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Distribution of Macrolide, Lincosamide, and Streptogramin B and Detection of *erm* Genes in *Staphylococcus aureus* from Wounds in Uyo, Nigeria

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Abstract

The increasing resistance to macrolide, lincosamide, and streptogramin B (MLSB) among methicillin-resistant *Staphylococcus aureus* (MRSA) is a challenge. The study determined the constitutive and inducible MLSB resistance and *erm* genes in *S. aureus* from wounds using the erythromycin-clindamycin D-zone test and a multiplex polymerase chain reaction assay, respectively. Of the 260 patients recruited in the study, *S. aureus* was isolated from the wounds of 102 patients, giving a prevalence rate of 39.2%. Of the 102 *S. aureus* isolates, 32.4% were methicillin-sensitive *S. aureus* (MSSA), and 67.6% were MRSA. The prevalence of *S. aureus* in wounds was higher in females (39.8%) than in males (38.4%). The highest prevalence of *S. aureus* was found in divorce patients (59.1%) and those residing in urban areas (39.7%). There was no statistical difference between the occurrence of MRSA and MSSA in wounds based on the sex ($p = 0.97$), age ($p = 0.08$), and marital status ($p = 0.41$) of the patients. All (100%) MRSA were resistant to Cefoxitin, and chloramphenicol, while < 50% of MRSA were resistant to Tetracycline, Ciprofloxacin, and Gentamycin. Of the 69 MRSA isolates from the wounds, 21.7% and 26.1% were iMLSB and cMLSB phenotypes, respectively. Nine (9) MSSA were iMLSB phenotypes, and eight MSSA were cMLSB phenotypes. Among the 12 representative isolates, three (3) MRSA and one (1) MSSA isolate possessed the *erm*C gene. This study has revealed that screening tests for iMLSB-resistant *S. aureus* strains are critical for the therapeutic management of wound infections caused by *S. aureus*.

Keywords: constitutive, inducible, methicillin, genes, *Staphylococcus aureus,* phenotypes

Резюме

Нарастващата резистентност към макролиди, линкозамиди и стрептограмин B (MLSB) сред метицилин-резистентните *Staphylococcus aureus* (MRSA) е сериозно предизвикателство. В настоящата статия е проучена конститутивната и индуцируемата резистентност към MLSB и *erm* гените при *S. aureus* от рани, като се използват съответно D-зоновият тест с еритромицин и клиндамицин и мултиплексен анализ с полимеразна верижна реакция. От 260 пациенти, набрани в проучването, *S. aureus* е изолиран от раните на 102 пациенти, което дава степен на разпространение от 39.2%. От всички 102 изолата на *S. aureus*, 32.4% са метицилин-чувствителни (MSSA), а 67.6% - MRSA. Разпространението на *S. aureus* в раните е по-високо при жените (39.8%), отколкото при мъжете (38.4%). Най-високо разпространение на *S. aureus* е установено при пациенти в развод (59.1%) и при живеещите в градски райони (39.7%). Не е установена статистическа разлика между появата на MRSA и MSSA в рани в зависимост от пола ($p = 0.97$), възрастта ($p = 0.08$) и семейното положение ($p = 0.41$) на пациентите. Всички MRSA са резистентни към цефокситин и хлорамфеникол, а <50% от MRSA са резистентни към тетрациклин, ципрофлоксацин и гентамицин. От 69-те изолата MRSA от раните 21.7% и 26.1% са съответно с фенотипове iMLSB и cMLSB. Девет MSSA са с фенотип iMLSB, а осем MSSA - с фенотип cMLSB. Сред 12-те представителни изолата, три изолата MRSA

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и един изолат MSSA притежават гена *erm*C. Това проучване установи, че скрининговите тестове за iMLSB-резистентни щамове *S. aureus* са от решаващо значение за терапевтичното лечение на рани от инфекции, причинени от *S. aureus*.

