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Burden of rifampicin resistance in methicillin-resistant *Staphylococcus aureus* among apparently healthy students at the University of Jos, Jos, Nigeria

Ocheme Julius Okojokwu[1], John Danjuma Mawak[5], Naomi Lawrence[1], Nanya Amos Cirfat[1], Ahmed Munirat Shaibu[2], John Chima Ndubuisi[3], Yusuf Amuda Agabi[1], Murna Ahmed Ali[1], Joseph Aje Anejo-Okopi[4]

[1]Department of Microbiology, University of Jos, Jos, Plateau State, [2]Department of Microbiology, Ahmadu Bello University, Zaria, [3]Department of Medical Laboratory Science, Federal University of Lafia, Nasarawa State, [4]Department of Microbiology, Federal University of Health Sciences, Otukpo, Benue State, [5]Department of Biological Sciences, Karl Kumm University, Vom, Plateau State, Nigeria.

ABSTRACT

Objectives: As a broad-spectrum antibiotic, rifampicin is used to treat staphylococcal infections. Due to its chemical makeup, it can easily get into tissues and abscesses, which majority of the other antibiotics (anti-staphylococcal drugs) have trouble doing. To treat these infections, methicillin-resistant *Staphylococcus aureus* (MRSA) isolates exhibit rapid evolution of rifampicin resistance, necessitating use of costly medicines. This study, therefore, assessed the burden of rifampicin resistance rate among MRSA in Jos, Nigeria.

Material and Methods: A total of 92 samples were collected from students at the University of Jos. *S. aureus* was isolated and identified by conventional methods. Susceptibility test was conducted to determine MRSA. After that, the MRSA was challenged with 30 µg of rifampicin using the Kirby–Bauer disk diffusion method.

Results: Out of the 92 samples that were isolated, 45 (48.91%) were from female students, while 47 (51.09%) were from male students. 57 (61.96%) samples were positive for *S. aureus*. Of the 57 (61.96%) *S. aureus* isolates recovered, 32 (56.14%) were found to be MRSA. These were subjected to rifampicin, and 18 (56.25%) showed resistance. The susceptibility patterns of *S. aureus* against antibiotics tested showed a susceptibility of 94.74, 77.19, 75.44, 73.68, 71.93, 64.91, 52.63, 43.86, and 31.58% to ofloxacin, clindamycin, chloramphenicol, ciprofloxacin, gentamycin, erythromycin, trimethoprim/sulfamethoxazole, cefoxitin, and tetracycline, respectively.

Conclusion: It was concluded that MRSA were present in the study population, and a substantial number (56.25%) of these were rifampicin resistant.

Keywords: antibiotics, MRSA, resistance, rifampicin, Staphylococcus aureus

INTRODUCTION

Gram-positive bacteria, known as Staphylococci, typically form sporadic clusters of cocci. Although they are mostly located on the skin, skin glands, and mucous membranes of mammals and birds, they are widely distributed in nature and, in some situations, can cause infections. Compared to the other frequent members of the genus (*Staphylococcus epidermidis, Staphylococcus saprophyticus, Staphylococcus haemolyticus*, and *Staphylococcus hominis*), *Staphylococcus aureus* is more detrimental and causes diseases more easily. It is a significant human pathogen responsible for a variety of clinical illnesses. It is the most common cause of osteoarticular, skin and soft tissue, pleuropulmonary, device-related infections, bacteremia, and infective endocarditis. The major cause of mortality and morbidity in hospitals, *S. aureus* has mostly been a nosocomial pathogen; nevertheless, community-associated Infections with these pathogens are increasing and are now a growing risk.[1] Antimicrobial resistance has grown due to the increase in use of antibiotics and its abusive use in treatment of staphylococcal infections, putting patients at risk for more severe infections.[2]

Corresponding author: Ocheme Julius Okojokwu, Department of Microbiology, Faculty of Natural Sciences, University of Jos, Jos, Plateau State, Nigeria. okojokwuoj@gmail.com; okojokwuo@unijos.edu.ng

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Methicillin-resistant *S. aureus* (MRSA) poses a significant public health challenge worldwide because it can cause a wide range of infections with limited treatment options. Rifampicin is one of the antibiotics used to treat MRSA infections; however, its efficacy is reduced when the bacteria become resistant. The burden of rifampicin resistance in MRSA among apparently healthy individuals is poorly reported in Nigeria, where the prevalence of MRSA is high. According to a previous study, the prevalence of MRSA among apparently healthy individuals in Nigeria ranges from 5 to 31.6%.[3] This prevalence is alarming, because MRSA is associated with increased morbidity, mortality, and healthcare costs.[4] Furthermore, the emergence of rifampicin-resistant MRSA strains complicates the treatment of infections, leading to treatment failures, longer hospital stays, and increased healthcare costs.

