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Nasal carriage of methicillin resistant *Staphylococcus aureus* among medical students of a private institution in Ilishan-Remo, Ogun State, Nigeria

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Abstract:

Background: Nasal carriage of methicillin resistant *Staphylococcus aureus* (MRSA) is a major factor for its transmission especially from the health workers and medical students to their patients. There are a number of published data on the prevalence of MRSA among health workers but data on nasal colonization of medical students by MRSA are sparse in Nigeria. The objectives of this study are to determine the prevalence of nasal carriage of MRSA among medical students of the Ben Carson School of Medicine, Babcock University, Ilishan-Remo, Ogun State, Nigeria, and identify risk factors associated with this nasal carriage.
Methodology: A case control study involving 100 clinical (study group) and 100 pre- clinical (control group) medical students was undertaken between March 2018 and October 2019. Structured questionnaire was administered to obtain socio-demographic information and potential risk factors. Nasal swab was collected from each student and cultured for isolation of *S. aureus* by standard microbiology techniques. Phenotypic MRSA was detected by the cefoxitin 30µg disk diffusion method according to the guideline of Clinical and Laboratory Standards Institute. The *mec*A gene was detected by conventional polymerase chain reaction (PCR) assay.
Results: The prevalence of *S. aureus* nasal carriage among the study group was 14% (14/100) while the prevalence among the control group was 6% (6/100) (p=0.097). The prevalence of phenotypic MRSA among the study group was 4% (4/100) and 1% (1/100) among the control group (p=0.3687) while *mec*A gene was detected in 3 of the 4 (75%) phenotypic MRSA positive study participants and in the only (100%) phenotypic MRSA positive (1%) control group. Antibiotics usage without prescription, antibiotic treatment of common cold, and use of antibiotics in the previous one year, were significantly associated with MRSA carriage among the study group.
Conclusion: Although the prevalence of nasal carriage of *S. aureus* and MRSA among clinical and pre-clinical medical students was not statistically significant, the risk factors identified with carriage of MRSA among the study group indicates the need for antimicrobial stewardship program to reduce carriage and transmission of MRSA by medical students.

Keywords: methicillin resistant, *Staphylococcus aureus*, *mec*A gene, nasal carriage, medical students

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Transport nasal de *Staphylococcus aureus* résistant à la méthicilline parmi les étudiants en médecine d'un établissement privé, Ilishan-Remo, État d'Ogun, Nigéria

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Abstrait:

Contexte: Le portage nasal de *Staphylococcus aureus* résistant à la méthicilline (SARM) est un facteur majeur pour sa transmission, en particulier des agents de santé et des étudiants en médecine à leurs patients. Il existe un certain nombre de données publiées sur la prévalence du SARM parmi les agents de santé, mais les données sur la colonisation nasale des étudiants en médecine par le SARM sont rares au Nigéria. Les objectifs de cette étude sont de déterminer la prévalence du portage nasal de SARM chez les étudiants en médecine de la Ben Carson School of Medicine, Babcock University, Ilishan-Remo, Ogun State, Nigeria, et d'identifier les facteurs de risque associés à ce portage nasal.
Méthodologie: Une étude cas-témoins portant sur 100 étudiants en médecine cliniques (groupe d'étude) et 100 précliniques (groupe témoin) a été entreprise entre mars 2018 et octobre 2019. Un questionnaire structuré a été administré pour obtenir des informations sociodémographiques et des facteurs de risque potentiels. Un écouvillon nasal a été prélevé sur chaque élève et cultivé pour l'isolement de *S. aureus* par des techniques de microbiologie standard. Le SARM phénotypique a été détecté par la méthode de diffusion sur disque de céfoxitine 30µg conformément aux directives de la Institut des normes cliniques et de laboratoire. Le gène *mec*A a été détecté par un essai classique de réaction en chaîne par polymérase (PCR).
Résultats: La prévalence du portage nasal de *S. aureus* dans le groupe d'étude était de 14% (14/100) tandis que la prévalence dans le groupe témoin était de 6% (6/100) (p=0,097) .La prévalence du SARM phénotypique parmi les groupe d'étude était de 4% (4/100) et 1% (1/100) dans le groupe témoin (p=0,3668) tandis que le gène *mec*A a été détecté chez 3 des 4 (75%) participants phénotypiques MRSA positifs à l'étude et dans le seul (100%) groupe témoin phénotypique SARM positif (1%). L'utilisation d'antibiotiques sans ordonnance, le traitement antibiotique du rhume et l'utilisation d'antibiotiques au cours de l'année précédente étaient significativement associés au portage du SARM dans le groupe d'étude.
Conclusion: Bien que la prévalence du portage nasal de *S. aureus* et du SARM chez les étudiants en médecine clinique et préclinique n'était pas statistiquement significative, les facteurs de risque identifiés avec le portage du SARM dans le groupe d'étude indiquent la nécessité d'un programme d'intendance antimicrobienne pour réduire le portage et transmission du SARM par les étudiants en médecine.