Introduction

The increasing prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) is of great public concern, and the emergence of MRSA and alterations in antibiotic resistance by *Staphylococcus* spp. have resulted in renewed interest in some groups of antibiotics, such as the macrolide, lincosamide, and streptogramin B (MLSB) antibiotics, for the treatment of related staphylococcal infections (Coutinho *et al*., 2010). The macrolide and lincosamide classes of antibiotics are represented by erythromycin and clindamycin, respectively (Akinjogunla *et al*., 2018). The macrolide, lincosamide, and streptogramin B antibiotics are chemically distinct families of antibiotics denoted by MLSB, but they have a similar inhibitory effect on bacterial protein synthesis by binding to the 23S rRNA of the bacterial 50S ribosomal subunits (Zarifan *et al.,* 2015). The ketolides are another member of the MLSB family, with telithromycin being the first commercially available Ketolides. Other newer classes of compounds, like the oxazolidinones (linezolid, sutezolid, eperezolid, and radezolid), bind to the 23S portion of the 50S ribosomal subunit (Foti *et al*., 2021), preventing initiation complex formation and possessing enhanced activity against MRSA and vancomycin-resistant *Enterococcus* spp.

The pharmacokinetic properties of clindamycin, such as good oral absorption, excellent penetration in the skin, tolerability, and its good alternative in penicillin-allergic patients, have made it a frequent treatment choice for staphylococci infections, particularly skin and soft tissue infections. However, this widespread use has increased the number of staphylococcal strains resistant to MLSB antibiotics (Moosavian *et al.,* 2014). The two mechanisms involved in the resistance of *Staphylococcus* spp. to MLSB antibiotics are the efflux of the antimicrobial agent by an ATP-dependent pump encoded by the *msr*A gene and the ribosomal binding site modification by 23S rRNA methylase mediated by *erm* genes (Ghanbari *et al.,* 2016). The mechanisms of ribosomal target site modification can be either constitutive or inducible (Adhikari *et al.,* 2017). The *S. aureus* with constitutive resistance exhibit *in vitro* resistance to erythromycin and clindamycin, whereas the *S. aureus* with inducible resistance show *in vitro* resistance to erythromycin but are sensitive to clindamycin (Pereira *et al*., 2016; Adhikari *et al.,* 2017; Modukuru *et al*., 2021). The risk for therapeutic failure is increased as constitutive MLSB (cMLSB) may arise from inducible MLSB (iMLSB) during clindamycin therapy in patients with severe staphylococci infections (Goudarzi *et al*., 2017). The wounds of patients may result from surgery, diabetic ulcers, hospital- or community-acquired injuries (Del-Core *et al*., 2018), and can cause sepsis, long or recurrent hospitalization periods (Akinjogunla *et al*., 2009), increased healthcare costs, and high mortality rates (Zervos *et al*., 2012). The widespread bacterial resistance to antibiotics and the higher occurrence of wound infections caused by MRSA and polymicrobial flora, especially in hospital environments, have made the control of wound infections seriously challenging. In Nigeria, most especially in the South-South, there is a paucity of information on constitutive and inducible MLSB resistance among *S. aureus*. The study determined the occurrence of resistant genes (*erm*A, *erm*B, and *erm*C), cMLSB, and iMLSB resistance among *S. aureus* from wound patients attending health facilities in Uyo, Nigeria.

Materials and Methods

Study design

This was a descriptive cross-sectional study of six months (September 2019 to February 2020), involving in- and out-patients with wound infections attending the University of Uyo Teaching Hospital (UUTH), the Primary Health Care Centre, and the Model Health Centre, in Uyo, Akwa Ibom State. The verbal informed consent of each patient to participate in the study was obtained prior to sample collection.

Study area

The study was carried out at three healthcare facilities in Uyo. Uyo is the capital city of Akwa Ibom State, located in the southern part of Nigeria, with a population of 554 906 (NPC, 2006). The people of Uyo are mainly the Ibibio-speaking tribe. Uyo is inarguably the business hub of the state, with good road networks and tourist centers. The city of Uyo lies between latitude 5°3' 0"N and longitude 7° 56' 0" E, at an altitude of 191 meters above sea level, with coordinates of 5° 2' 20.2668" N and 7°54' 34.0920″ E.