Though primarily used for treating tuberculosis, Rifampicin is a component of active combination therapy against MRSA and biofilms formed by these organisms.[5] Although MRSA strains are susceptible to rifampicin *in vitro*, the isolates rapidly develop resistance.[6]

This study was therefore conducted with the aim to determine the burden of rifampicin resistance in MRSA among apparently healthy students at the University of Jos, Nigeria.

MATERIALS AND METHODS

Study area

This study was conducted at the University of Jos, Jos North Local Government Area, Plateau State, North-Central Region of Nigeria. Jos has a population of about 900,000 residents based on the 2006 census.[7] It is about 1238 m or 4,062 feet above sea level. It has a latitude of 9°56'N and longitude of 8°53'E with monthly mean temperature of 21–25°C and 179 km (111 miles) from Abuja, the Nation's Federal Capital Territory.[8]

Inclusion and exclusion criteria

The inclusion criteria for the study were healthy students of the University of Jos, aged ≥16 years with no history of hospital admission in the past 6 months, no history of use of antibiotics in the past 2 weeks, and who provided a written informed consent. Students who had a history of antibiotics usage within the last 2 weeks, those who had received any form of immunosuppressive therapy in the past 6 months, with a history of chronic diseases, and those who declined to provide informed consent were excluded from the study. These criteria were set to ensure that the study population was representative of apparently healthy individuals without any confounding factors that could affect the study results.

Ethical approval

This study was approved by the Health Research Ethics Committee (HREC) at Plateau State Specialist Hospital, Jos via a notice of approval with reference number PSSH/ADM/ETH.CO/2019/005. Participant information was confidential throughout the study, and all data were collected and analyzed anonymously.

Determination of sample size

The sample size was determined to be 92 using the OpenEpi statistical software (version 3.01). The expected prevalence of rifampicin resistance among MRSA was estimated to be 6.4% based on the previous studies,[9] and the desired level of precision was set at 5%.

Sample collection

The nasal swab samples were collected from the anterior nares of the participants using sterile cotton swabs, and the swabs were transported to the microbiology laboratory of the Department of Microbiology, University of Jos, for processing.

Isolation and identification of *Staphylococcus aureus*

Within 1–2 h of sample collection, the swab sticks containing different samples were inoculated into mannitol salt agar (MSA) plates and incubated aerobically at 37°C for 24 h. Isolated colonies that seemed to be *S. aureus* after incubation were allowed to expand on nutrient agar plates before being identified biochemically and under a microscope.[10,11] A pure colony was chosen and gram stained for microscopic examination. Under a light microscope with a magnification of 100X, the form, organization, and gram responses of the isolates were examined.[12] Following established protocols, necessary confirmatory physiological and biochemical tests, such as catalase, coagulase, hemolytic test, and triple sugar iron agar tests, were carried out to determine probable *S. aureus*.[12]

Antibiotics susceptibility test

Disk agar diffusion test

In accordance with the recommendations of the Clinical and Laboratory Standards Institute (CLSI), the Kirby–Bauer disk diffusion technique was used to ascertain the antibiotic resistance profile of the isolates. Mueller–Hinton agar in 20 mL sterile aliquots was placed on to sterile petri dishes and allowed to set. Mueller–Hinton agar plates were flooded with overnight cultures of the isolates diluted in normal saline to 0.5 McFarland turbidity standard (1.5×10^8 cfu/mL). With sterile forceps, the different antibiotics disks: ofloxacin (5 μg),

chloramphenicol (30 µg), clindamycin (2 µg), gentamicin (10 µg), erythromycin (15 µg), penicillin (10 units), tetracycline (30 µg), cefoxitin (30 µg), ciprofloxacin (30 µg), and trimethoprim/sulfamethoxazole (co-trimoxazole) (1.25/23.75 µg) were aseptically placed on the inoculated agar plate and incubated at 37°C for 24 h. After 24 h of incubation, zones of inhibition were measured to the nearest millimeter, and interpreted accordingly as sensitive, intermediate, or resistant based on the CLSI zone diameter interpretative standards.[13] Isolates resistant to cefoxitin were considered to be resistant to methicillin.[13] Isolates resistant to at least two classes of antibiotics were reported as multidrug-resistant (MDR). To determine the multiple antibiotic resistance (MAR) indexes for each isolate, we used a formula that involved two variables, a and b. Variable "a" represents the number of antibiotics the isolate resisted, while variable "b" represents the total number of antibiotics tested to evaluate the susceptibility of the isolate. The formula we used to calculate the MAR index was MAR = a/b, where a is divided by b. This helped us to determine the degree of resistance of each isolate to multiple antibiotics. The higher the MAR index, the more resistant was the isolate to antibiotics.[14]

