of people in the area under study were farmers (41.9%), 27.2% were heath care workers, 24.4% were students and a few (6.5%) were traders. A total of 47.38% of the participants were patients, 26.4% were Health Care workers where as 26.18% were patients' care takers. The highest percentage of the participants (28.8%) had primary as their highest level of Education, 23.82% were for Tertiary training, 19.37% were for secondary, 15.97% had no education at all and the least percentage (12.04%) had completed their university.

Most of the participants (90.31%) were Christians, 3.14% were Muslims and 6.5% were of other religions. 83.7% of the participants were married, 10.21% were single and a few of them had divorced (6.02%). A total of 97.9% of the participants were residing in their homes, 1.83% were from the barracks and a few (0.26%) were from school. Most of the study participants (43.46%) had been admitted in the hospital for 1 day where as 8.12% of the patients were admitted for 2 and 3 days, 34.29% had been admitted for 5 days while only 6.2% of the patients were admitted in the hospital for 4 days.

Majority of the participants 57.32%) had no history of a surgical procedure and 42.67% had a history of a surgical procedure. A total of 30.10% had a skin infection where as 69.89% did not have a skin infection. Most of the participants (72.25%) had a history of using antibiotics and 27.74% had not used any antibiotics. A total of 20.41% had gotten involved in a contact sport. Most of the participants 69.89% had no history of self-prescription of the drug where as 30.10% had a history of self-prescription of the drugs. A total of 53.92% of the participants had a history of contact with animals and the rest (46.07%) had had no contact with animal as shown in table 2.

5.2 MRSA Nasal Carriage Prevalence Among HCWs, Patients and Patients Caretakers.

Characteristic	Number of participants	Frequency		Test of significance	
		No MRSA carriers Number (%)	Confirmed MRSA carriers Number (%)	P-value	Chi^2
Participants	382	267(69.89%)	115(30.10%)		
Sex					
Females	237	171(72.15%)	66(27.84%)	0.219	1.5110
Males	145	96(66.20%)	49(33.79%)		
Participant Category					
Heath care workers	101	71(70.29%)	30(29.70%)	0.825	0.3838
Patients	181	124(68.50%)	57(31.49%)		
Patients' Caretakers	100	72(72.0%)	28(28.0%)		
Department					
Dental	7	5(71.42%)	2(28.57%)	0.021	29.3958
ENT	2	2(100%)	0(0%)		
Eye	2	2(100%)	0(0%)		
Gynecology & Obstetrics	50	38(76.0%)	12(24.0%)		
MCH	30	21(70.0%)	9(30.0%)		
Maternity	40	34(85.0%)	6(15.0%)		
Medical ward	47	35(74.46%)	12(25.53%)		
OPD	125	81(64.8%)	44(35.2%)		
Occupational therapy	2	2(100%)	0(0%)		
Orthopedics Unit	5	5(100%)	0(0%)		
Pediatric ward	10	4(40.0%)	6(60.0%)		
Physiotherapy	3	1(33.33%)	2(66.66%)		
Private Wing	10	5(50.0%)	5(50.0%)		
Psychiatric ward	5	5(100%)	0(0%)		
Surgical ward	40	25(62.5%)	15(37.5%)		
TB ward	4	0(0%)	4(100%)		
District					
Kabale	275	193(70.18%)	82(29.81%)		
Kanungu	8	8(100%)	0(0%)		
Kisoro	3	2(66.66%)	1(33.33%)		

Mbarara	1	1(100%)	0(0%)	0.566	
Ntungamo	18	11(61.11%)	7(38.88%)		
Rubanda	51	36(70.58%)	15(29.41%)		
Rukiga	25	15(60.0%)	10(40.0%)		
Rukungiri	1	1(100%)	0(0%)		
Other religion	25	17(68.0%)	8(32.0%)		
Marital status					
Married	320	224(70.0%)	96(30.0%)	0.238	1.0904
Single	39	30(76.9%)	9(23.07%)		
Divorced	23	13(56.52%)	10(43.47%)		
Residence of participants					
Home	374	260(69.51%)	114(30.48%)	0.525	1.288
Barracks	7	6(85.71%)	114.28%)		
School	1	1(100%)	0(0%)		
Occupation of the participants					
Health care work	104	72(69.23%)	32(30.76%)	0.723	1.3273
Trader	25	18(72.0%)	7(28.0%)		
Farmer	160	108(67.50%)	52(32.5%)		
Others	93	69(74.19%)	24(25.80%)		

Table 2: MRSA Nasal Carriage Prevalence Among Study Participants.