Mots-clés: résistant à la méthicilline, Staphylococcus aureus, gène mecA, portage nasal, étudiants en médecine

Introduction:

Staphylococcus aureus is one of the most common bacterial causes of infection in the community and healthcare settings (1). However, the emergence of the drug resistant strains of *S. aureus* especially, methicillin resistant *S. aureus* (MRSA), has become a global threat (2,3) with reports from many United State hospitals and communities (4). According to a report on antimicrobial resistance by the World Health Organization (WHO), patients with MRSA infections are estimated to be 64% more likely to die than people infected with the non-resistant *S. aureus* strain (5).

In addition, patients with MRSA infections have twice as much hospital bills paid than those who are infected with methicillin sensitive *S. aureus* (MSSA) due to longer duration of illness, additional tests and use of more expensive drugs (3). In Nigeria, MRSA prevalence of 28.6% to 81% has been reported in different parts of the country among hospital in-patients (2,6,7). Methicillin resistance in *S. aureus* is due to acquisition of *mec*A gene that encodes an abnormal penicillin-binding protein 2a (PBP2a), and recently a similar gene called *mec*C gene has been described as a cause of MRSA (8).

The anterior nares are the main reservoirs of MRSA, although other body sites are frequently colonized such as the hands, skin, axillae, and intestinal tract (9,10). Nasal carriage of MRSA is a major factor for transmission of this pathogen (9). Healthcare workers (HCWs) are important in the transmission of MRSA because they are at higher risk of colonization than the general public, apparently due to increased exposure to this organism (2,11,12). Transmission occurs during contact with patients when infection control measures are not adhered to (12).

Aside HCWs, medical students can be

potential nasal carriers of MRSA and can aid in transmission of this pathogen within hospitals because of exposure to patients and other healthcare workers during clinical rotations (13). In Louisiana, exposure of medical students to the hospital environment was reported to have increased the prevalence of MRSA nasal colonization from 0% to 3.2% (14). In Taiwan, the MRSA carriage rate amongst medical students was reported to be 2.2% (15) while in Saudi Arabia, the prevalence rate was 6.7% (13). It has been reported that the prevalence of MRSA in health institutes is directly proportional to the morbidity and mortality caused by the strains (16). Therefore, screening for MRSA in hospitals is an important factor for building up successful infection control strategies.

In Nigeria, data on MRSA nasal colonization of medical students are sparse. Therefore, the objectives of this study are to determine the prevalence of MRSA nasal carriage rate among medical students of the Ben Carson School of Medicine, Ilishan-Remo, Ogun State, Nigeria, and identify risk factors associated with MRSA nasal carriage among the study participants.

Materials and method:

Study setting, design, subjects and sampling method

This was a case control study in which 100 clinical (study group) and 100 pre-clinical (control group) medical students of the Ben Carson School of Medicine, Babcock University were recruited for the study. The sample size of 100 was calculated for the study based on 6.7% prevalence of MRSA among medical students in Saudi Arabia to give a 95% confidence level and margin of error of 5%.

The controls were matched and recruited in ratio 1:1 with the study group. A simple random sampling using ballot without replacement technique was used to recruit the participants for the study. The study was conducted between March 2018 and October 2019.

Ethical approval

Ethical approval was obtained from Babcock University Ethical review committee before commencement of the study. Informed consent of each participant was also obtained.

Data and sample collection

A semi structured, pre-tested questionnaire was interviewer-administered to each student participant to obtain socio-demographic information and attributes considered risk factors for MRSA nasal colonization. Nasal swab was collected from each participant using moist sterile cotton tip swab and transported to the laboratory for analysis.