Rifampicin resistance

MRSA isolates identified by an agar diffusion test were emulsified in normal saline. The turbidity was corrected to 0.5 McFarland standard as per the CLSI guidelines.[13] MRSA was streaked onto the surface of Mueller–Hinton agar with a sterile swab stick in three different directions, with the plate being rotated by about 60 degrees to ensure even dispersion. The antibiotic disk (Rifampicin 30 g) was then aseptically placed on the agar surface after the plates had dried for 10 min. After another 30 min of drying, the samples were incubated at 35°C for 24 h. Following the measurement and recording of the diameter of the zone of inhibition to the nearest millimeter, isolates were categorized as resistant, intermediate, or sensitive as per the CLSI guidelines.[13]

Statistical data analysis

The data collected from this study were entered into a Microsoft Excel spreadsheet and analyzed using SPSS version 23.0 software. The prevalence of rifampicin-resistant MRSA was determined by calculating the proportion of MRSA isolates resistant to rifampicin. Descriptive statistics, such as percentages, were used to summarize the data. The chi-square test determined the association between rifampicin resistance and categorical variable, for example, sex. $P < 0.05$ was considered statistically significant.

RESULTS

Fifty-seven (62.0%) *S. aureus* was isolated in this study; 33 (70.2%) were obtained from male students, and 24 (53.3%) were acquired from female students [Table 1]. [Table 2] presents the results obtained from the antibiotic susceptibility test of all 57 confirmed *S. aureus*. According to the result of susceptibility test using the cefoxitin disk, 32 (56.1%) of the *S. aureus* isolates were resistant to cefoxitin and therefore considered methicillin-resistant. In comparison, all the isolates were resistant (100%) to penicillin. The resistance to other antibiotics was as follows: 19.3% to chloramphenicol, 22.8% to clindamycin, 28.1% to gentamicin, 31.6% to erythromycin, 49.1% to tetracycline, 21.0% to ciprofloxacin, and 38.6% to trimethoprim/sulfamethoxazole.

The MDR pattern observed in the study was the three-antibiotic patterns of cefoxitin, penicillin, and tetracycline,

Table 1: Occurrence of *Staphylococcus aureus* based on sex.

Source of isolates	Number of samples (%)	Positive on mannitol salt agar (%)	Negative on mannitol salt agar (%)
Female	45 (48.9)	24 (53.3)	21 (46.7)
Male	47 (51.1)	33 (70.2)	14 (29.8)
Total	**92**	**57 (62.0)**	**35 (38.0)**

Table 2: Antimicrobial susceptibility profile of *Staphylococcus aureus.*

Class of antibiotics	Antibiotics (concentration)	Sensitive (%)	Intermediate (%)	Resistant (%)
Fluoroquinolones	Ofloxacin (5 µg)	54 (94.7)	3 (5.3)	0 (0.0)
Fluoroquinolones	Ciprofloxacin (5 µg)	42 (73.7)	3 (5.3)	12 (21.0)
Phenicols	Chloramphenicol (30 µg)	43 (75.4)	3 (5.3)	11 (19.3)
Lincosamides	Clindamycin (2 µg)	44 (77.2)	0 (0.0)	13 (22.8)
Aminoglycosides	Gentamicin (5 µg)	41 (71.9)	0 (0.0)	16 (28.1)
Macrolides	Erythromycin (15 µg)	37 (64.9)	2 (3.5)	18 (31.6)
β-Lactams	Penicillin (10 units)	0 (0.0)	0 (0.0)	100 (100.0)
Tetracyclines	Tetracycline (30 µg)	18 (31.6)	11 (19.3)	28 (49.1)
Cephalosporins	Cefoxitin (30 µg)	25 (43.9)	0 (0.0)	32 (56.1)
Antifolates/Sulphonamides	Trimethoprim/Sulfamethoxazole (1.25/23.75 µg)	30 (52.6)	5 (8.8)	22 (38.6)

which was seen in 7 out of 27 (25.9%) of the MDR *S. aureus* isolates. The subsequent most common patterns were the three-antibiotic pattern of penicillin, tetracycline, and trimethoprim/sulfamethoxazole, and the four-antibiotic pattern of chloramphenicol, clindamycin, gentamicin, and tetracycline, each of which was seen in 5 out of 27 (18.5%) of the MDR *S. aureus* isolates [Table 3]. Several MDR patterns involving five or more antibiotics were seen. The least common was the 7-antibiotic pattern of cefoxitin, chloramphenicol, clindamycin, erythromycin, penicillin, tetracycline, and trimethoprim/sulfamethoxazole, which was seen in 1 out of 27 (3.7%) of the MDR *S. aureus* isolates. Twenty-seven (27) isolates (47.4%) had an MAR index ≥ 0.3, indicating resistance to at least 30% of the antibiotics tested. The most common MAR index was 0.3, which was observed in 12 isolates (44.44%).