According to the study, 130 participants had MRSA identified phenotypically by growth on Mannitol Salt Agar and resistance to Cefoxitin antibiotic disc. A total of 267 participants were identified as having no MRSA. A total of 115 out of the 130 of the participants who had MRSA identified phenotypically, had MRSA as confirmed by the mecA gene. All the 130 participants who had MRSA identified phenotypically, had no Pvl gene.

The figure 1: Below Shows the Number of MRSA Cases as Confirmed by mecA Gene in KRRH

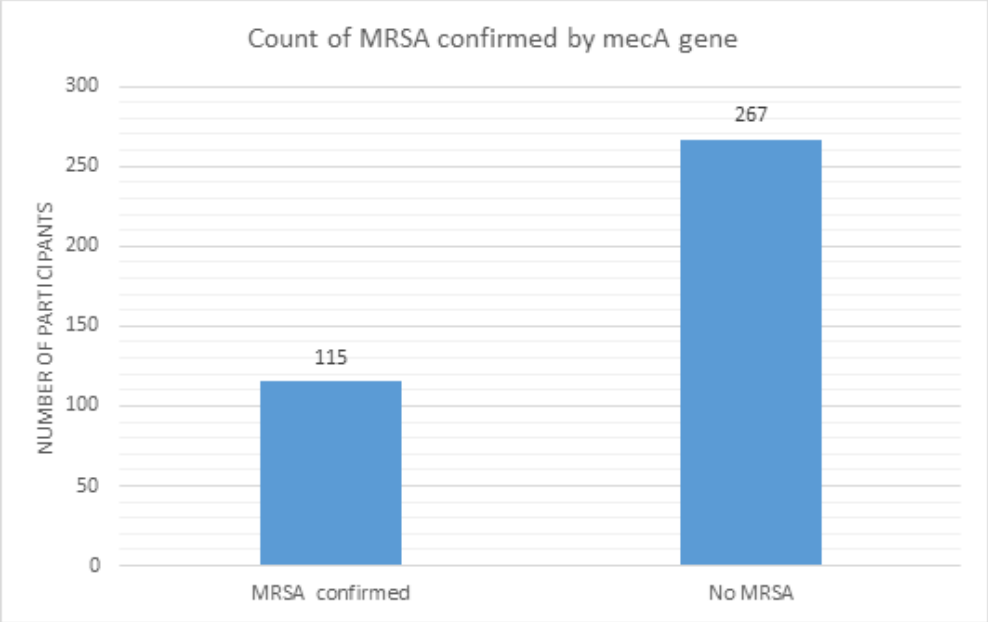

Figure 1: MRSA as confirmed by mec A gene.

The figure 2 below shows nasal carriage prevalence of MRSA among Patients, Health care workers and patients' caretakers at KRRH.