Isolation and identification of *Staphylococcus aureus*

The nasal swabs were inoculated onto Blood agar plates and incubated aerobically at 37°C for 24 hours. Colonies on culture plates were identified and confirmed as *S. aureus* by Gram stain reaction, catalase test, tube coagulase test and growth on mannitol-salt agar (a selective medium for *S. aureus*) (17). Antibiotic susceptibility test on each *S. aureus* isolate was done by the modified Kirby Bauer disk diffusion method (18).

Phenotypic methicillin resistance detection

All identified *S. aureus* isolates were screened for methicillin resistance by the cefoxitin disk diffusion test using 30µg disk on Mueller Hinton agar. Inhibition zone diameter of ≤21 mm was reported as oxacillin (methicillin)-resistant and >21 mm as oxacillin (methicillin)-sensitive (19). Methicillin sensitive *S. aureus* (MSSA) ATCC 25923 was used as negative control and MRSA ATCC 43300 served as positive control strain (19).

Detection of *mec*A gene by PCR assay

DNA extraction was done using DNA extraction kit (Zymo Research Quick-DNA Fungal/ Bacteria Miniprep Kit) following the manufacturer's instruction. The *mec*A gene was amplified using previously described primers *mec*A-F-5'-GTGGAATTGGCCAATACAGGAAC-3' and *mec*A-R-5'-GTTAGTTGAATATCTTTGCCATC-3' (Inqaba) which produced a 502 bp amplicon (13). The 25µl PCR volume consist of 12.4µl nuclease free water, 2.5µl of 10xPCR buffer, 2µl of 25mM $MgCl_2$, 1µl each of forward and reverse primers, 1µl of DMSO, 2µl of 2.5mM dNTPs, 0.1µl of 5µ/µl Taq DNA polymerase and 3µl of extracted template DNA.

The reaction was amplified in a PCR thermal cycler (Applied Biosystems Gene Amp PCR system 9700) of 9 cycles of initial denaturation at 94^0C for 5 minutes, denaturation at 94^0C for 15 seconds, annealing temperature of 65^0C for 20 seconds, extension at 72^0C for 30 seconds and holding temperature at 10^0C. This was followed by 35 cycles of denaturation at 94^0C for 15 seconds, annealing temperature of 55^0 C for 20 seconds, extension at 72^0C for 30 seconds and final extension at 72^oC for 7 minutes. The PCR products were run on 2.5% agarose gel with ethidium bromide dye and visualized under ultraviolet transilluminator and photographed. Amplicon size of 502 bp was considered positive for *mec*A gene (13).

Results:

A total of 200 medical students were studied; 100 in the clinical (study group) and 100 in the pre-clinical (control group) rotation. The socio-demographic characteristics of the participants as depicted in Table 1 showed that the two groups were well matched in gender and other characteristics. Expectedly however, majority of the participants in the study group (67%) were aged 21-25 years while majority of participants in the control group (92%) were aged 15-20 years.

The prevalence rate of *S. aureus* nasal carriage among the study group was 14% (14/100) while the rate was 6% (6/100) among the control group (p=0.0970). Similarly, the prevalence rate of MRSA nasal carriage among the study group was 4% (4/100) while the rate was 1% (1/100) among the control group (p=0.3687) (Table 2). There was no statistically significant relationship between the prevalence rate of *S. aureus* and MRSA nasal carriage in the study and that of the control group. The prevalence rate of methicillin resistance in the *S. aureus* population from the study group was 28.6% (4/14) while the rate in the control group was 16.7% (1/6). Out of the 4 phenotypic MRSA isolates from the study group, 3 (75%) were *mec*A positive while the only MRSA isolate (100%) from the control group was *mec*A positive (p=0.312) (Fig 1).

Table 1: Socio-demographic characteristics of the clinical (study group) and pre-clinical (control) medical students of Ben Carson School of Medicine, Ilishan-Remo, Ogun State, Nigeria

Socio-demographic variables	Study group No (%)	Control group No (%)
Age group (years)		
15-20	32 (32)	92 (92)
21-25	67 (67)	7 (7)
26-30	1 (1)	1 (1)
Gender		
Male	40 (40)	44 (44)
Female	60 (60)	56 (56)
Marital status		
Single	94 (94)	97 (97)
Married	6 (6)	3 (3)
Family type		
Monogamous	97 (97)	94 (94)
Polygamous	3 (3)	6 (6)
Religion		
Christianity	93 (93)	95 (95)
Islam	7 (7)	5 (5)

Table 2: Comparisons of the prevalence of nasal carriage of *Staphylococcus aureus* and MRSA between clinical and pre-clinical medical students of Ben Carson School of Medicine, Ilishan-Remo, Ogun State, Nigeria