[Table 4] provides the proportion of rifampicin resistance among methicillin-sensitive *S. aureus* (MSSA) and MRSA. The prevalence of rifampicin resistance in MSSA was 8.0%, all occurring among males (2 [13.3%]). There was a statistically significant association (χ^2 = 11.458, P = 0.001) between the resistances of *S. aureus* to methicillin and rifampicin. Of the 32 (56.1%) MRSA samples obtained, 18 (56.3%) were resistant to rifampicin. Five (35.7%) MRSA samples isolated from female subjects were rifampicin-resistant, and the remaining 13 (72.2%) rifampicin-resistant isolates were obtained from male students, resulting in 18 (56.3%) rifampicin-resistant *S. aureus*. There was no statistically significant association (P > 0.05) between the sex of the students and the resistance of *S. aureus* to rifampicin and methicillin [Table 5].

DISCUSSION

This study was designed to determine the burden of rifampicin resistance in MRSA among apparently healthy undergraduate students at the University of Jos, Nigeria. Because of this, 62.0% of the study subjects were discovered to have nasal carriage of *S. aureus*. This implies that the students with nasal colonization could suffer opportunistic and possibly life-threatening infections, including but not limited to surgical-site infection, otitis media, or other infections that increase morbidity as well as mortality.[14]

The relatively high prevalence of *S. aureus* found in the study was consistent with the results of the study conducted by

Table 3: Multidrug resistance pattern and multiple antibiotic resistance index of *Staphylococcus aureus* (N = 57).

Number of antibiotics	MDR pattern	No. of antibiotics classes	Number of MDR *S. aureus* (%)	MAR Index
3	CFX, PEN, TET	3	7 (25.9)	0.3
3	PEN, TET, TMP/SMX	3	5 (18.5)	0.3
4	CHL, CLI, GEN, TET	4	5 (18.5)	0.4
5	CFX, CLI, GEN, PEN, TMP/SMX	5	3 (11.1)	0.5
6	CFX, CIP, GEN, PEN, TET, TMP/SMX	6	4 (14.8)	0.6
7	CFX, CHL, CIP, ERY, GEN, TET, TMP/SMX	7	1 (3.7)	0.7
7	CFX, CHL, CLI, ERY, PEN, TET, TMP/SMX	7	2 (7.4)	0.7
Total			**27 (47.4)**	

CFX: cefoxitin, CHL: chloramphenicol, CIP: ciprofloxacin, CLI: clindamycin, ERY: erythromycin, GEN: gentamicin, PEN: penicillin, TMP/SMX: trimethoprim/sulfamethoxazole, TET: tetracycline, MDR: Multidrug resistance, MAR: Multiple antibiotic resistance.

Table 4: Prevalence of rifampicin resistance in methicillin-resistant *S. aureus* based on sex.

Sex	Cefoxitin	RIF-S (%)	RIF-R (%)	Total	χ^2	P
Overall	MSSA	23 (92.0)	2 (8.0)	25	11.458	0.001**
	MRSA	16 (50.0)	16 (50.0)	32		0.001*†
	Total	**39 (68.4)**	**18 (31.6)**	**57**		
Female	MSSA	10 (100.0)	0 (0.0)	10	4.511	0.034*
	MRSA	9 (64.3)	5 (35.7)	14		0.053*†
	Total	**19 (79.2)**	**5 (20.8)**	**24**		
Male	MSSA	13 (86.7)	2 (13.3)	15	7.823	0.005**
	MRSA	7 (38.9)	11 (61.1)	18		0.011*†
	Total	**20 (60.6)**	**13 (39.4)**	**33**		

*: Significant association exists at P ≤ 0.05, **: Significant association exists at $p \leq 0.01$, †: Fisher's exact test, χ^2: Chi square, MSSA: Methicillin-sensitive *Staphylococcus aureus*, MRSA: Methicillin-resistant *Staphylococcus aureus*, RIF-S: Rifampicin sensitive, RIF-R: Rifampicin resistant.