Ethical approval and informed consent

Ethical approval for the study was obtained from the Ethical Review Board of the University of

Uyo Teaching Hospital and the Akwa Ibom State Ministry of Health before the study commenced.

Inclusion criteria

Males and females, inpatient and outpatient patients of all ages agreed and verbally consented to participate in the study. Also included were patients who had been on antibiotics for the past two weeks and were referred to the orthopedic, burns, and accident and emergency units of the health care facilities for medical care.

Exclusion criteria

The patients who were not on antibiotic therapy within two weeks prior to their presentation at the general outpatient department of the health facilities and in-patients not on antibiotic medication and/or those that declined to participate in the study.

Data collection

The relevant data for this study were gathered from consenting patients' biodata. The data obtained included socio-demographic data (sex, age, marital status, and place of residence) and clinical data (history of previous infection, site of infection, source of wound, clinical disease, antibiotic use, and period of hospital stay).

Sample size determination

The minimum sample size was calculated using the formula by Bill Godden (2004), based on the prevalence rate of 21.5% for MLSB resistance obtained from a study carried out in Port Harcourt, South-South, Nigeria, by Nwokah and Abbey (2016), with a precision of 5% and a confidence level of 95%.

$$s = \frac{Z^2 \; x \; P \; x \; (1 - P)}{C^2}$$

Where: Ss: sample size; Z: z-value (1.96 for a 95 percent confident interval); P: percentage of population, picked based on previous studies (21.5%); C: Confidence interval (0.05).

$$Z^2 = 1.962$$

$$1 - p = 1 - 0.215 = 0.785$$

$$C^2 = 0.05 = 0.0025$$

$$Sample\ Size\ (Ss) = \frac{3.8416 \; x \; 0.215 \; x \; 0.785}{0.0025}$$

$$Sample\ Size\ (Ss) = \ 259.35 = \sim 259.4$$

= 259.4; thus 260 was used as the sample size.

Sample collection

The wound swabs from in- and out-patients (n = 260) were aseptically obtained after the wound's immediate surface exudates and contaminants were cleansed off with moistened sterile gauze and normal saline solution. Each wound swab was collected by firmly rotating the sterile, moistened swab stick over the wound area so that the head of the swab was in contact with the wound surface. Two samples were collected from each wound site, one for Gram staining and microscopic examination and the other for culture. All the wound swab samples were labeled and transported in an icebox to the Medical Microbiology Laboratory, University of Uyo Teaching Hospital for processing.

Isolation and identification of Staphylococcus spp.

Each of the wound swab samples was placed into test tubes containing 2 mL of sterile nutrient broth (Oxoid, England) for 4-6 h. These were then inoculated onto Mannitol Salt Agar (Oxoid) plates and incubated at 37°C for 24 h. After incubation, the yellow colonies on each plate were subcultured onto each nutrient agar (Oxoid) plate and incubated at 37°C for 24 h. A pure culture of the isolate was streaked onto nutrient agar slants, incubated at 37°C for 24 h, and stored in the refrigerator at 4°C for characterization and identification. All isolates were examined under the microscope, Gram stained, and identified using the Vitek 2 automated system (Biomeriux, Inc., France)**.**

Phenotypic detection of methicillin-resistant Staphylococcus aureus (MRSA)

Phenotypic detection of MRSA from wound samples was done using the disc diffusion method (CLSI, 2020). Exactly 0.1 mL of *S. aureus*, prepared directly from the overnight culture and adjusted to the 0.5 McFarland turbidity standard, was inoculated onto a plate of MHA using a sterile micropipette. Cefoxitin discs (30 µg) were placed on MHA plates and incubated at 37ºC for 18 h. Inhibitory zones after incubation were observed and measured in millimeters (mm). The interpretation of the measurements as methicillin-sensitive (MS: ≥ 22 mm) and methicillin-resistant (MR: ≤ 21mm) was done according to the CLSI guideline (CLSI, 2020). *S. aureus* ATCC 25923 was used as the standard strains for quality control.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed using the modified Kirby-Bauer disc diffusion method (CLSI, 2020). The antibiotic discs used were: Penicillin (10 U), Ciprofloxacin (5 µg), Cefoxitin (30 µg), Gentamicin (10 µg), Clindamycin (2 µg), Erythromycin (15 µg), Tetracycline (30 µg), and Chloramphenicol (30 µg) (Oxoid, UK). A