Variable	Groups		Fisher Exact Statistics	
	Study (%)	Control (%)	OR	*p* value
Staphylococcus aureus			2.550	0.0970
Positive	14 (14)	6 (6)		
Negative	86 (86)	94 (94)		
MRSA			4.125	0.3687
Positive	4 (4)	1 (1)		
Negative	96 (96)	99 (99)		

MRSA=methicillin resistant *Staphylococcus* aureus; OR = Odds Ratio

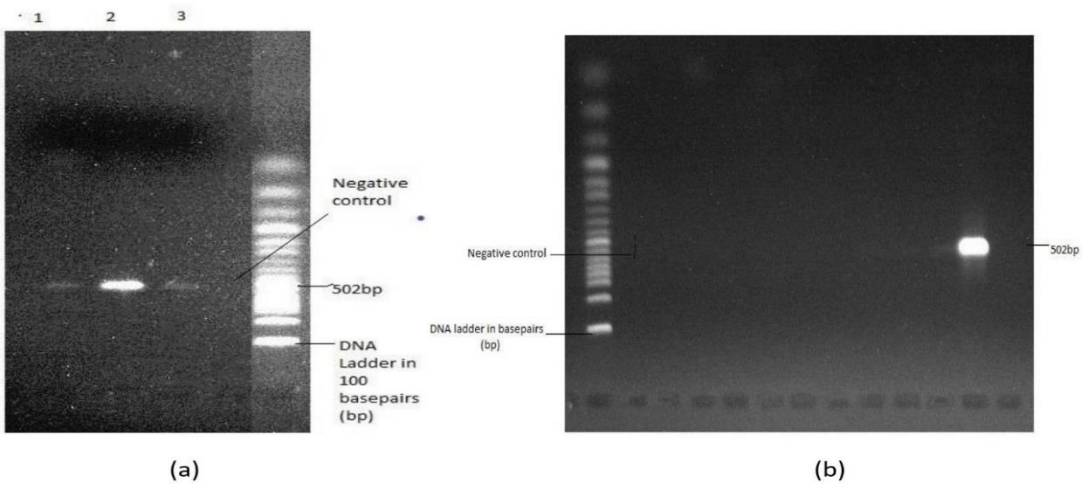

Fig 1: Gel electrophoresis picture of the amplified *mec*A gene of the 3 *S. aureus* isolates in the study group (a) and one *S. aureus* isolate in the control group (b)

Table 3: Factors associated with MRSA nasal carriage among clinical medical students of Ben Carson School of Medicine, Ilishan-Remo, Ogun State, Nigeria

Factors	Sub-variables	MRSA (n=100)		Statistics	
		Yes (%)	No (%)	X^2	*p*-value
Age in years	15-25	3 (2.4)	29 (90.6)	3.546	0.1698
	26-30	1 (1. 5)	66 (98.5)		
	31-35	0	1 (100)		
				OR	*p* value
Gender	Male	2 (5) .0	38 (95)	1.526	1.0000
	Female	2 (3.3)	58 (96.7)		
Marital status	Single	4 (4.3)	90 (95.7)	0.6464	1.0000
	Married	0	6 (100)		
Family type	Monogamous	4 (4.3)	90 (95.7)	0.6464	1.0000
	Polygamous	0	6 (100)		
Religion	Christianity	3 (3.2)	90 (96.8)	0.2000	0.2554
	Islam	1 (14.3)	6 (85.7)		
Antibiotics in the last three months	Yes	1 (20)	4 (80.0)	7.667	0.1881
	No	3 (3.2)	92 (96.8)		
Antibiotics without prescription	Yes	3 (25)	9 (75)	29.00	0.0051*
	No	1(1.1)	87 (98.9)		
Recent hospital admission	Yes	0	2 (100)	4.200	1.0000
	No	4 (4.1)	94 (95.9)		
Antibiotics for common cold	Yes	3 (60)	2 (40)	141.00	0.0002*
	No	1 (1.05)	94 (98.05)		
Recent surgery	Yes	0	8 (100)	1.157	1.0000
	No	4 (4.3)	88 (95.7)		
Use antibiotics in the last one year	Yes	3 (15)	17 (85)	13.942	0.0245*
	No	1 (1.25)	79 (98.75)		

X^2=Chi square; OR = Odds Ratio; **p* value less than 0.05 is considered significant

Antibiotic usage without prescription, usage of antibiotics to treat common cold and use of antibiotics in the last one year were all significantly associated with MRSA nasal carriage among the study group (Table 3).