Table 5: Rifampicin resistance among methicillin-resistant *Staphylococcus aureus.*

Source of samples	Total number of samples (%)	Resistant to cefoxitin (%)	Resistant to rifampicin (%)
Female	24 (42)	14 (58.3)	5 (35.7)
Male	33 (58)	18 (54.5)	13 (72.2)
Total	**57 (100)**	**32 (56.1)**	**18 (56.3)**
χ^2		0.081	2.215
P		0.776	0.137

χ^2: Chi-square.

Nsofor *et al.*[15] in Rivers State, Nigeria, which found that 62.9% of learners in Elele had a nasal carriage. However, the results contrasted with the 16% found in a study conducted at the Federal University of Birnin Kebbi in Nigeria and published by Aliyu *et al.*[16] Additionally, *S. aureus* nasal colonization rates of 33.3% in healthy Amassoma residents in the Niger Delta region of Nigeria and 14.0% among medical students in Lagos, Nigeria, were reported by Onanuga and Temedie[17] and Adesida *et al.*,[18] respectively.

The prevalence of MRSA obtained in this study contrasts with the findings of the studies carried out by Osinupebi *et al.*[19] in Abeokuta and Udobi *et al.*[20] in Zaria, Nigeria, where prevalence rates of 41 and 85.25%, respectively, were recorded. This study showed a lower prevalence than the study by Jean-Marie *et al.*,[21] who recorded a prevalence of 63.5% on surgical site infection in Kinshasa. However, Barkin *et al.*[22] reported a prevalence of 53.4%, which was congruent with the report of this study. The difference in sample size, study sites, and geographical variations may be the cause of this variation.

One unexpected finding was the extent to which the *S. aureus* isolates were resistant to penicillin (100%). It suggests that *S. aureus* circulating in the student population cannot be treated with penicillin if the organism causes a disease. In addition, the organism was found to be resistant in varying degrees to commonly used antibiotics such as cefoxitin, chloramphenicol, tetracycline, gentamicin, ciprofloxacin, and trimethoprim/sulfamethoxazole (co-trimoxazole). A possible explanation for this might be the indiscriminate use of antibiotics abuse where patients fail to complete the entire course of the prescribed dosage of antibiotics. It could also be due to the problematic practice of self-medication prevalent in the student community.[9,23] These results agree with the findings of other studies, in which all *S. aureus* isolated were resistant to penicillin.[24]

The results showed that 47.4% of the isolates had a MAR index of 0.3 or higher, indicating a high level of antibiotic resistance. The high levels of antibiotic resistance would make it difficult to treat infections with common antibiotics. The MAR index can also help identify the source of antibiotic resistance, possibly due to the indiscriminate use of antibiotics in clinical settings, agriculture, and animal husbandry. These findings highlight the need for more responsible use of antibiotics to reduce the emergence of antibiotic-resistant bacterial strains. Additionally, there is a need for the development of new antibiotics to combat antibiotic-resistant bacteria.

The higher prevalence of rifampicin resistance in male students than in females in this study was consistent with that of the previous studies that reported higher rates of MRSA and antimicrobial resistance in male individuals.[25,26] The reasons for this gender difference are unclear, but they may be related to differences in behavior, lifestyle, or biological factors.

In contrast to previously reported findings where rifampicin resistance in MRSA varied between 3.26 and 46.4%,[27,28] 50.0% prevalence of rifampicin in MRSA was recorded in this study. The high prevalence of rifampicin resistance among MRSA is most likely a result of the frequent use of the drug to treat tuberculosis in this setting.[9] In developing countries such as Nigeria, many people rely on self-medication, which is also associated with an incomplete dosing regimen of antibiotics and failure to comply with treatment. This renders the antibiotic completely ineffective in treatment. The findings confirm the association between rifampicin and methicillin resistance. This suggests that intrinsic factors in the organism could be responsible for the resistance and not the gender of the study subjects.

CONCLUSION

It was concluded that the prevalence of *S. aureus* among the students at University of Jos, Nigeria was found to be 62%. In addition, MRSA was present in this study population with a prevalence of 56.1%, and a substantial number (56.3%) of these were rifampicin resistant. This study provides important insights into the burden of rifampicin resistance in MRSA among apparently healthy students at the University of Jos, Jos, Nigeria. The high prevalence of MRSA and rifampicin resistance underscore Nigeria's urgent need for infection prevention and control measures and antimicrobial stewardship programs. Further studies are needed to identify the risk factors for MRSA and antimicrobial resistance in Nigeria and to develop effective strategies for their prevention and control.

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Declaration of patients consent

The authors certify that they have obtained all appropriate patient consent.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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