loopful of *S. aureus* was taken, transferred to a tube containing 5 mL of sterile distilled water, and gently mix until it formed a homogenous suspension and was adjusted to the 0.5 McFarland turbidity standard. The entire surface of the MHA (Oxoid) plate was uniformly flooded with *S. aureus* suspension using sterile swab sticks and allowed to dry for 5 min. The antibiotic discs were aseptically placed on the surface of inoculated plates and were incubated at 37ºC for 18 h. Inhibitory zones after incubation were observed and measured in millimeters. The interpretation of the measurement as sensitive and resistant was made according to the standard interpretative zone sizes given by CLSI guidelines. *S. aureus* ATCC 25923 was used as a standard strain for quality control.

Detection of iMLSB and cMLSB S. aureus phenotypes

The iMLSB and cMLSB *S. aureus* phenotypes were detected using a double-disc diffusion test (CLSI, 2020). A loopful of *S. aureus* was taken, transferred to a tube containing 5 mL of sterile distilled water, and gently mix until it formed a homogenous suspension and was adjusted to the 0.5 McFarland turbidity standard. The entire surface of the MHA (Oxoid) plate was uniformly flooded with *S. aureus* suspension using sterile swab sticks and allowed to dry for 5 min. Clindamycin (CLI, 2 µg) and Erythromycin (ERY, 15µg) (Oxoid UK) discs were aseptically placed 15 mm apart, edge to edge, on the surface of inoculated plates and were incubated at 37ºC for 18 h. Inhibitory zones after incubation were measured in millimeters (mm), and the results were interpreted as follows: CLI (sensitive: 21 mm; resistant: 14 mm) and ERY (sensitive: 23 mm; resistant: 13 mm). *S. aureus* that was resistant to ERY but sensitive to CLI and gave a circular zone of inhibition around CLI was MS (Macrolide-Streptogramin) phenotypes. *S. aureus* that was resistant to both ERY and CLI were considered constitutive MLSB (cMLSB) phenotypes. *S. aureus* with the inducible MLSB phenotype (iMLSB) showed resistance to ERY, sensitivity to CLI, and a D-shaped zone of inhibition around CLI with flattening towards the ERY disc (Deotale *et al*., 2010; Akinjogunla *et al*., 2018).

Extraction of genomic DNA of Staphylococcus aureus

The genomic DNA was extracted from MSSA and MRSA isolates with MLSB resistance using QIAamp® DNA Mini kits from QIAGEN (Germany) according to the producer's guidelines. The isolates were subcultured on tryptic soy agar (TSA) at 37ºC for 24 h. TENT buffer (10 mM Tris-HCL, 0.1 m NaCl, 1 mM EDTA, 5% (v/v) Triton X 100, pH 8.0) was used to suspend pure colonies. The cell suspension was boiled at 100ºC and centrifuged at 10000 rpm for 5 min. The supernatant fluid was transferred into a new sterile tube. Approximately 95% cold ethanol was added at 20ºC for 20 min. The solution was centrifuged, and the DNA template was dissolved in 50 µL sterile dH_20 and stored at 20ºC until PCR amplification.