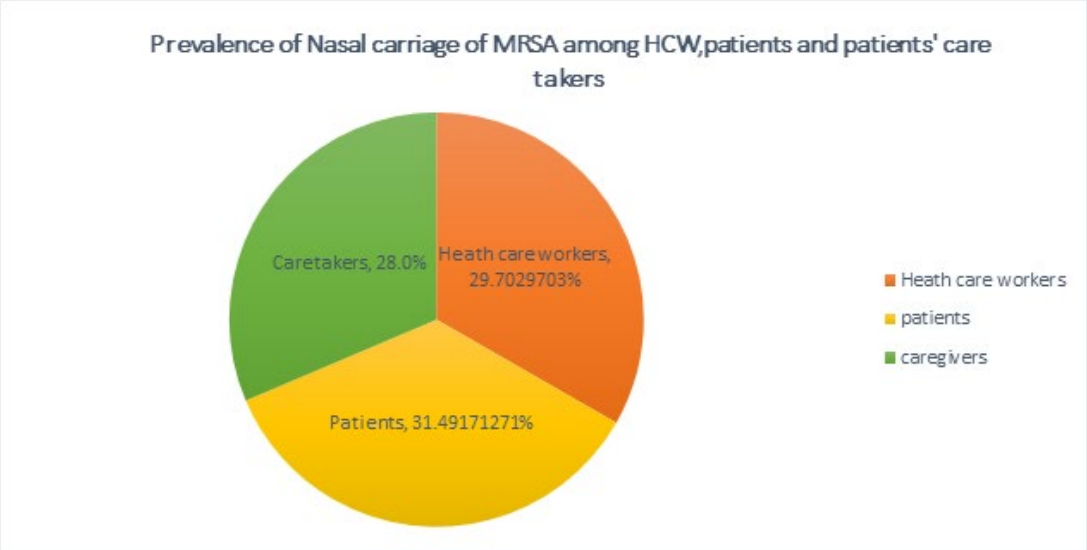

Figure 2: Prevalence MRSA Among Heath Care workers, Patients and Patients' caretakers.

According to the study, nasal carriage prevalence of MRSA in KRRH generally was 30.1% and it was 28% in patients' caretakers, 29.7% in Heath care workers and 31.49% in patients.

5.3 MRSA Nasal Carriage Antibiotic Sensitivity Patterns of Isolates from HCWs, Patients and Patients' Caretakers at KRRH.

A total of 130 isolates for MRSA were tested on 12 antibiotic discs according to Clinical and Laboratory Standards Institute (CLSI) 2020 guidelines. Antibiotic resistance patterns among the isolates showed the pattern as per the findings in table 3 below: The phenotypic resistance profile for MRSA from nasal samples when subjected to the above antibiotics revealed the highest resistance to Cefoxitin (100%) and Penicillin (100%). This was followed by Trimethoprim/Sulfamethoxazole (76.9%), Erythromycin (52.3%), Gentamycin (48.46%), Levofloxacin (46.9%), Tetracycline (40.76%). On the contrary, MRSA was more susceptible when using Clindamycin (70.7%), Ceftaroline (69.2%), Linezolid (61.5%), Chloramphenicol (60.0 %), Ciprofloxacin (56.9 %), and Tetracycline (56.9 %).

Antibiotic Name	Breakpoints	Number	Percentage Resistance(%R)	Percentage Intermediate(%I)	Percentage Susceptible(%S)
Penicillin G(P)10µg	S >= 29	130	100	0	0
Ceftaroline (CEF)30µg	20 - 24	130	30.7	0	69.2
Cefoxitin (FOX)30µg	S >= 22	130	100	0	0
Gentamycin (CN)10µg	13 - 14	130	48.46	2.3	49.2
Ciprofloxacin (CIP)5µg	16 - 20	130	39.2	3.8	56.9
Levofloxacin (LEV)5µg	16 - 18	130	46.9	3.8	49.2
Trimethoprim Sulfamethoxazole (SXT)25µg	11- 15	130	76.9	14.6	8.46
Clindamycin (DA)2µg	15 - 20	130	3.07	26.15	70.7
Erythromycin (ERY)15µg	14 - 22	130	52.3	17.6	30.0
Linezolid (LIZ)30µg	S >= 21	130	38.46	0	61.5
Chloramphenicol(C)30µg	13 - 17	130	30.76	9.2	60.0
Tetracycline (TE)30µg	15 - 18	130	40.76	2.3	56.9

Table3: The Table Below Shows the Break Points that Help in Determining the Sensitivity Patterns of the Different Antibiotics to MRSA.