Discussion:

MRSA cause infections in both the hospital and community, and healthcare workers and medical students can be the route

of infection especially when infection control practices are not adhered to (13). In this present study, the prevalence of MRSA nasal carriage among clinical medical student was 4%. In Nigeria, data on the prevalence of MRSA nasal carriage among the clinical students are sparse, however in other parts of the world, our finding is similar to that of Baliga et al., (20) in Turkey who reported a prevalence of 4.4% among medical students on clinical rotation and Piechowicz et al., who reported a prevalence of 4.5% (21). Other studies across the world have reported different prevalence rates among clinical students; Bettin et al., (14) in Columbia reported 1.6%, Bellows et al., (15) reported 3.2% in New Orleans, Chen et al., (22) reported 1.9% in Taiwan, and Jujena et al., (23) reported a prevalence of 9% in India. The different rates reported from different parts of the world might be related to the level and degree of adherence to standard infection control practices in hospitals where medical students undergo their clinical rotations.

In this present study, the prevalence of 4% for MRSA nasal carriage among the clinical students was higher than the 1% among the pre-clinical students, and similarly with *S. aureus* nasal carriage (14% versus 6%) but the differences in rates were not statistically significant, probably due to small number of cases. However, frequent exposure to the hospital is known to play a role in nasal colonization by MRSA (13), which could be responsible for the higher colonization, albeit statistically insignificant, of the clinical students who are more exposed to hospital at this stage of their training.

Other studies have reported higher colonization rate in clinical than pre-clinical students, Bellows et al., (14) reported that exposure of students to hospital clinical rotation increased the prevalence of MRSA nasal carriage from 0 to 3.2%, and Slifka et al., (24) in the USA reported the prevalence of MRSA among students with and without significant healthcare associated exposure to be 3.4% versus 2.1%. Also, Peichowicz et al., (21) in their study reported that 21% of clinical students were colonized by MRSA while all preclinical students were negative. In Saudi Arabia, Zakai et al., (13) reported that medical interns carry MRSA more than 6th year clinical students and students who were not exposed to clinical work. Although some other studies reported contrary results in which pre-clinical students had more nasal colonization than clinical students (14,15), other risk factors responsible for nasal colonization such as antibiotic mis-use might be responsible for these contrary reports. All the same, studies conducted among students un-exposed to clinical duties in Thailand and Hungary have reported low prevalence rates of MRSA (25,26). Therefore, considering majority of the data, frequent hospital exposure among medical students might increase the prevalence of MRSA nasal colonization and education on standard infection control practices will help to reduce colonization among these students than the recommended mupirocin for decolonization because of some reported drawbacks of the decolonizer (27).

The *mec*A gene mediates methicillin resistance in *S. aureus* but one phenotypic MRSA isolate from the study group did not carry *mec*A gene, which may indicate that other *mec* genes aside *mec*A may be responsible for resistance in this isolate (8). Antibiotic usage without prescription, usage of antibiotics to treat common cold and use of antibiotics in the last one year were all significantly associated with MRSA nasal colonization among the study group. This finding is similar to previous studies in which recent use of antibiotics and use of antibiotics in the last six months were significantly associated to MRSA (15,23). These data showed that mis-use of antibiotics is a factor to consider in MRSA nasal colonization.

A major limitation in our study is the non-longitudinal nature of the design in which only one nasal swab sample was collected from the participants hence the new nomenclature of MRSA nasal carriage of persistent carriers and others (28), as distinct from the old nomenclature of persistent, intermittent and non-carrier, cannot be fulfilled with only one simple swab test.

Conclusion:

In conclusion, although the difference between the prevalence of nasal carriage of *S. aureus* and MRSA did not reach a statistically significant level, the prevalence rates were higher among the clinical than the pre-clinical students. Antibiotic usage without prescription, usage of antibiotics to treat common cold, and usage of antibiotics in the last one year, were all significantly associated with MRSA nasal colonization among the clinical (study group) students.

With these findings, awareness should

be raised among medical students of the need to adhere to standard infection prevention and control (IPC) practices to limit the spread of MRSA in the hospital. Emphasis on antimicrobial stewardship is pivotal to preventing emergence of antimicrobial resistant organisms such as MRSA.

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