PCR amplification of ermA, ermB and ermC genes in Staphylococcus aureus

Erm genes were amplified by PCR using specific primers for the *erm* A, B, and C genes (Coutinho *et al.,* 2010). Each reaction contained 5 L of DNA template, 2.5 L of PCR buffer (X10), 1 L MgCl2 (50 mM), 0.5 L of dNTPs (10 mM), 5 M of each of the forward and reverse *ermA*, ermB, and *ermC* primers, 0.25 L of Taq DNA polymerase (5 u/L), and 11.25 L of water. Amplification of the target genes was done under the following PCR conditions: Initial denaturation at 94°C for 10 min, followed by 35 cycles of denaturation at 94°C for 30 sec, annealing at 53°C for 30 sec, and extension at 72°C for 60 sec, followed by a final extension at 72°C for 10 min (Coutinho *et al.,* 2010). Amplicons were analyzed after running on a 2% agarose gel containing ethidium bromide in comparison to a 100 bp molecular size standard ladder. The bands were visualized using a UV transilluminator (312 nm), photographed, and analyzed.

Statistical analysis

The data were analyzed using IBM SPSS version 20. The distribution of variables was reported in terms of frequency and percentages. The comparison of the proportion of the distribution of the dependent variable across the independent variables was analyzed using Chi-square. A statistically significant difference was considered at a p-value < 0.05.

Results

The percentage occurrences of MRSA and MSSA in wound samples of patients based on hospitals are presented in Table 1. One hundred and two *S. aureus* were isolated from the wound samples (n = 260) of patients attending the Primary Health Care Center (PHCC), Model Health Center (MHC), and the University of Uyo Teaching Hospital (UUTH). Of the 102 *S. aureus* isolates, 33 (32.4%) were MSSA, and 69 (67.6%) were MRSA. The prevalence of MRSA was highest in patients

attending PHCC (72.7%), compared to other health facilities. while the highest prevalence of MSSA (35.3%) was recovered from patients attending MHC (Table 1).

Table 1. Methicillin-resistant *S. aureus* in wound samples based on hospitals

Hospital	No. of *S. aureus* isolated	MSSA	MRSA
		No. (%)	No. (%)
PHCC	22	6 (27.3)	16 (72.7)
MHC	17	6 (35.3)	11 (64.7)
UUTH	63	21 (33.3)	42 (66.7)
Total	102	33 (32.4)	69 (67.6)

Keys: PHCC: Primary Health Care Center; UUTH: University of Uyo Teaching Hospital; MHC: Model Health Center; MSSA: Methicillin-sensitive *S. aureus*; MRSA: Methicillin-resistant *S. aureus*

The distribution of *S. aureus* among the subjects based on their socio-demographic characteristics is shown in Table 2. Of the 260 patients recruited in the study, *S. aureus* was isolated from 102 patients, giving a prevalence rate of 39.2%. The prevalence of S. *aureus* was slightly higher in female patients 59 (39.8%) than in male patients 43 (38.4%), as well as in the age group of 41–60 years (43.0%). Based on the marital status and place of residence, the occurrence of *S. aureus* was highest among divorce patients with wounds (13/22; 59.1%) and those residing in urban areas (73/184; 39.7%). There was no statistically significant difference in the occurrence of *S. aureus* among the patients based on sex, age, marital status, or place of residence ($p > 0.05$) (Table 2).

The occurrence of MRSA and MSSA in the wound samples of subjects based on their socio-demographic characteristics is presented in Table 3.

Of the 102 *S. aureus* isolates from the wound samples, 69 (67.6%) and 33 (32.4%) were MRSA and MSSA phenotypes, respectively. Of the 69 samples with MSRA, 29 were males and 40 were females, giving a gender ratio of 1:1.3. The subjects that were residents of urban areas had a higher prevalence of MRSA (52/73; 71.2%) than subjects that were residents of rural areas (17/29; 58.6%). There was no statistical difference between the occurrence of MRSA and MSSA in wound samples based on sex ($p = 0.97$), age ($p = 0.08$), marital status ($p = 0.41$), and place of residence ($p = 0.22$) of the patients.