6. Discussion

The subject matter under this chapter entail the discussion of the study findings, conclusion, limitations and recommendations in a chronological manner as per the study objectives including (1) To determine the prevalence of nasal carriage of MRSA among HCWs, Patients and Patients' caretakers at KRRH (2) To determine antibiotic sensitivity patterns of MRSA isolated from nasal carriage of HCWs, Patients and Patients' caretakers at KRRH. According to the study, it was found out that most numbers that were identified as having MRSA were mainly concentrated in surgical ward 15(37.5%), OPD 44(35.2%), medical ward 12(25.53%), Gynecology &Obstetrics 12(24.0%), and MCH 9(30.0%). This was attributed to high number of patients who may not have signs and symptoms of MRSA but are carriers and spread the infection through direct contact with contaminated hands and contaminated wounds especially those from surgical and gynecology wards.

Generally, MRSA nasal carriage prevalence in Kabale Regional Referral Hospital was found out to be 30.1%. The findings were corroborated by who reported that the prevalence of nasal carriage of MRSA differed from one health care facility to another and that it ranged from 32%-52%. It was also corroborated by who found out that the prevalence of MRSA was 43.14% at Kawolo general hospital [2,8]. It was also in agreement with a study conducted by Wangai et al., 2019, that showed the prevalence of MRSA was between 31% and 42% in Uganda [9]. However, the findings contrast with studies done in Mbale Regional Referral Hospital Eastern, Uganda by, that indicated the prevalence of MRSA to be 13.3% among HCWs and patients [10].

The prevalence of MRSA was found to be 31.49% in patients, 29.7% in HCWs, and 28% among patients' caretakers. This correlated with who conducted a study and indicated the prevalence of MRSA to be 31.5% in patients in Kampala International teaching Hospital, and who conducted studies in Ghana that indicated a prevalence of MRSA nasal carriage in Inpatients to be 30.0%, HCWs 27.8% and caretakers 10%. According to the study, antibiotic susceptibility patterns indicated that MRSA was more susceptible to Clindamycin, Ceftaroline, Linezolid, Chloramphenicol, Ciprofloxacin and Tetracycline [11,12]. This was corroborated by that reported Clindamycin, Ceftaroline, Teicoplanin and Telavancin are effective drugs that can be used to manage MRSA cases [13,14].

7. Conclusion

Generally, the prevalence of nasal carriage of MRSA in the study area was found to be 30.1%, and 31.49% in patients, 29.7% in HCWs, and 28% among patients' caretakers. The highest nasal carriage rate of MRSA was found in patients (31.49%). MRSA was more common in OPD department, followed by medical ward, Gynecology and Obstetrics wards. MRSA strains were sensitive to Ceftaroline, Clindamycin, Ciprofloxacin, Chloramphenicol, Linezolid and Tetracycline. Most of MRSA isolates were multidrug resistant to antibiotics such as Cefoxitin, Sulfamethoxazole-Trimethoprim, and Penicillin [15-41].

Recommendations

This study has been the first ever to be conducted in Kabale Regional Referral Hospital, Uganda, thus providing baseline data and information in regard to MRSA nasal carriage in patients, HCWs and Patients' caretakers. MRSA strain typing should be carried out to ascertain the cause of resistance. Phenotypic and genotypic studies are needed to establish and clarify the genetic mechanism behind susceptibilities to antibiotics. Further studies should aim at conducting the studies not only in healthcare settings but also in the community to ascertain transmission and MRSA strains patterns. Future studies should aim at detecting other genetic markers other such mecC gene, spa gene as well as whole genome sequencing and not only focusing on mecA gene and pvl gene.

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Authors' Contributions

JB and FB and were involved in the conception and study design frame work, TS and PN carried out data analysis, Presentation and interpretation. The first draft of the manuscript was done by BM and JB while FB and JB reviewed the first draft of the manuscript.

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Availability of Data and Materials

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Consent for Publication

Not applicable.

Competing Interests

The authors declare that they have no competing interests.

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