All (100%) MRSA were resistant to Cefoxitin, Penicillin, and Chloramphenicol; between 51.5% and 59.4% of MRSA and MSSA were resistant to Clindamycin and Erythromycin, while < 50% of MRSA were resistant to Tetracycline, Ciprofloxacin, and Gentamycin (Table 4). There was a significant difference between the resistance profiles of MRSA and MSSA to Cefoxitin ($p = 0.001$). The distribution of macrolide, lincosamide, and streptogramin B (MLSB) resistance phenotypes in MSSA and MRSA isolates is presented in Table 5. Of the 69 MRSA isolates from the wound samples, 15 (21.7%), 18 (26.1%), and 1 (1.4%) were iMLSB,

Table 2. Socio-demographic characteristics of the subjects based on the wound samples with *S. aureus*

Variables	Categories	No of Samples Collected	No (%) of Wound Samples		p-value	χ^2
			With *S. aureus*	Without *S. aureus*		
Sex	Female	148	59 (39.9)	89 (60.1)	0.81	0.058
	Male	112	43 (38.4)	69 (61.6)		
	Total	260	102 (39.2)	158 (60.6)		
Age group (yrs)	20-40	102	36 (35.3)	66 (64.7)	0.53	1.263
	41-60	100	43 (43.0)	57 (57.0)		
	61-80	58	23 (39.7)	35 (60.3)		
	Total	260	102 (39.2)	158 (60.6)		
Marital Status	Divorce	22	13 (59.1)	9 (40.9)	0.24	4.154
	Married	169	62 (36.7)	107 (63.3)		
	Single	37	14 (37.8)	23 (62.2)		
	Widow	32	13 (40.6)	19 (59.4)		
	Total	260	102 (39.2)	158 (60.6)		
Residence	Rural	76	29 (38.2)	47 (61.8)	0.82	0.052
	Urban	184	73 (39.7)	111 (60.3)		
	Total	260	102 (39.2)	158 (60.6)		

Table 3. Socio-demographic characteristics of the subjects based on the wound samples with methicillin-resistant and methicillin-sensitive *S. aureus*

Variables	Categories	No of *S. aureus*	No (%) of Wound Samples		p-value	χ^2
			With MSRA	Without MSSA		
Sex	Female	59 (39.9)	40 (67.8)	19 (32.2)	0.97	0.001
	Male	43 (38.4)	29 (67.4)	14 (32.6)		
	Total	102 (39.2)	69 (67.6)	33 (32.4)		
Age group (yrs)	20-40	36 (35.3)	22 (61.1)	14 (38.9)	0.08	5.084
	41-60	43 (43.0)	27 (62.8)	16 (37.2)		
	61-80	23 (39.7)	20 (86.9)	3 (13.1)		
	Total	102 (39.2)	69 (67.6)	33 (32.4)		
Marital Status	Divorce	13 (59.1)	11 (84.6)	2 (15.4)	0.41	2.931
	Married	62 (36.7)	39 (62.9)	23 (37.1)		
	Single	14 (37.8)	9 (64.3)	5 (35.7)		
	Widow	13 (40.6)	10 (76.9)	3 (23.1)		
	Total	102 (39.2)	69 (67.6)	33 (32.4)		
Residence	Rural	29 (38.2)	17 (58.6)	12 (41.4)	0.22	1.508
	Urban	73 (39.7)	52 (71.2)	21 (28.8)		
	Total	102 (39.2)	69 (67.6)	33 (32.4)		

Table 4. Antibiotic resistant profile of methicillin-resistant and methicillin-sensitive *S. aureus*

Variables	Categories	No of *S. aureus*	No (%) of Wound Samples		p-value	χ^2
			With MSRA	Without MSSA		
Sex	Female	59 (39.9)	40 (67.8)	19 (32.2)	0.97	0.001
	Male	43 (38.4)	29 (67.4)	14 (32.6)		
	Total	102 (39.2)	69 (67.6)	33 (32.4)		
Age group (yrs)	20-40	36 (35.3)	22 (61.1)	14 (38.9)	0.08	5.084
	41-60	43 (43.0)	27 (62.8)	16 (37.2)		
	61-80	23 (39.7)	20 (86.9)	3 (13.1)		
	Total	102 (39.2)	69 (67.6)	33 (32.4)		
Marital Status	Divorce	13 (59.1)	11 (84.6)	2 (15.4)	0.41	2.931
	Married	62 (36.7)	39 (62.9)	23 (37.1)		
	Single	14 (37.8)	9 (64.3)	5 (35.7)		
	Widow	13 (40.6)	10 (76.9)	3 (23.1)		
	Total	102 (39.2)	69 (67.6)	33 (32.4)		
Residence	Rural	29 (38.2)	17 (58.6)	12 (41.4)	0.22	1.508
	Urban	73 (39.7)	52 (71.2)	21 (28.8)		
	Total	102 (39.2)	69 (67.6)	33 (32.4)		

Keys: MSSA: Methicillin Sensitive *S. aureus*; MRSA: Methicillin Resistant *S. aureus*

cMLSB, and MS phenotypes, respectively. The results showed that 9 of 33 (27.3%) MSSA were iMLSB phenotypes and 8 of 33 (24.2%) MSSA were cMLSB phenotypes (Table 5). There was no statistically significant difference in the occurrences of iMLSB, cMLSB, and MS phenotypes among the MRSA and MSSA at $p > 0.05$.

The distribution of *erm* genes among MSSA and MRSA isolates is presented in Table 6. Of the 12 MRSA and MSSA representative isolates, three (3) MRSA, comprised of iMLSB (n = 2) and cMLSB (n = 1) *S. aureus,* possessed *erm*C, One (1) MSSA possessed *erm*C, while none possessed either *erm*A or *erm*B.

Discussion

The increasing prevalence of antibiotic resistance, particularly among community- and hospital-acquired pathogens, is of grave public health

Table 5. Inducible - and constitutive - MLSB resistant MRSA and MSSA phenotypes in wound samples

MLSB Status	No (%)		p-value
	MRSA (n, 69)	MSSA (n, 33)	
iMLSB	15 (21.7)	9 (27.3)	0.61
cMLSB	18 (26.1)	8 (24.2)	0.84
MS	1 (1.4)	0 (0.0)	1.00

Keys: iMLSB: inducible MLSB; cMLSB: constitutive MLSB: MS: Macrolide-Streptogramin; MSSA: methicillin-sensitive *S. aureus*; MRSA: methicillin-resistant *S. aureus*

Table 6. Resistant genes (*erm*) in representative (n=12) methicillin-resistant and methicillin-sensitive *S. aureus* from wound samples

MLS Phenotype		No (%) Positive		
		*erm*A	*erm*B	*erm*C
	MSSA (2)	0 (0.0)	0 (0.0)	0 (0.0)
iMLSB	MRSA (3)	0 (0.0)	0 (0.0)	2 (66.7)
	Total (5)	0 (0.0)	0 (0.0)	2 (40.0)
	MSSA (2)	0 (0.0)	0 (0.0)	1 (50.0)
cMLSB	MRSA (4)	0 (0.0)	0 (0.0)	1 (25.0)
	Total (6)	0 (0.0)	0 (0.0)	2 (33.3)
	MSSA (0)	0 (0.0)	0 (0.0)	0 (0.0)
MS	MRSA (1)	0 (0.0)	0 (0.0)	0 (0.0)
	Total	0 (0.0)	0 (0.0)	0 (0.0)

Keys: iMLSB: inducible MLSB; cMLSB: constitutive MLSB: MS: Macrolide-Streptogramin; MSSA: methicillin-sensitive *S. aureus*; MRSA: methicillin-resistant *S. aureus*

concern worldwide (Akinjogunla *et al*., 2011; Karimi *et al.,* 2017). *S. aureus,* especially MRSA strains, has emerged as a leading pathogen in a wide range of wound infections (Akinjogunla *et al*., 2014; Al-Kasaby and Abou El-khier, 2017), poses serious therapeutic problems, and worsens the prognosis of wound patients (Wang *et al.,* 2010; Khoshnood *et al.,* 2019). The widespread increase in methicillin-resistant *S. aureus* has resulted in a renewed interest in the use of MLSB antibiotics to treat infections caused by *S. aureus*. However, indiscriminate use of MLSB antibiotics has resulted in the development and spread of *S. aureus* strains harboring resistant genes to MLSB antibiotics (Cetin *et al.,* 2010).

In our study, the overall prevalence of *S. aureus* colonization in the wound samples of patients was 39.2%. This result agrees with the 32.5% previously obtained by Akinjogunla *et al.* (2018) in Uyo, but was lower than the 52% obtained in a study conducted in Lagos, Nigeria (O'Malley *et al.,* 2015) and the 49.7% reported in Ethiopia (Mahammedaman *et al.*, 2019). The females had a slightly higher prevalence rate (39.9%) of *S. aureus* wound colonization than males (38.4%). This may be attributed to a decrease in immunity with age, as most women recruited in the study were of menopausal age, and women are more likely to be exposed to domestic injuries and burns at home than men. In our study, the highest prevalence of *S. aureus* was found in divorce patients and those residing in urban areas. This corroborated the report of Mohammedaman *et al.* (2019), who recorded a higher colonization rate of *S. aureus* (MRSA) among urban dwellers than among rural dwellers. The overall prevalence of MRSA and MSSA was 67.6% and 32.4%, respectively. The prevalence of MRSA obtained in this present study was higher than the 46.2% MRSA obtained in Uyo by Akinjogunla *et al.* (2018), 82.27% MRSA in Ethiopia (Mohammedaman *et al.*, 2019), and 82.27% MRSA in Iran (Khoshnood *et al.,* 2019). Variations in MRSA prevalence could be attributed to geographical distributions and study periods (Akinjogunla and Enabulele, 2010).

In our results, between 51.5% and 59.4% of MRSA and MSSA were resistant to Clindamycin and Erythromycin. The high Erythromycin resistance by MRSA in this present study agrees with the >40% Erythromycin resistant MRSA reported by Mittal *et al.* (2013) in India; Bulgaria (Lesseva and Hadjiiski, 1996), and Akinjogunla *et al.* (2018) in Nigeria. Increased resistance of *S. aureus* to MLSB antibiotics and the concomitant spread of MLSB strains in infected wound patients have been reported (Fiebelkorn *et al.,* 2003; Vandana *et al.,* 2009). In our study, 21.7% and 26.1% of MRSA were iMLSB and cMLSB phenotypes, respectively. These values were higher than the 14.7% iMLSB and 17.6% cMLSB-resistant MRSA obtained by Gadepalli *et al.* (2006) and Dardi and Khare (2013).

Resistance of *S. aureus* to MLSB antibiotics is either due to an active efflux pump encoded by the *msrA* gene or ribosomal target modification mediated by *erm* genes. Of the 12 MRSA and MSSA representative isolates, MRSA (n = 3) and MSSA (n = 1) isolates possessed *erm*C, while none possessed either *erm*A or *erm*B. The detection of only the *erm*C gene in MSSA and MRSA isolates corroborated the findings of studies conducted in Iran (Aktas *et al.,* 2007); Bulgaria (Gergova *et al*., 2019); Turkey (Ghanbari *et al.,* 2016); and Brazil (Pereira *et al.,* 2016); but the result differs from the

studies in Egypt, where *erm*A was the predominant gene detected in MRSA and MSSA (Al-kasaby and Abou El-khier, 2017). The *erm* genes encode enzymes that confer inducible constitutive resistance to MLSB antibiotics through methylation of the 23S rRNA, thereby reducing the binding of MLSB to the ribosomal target (Shantala *et al.,* 2011).

Conclusion

This study has revealed the prevalence of constitutive and inducible MLSB resistance among MRSA obtained from patients with wound infections and similarly shown the *ermC* gene as the principal erythromycin-resistant gene in *S. aureus* from the wound samples in our localities. Consequently, routine screening tests for iMLSB-resistant *S. aureu*s strains are critical for the therapeutic management of wound infections caused by this pathogen.

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