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MOLECULAR TYPING OF METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* OBTAINED FROM CLINICAL SPECIMENS IN TERTIARY HOSPITALS IN JOS, NIGERIA

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ABSTRACT

Background: Methicillin resistant *S. aureus* (MRSA) constitutes a serious public health problem in hospitals and community settings. Knowledge of local data on antibiotic resistance of MRSA isolates is important for empiric antibiotic prescription. **Aim:** To determine the prevalence, clonal composition, antibiotic resistance and virulence profiles of MRSA obtained from patients in tertiary hospitals in Jos, Nigeria. **Method:** A total of 214 *S. aureus* isolates were tested for susceptibility to antibacterial agents by the disc diffusion method and determination of the minimum inhibitory concentration (MIC). Methicillin resistance was confirmed by the PCR amplification of *mecA* gene. The MRSA were genotyped using *spa* typing, multilocus sequence typing (MLST) and DNA microarray analysis. **Results:** Forty-seven (21.9%) of the 214 isolates were positive for *mecA.* Most of the MRSA isolates were cultured from wound 27 (57.4%) and urine 11 (23.4%) specimens of males (n=27 (57.4%) of age group 11-29 (n=30; 63.8%). The isolates were resistant to penicillin G and trimethoprim mediated by *blaZ* and *dfrS1*, respectively. All MRSA were identified as CA-MRSA (SCCmec type IV (n=4); SCCmec type V (n=43) by SCC *mec* typing. The MRSA belonged to 10 *spa* types with t008 (n=14; 29.8%) as the dominant *spa* type, followed t064 and t2658 (n=7; 14.9%), and six sequence types, ST8 (n=28), ST772 (n=6), ST7 and ST152 (n=5), ST22 (n=2) and ST88 (n=1). The common genotypes were ST8-MRSA-V-t008/t064/t2658 (n=27), ST772-MRSA-V, [PVL$^+$]/t657/t345 (n=6), ST7-MRSA-V-t091 (n=5) and ST152-MRSA-V//t355/t4690 (n=5), whereas ST22-MRSA-IV [tst1$^+$]/t032 (n=2) and ST88-MRSA-IV /t786 (n=1) were less common. **Conclusion:** The study revealed a MRSA prevalence of 21.9%. The MRSA isolates belonged to diverse genetic backgrounds dominated by ST8-MRSA-V-t008/t064/t2658 genotype. The study has provided a platform for future studies on the molecular characteristics of MRSA in the central region of Nigeria.

KEYWORDS: Antimicrobial sensitivity testing, polymerase chain reaction, MRSA.

INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major cause of healthcare and community-associated infections worldwide.[1,2] Since its emergence in UK in 1960s[3], the prevalence of isolation of MRSA has been on the increase worldwide,[4,5,6] including Nigeria.[7,8,9,10,11,12] Initially, MRSA isolates caused infections in elderly patients admitted to healthcare facilities, such as nursing homes and long-term care facilities with previous history of antibiotic use and surgery. These strains were designated Healthcare-associated MRSA (HA-MRSA).[13,14,5] However, since the 1990s, MRSA, described as community-acquired or community-associated MRSA (CA-MRSA), have been isolated from apparently healthy individuals in communities who had no previous history of hospital admission or antibiotic treatment.[13,5] Since then, CA-MRSA isolates have become the major causes of infections in the community and healthcare facilities worldwide.[5, 2]

Methicillin-resistant *Staphylococcus aureus* evolved through the acquisition of the *mecA* gene by previously susceptible isolates. The *mecA* gene is responsible for the synthesis of a novel penicillin-binding protein known as penicillin-binding protein 2a, which has decreased binding affinity for penicillin and cephalosporins and therefore confers resistance to beta-lactam antibiotics except the 5th generation cephalosporin, Ceftaroline.[15] The *mecA* is located on a mobile genetic island called staphylococcal cassette chromosome *mec* (SCC *mec*).[16] The SCC*mec* genetic element differ in size and structural organisation and based on these differences 13 SCC*mec* types (types I-XIII) have been described.[5] The differences in the SCC*mec* types carried by MRSA strains formed the basis of the Staphylococcal Cassette Chromosome mec (SCC*mec*) typing which genotypically differentiates MRSA strains as either health care-associated (HA-MRSA) or community-associated (CA-MRSA).[17, 18] HA-MRSA strains usually harbor SCC *mec* I, II, and III, while CA-MRSA strains harbor SCC*mec* IV, V, and VI and others.[5]

It has been established that the epidemiology of MRSA strains is constantly changing in different geographical locations.[19] It is therefore necessary to study MRSA isolates from local healthcare facilities to obtain local data that can be used for empirical treatment of infections and design appropriate infection control protocols.

In Nigeria, the prevalence and molecular characteristic of MRSA isolated in the Southwest[20, 21], Northeast[10, 22] and Northwest[23] of the country have been studied. However, there are no data on the molecular characteristics and virulence gene profiles of MRSA colonizing or infecting patients in Jos, North Central Nigeria. This study was undertaken to provide information that will assist in better understanding of epidemiology, genetic diversity, clonal composition and virulence profiles of MRSA circulating in Jos metropolitan hospitals.

MATERIALS AND METHODS

Bacterial isolates

S. aureus isolates were obtained from clinical specimens at three tertiary hospitals in Jos. These were Jos University Teaching Hospital (JUTH) (600-beds), Plateau State Specialist Hospital (PSSH) (177-beds) and Bingham University Teaching Hospital (BUTH) (150-beds). During the study period from December 2017 to July 2019, 1024 presumptively identified *S. aureus* isolates were obtained as part of routine microbiology diagnostic investigations in the microbiology laboratories of the afore-mentioned tertiary hospitals. A total of 214 isolates were confirmed as *S. aureus* after the 1024 isolates were re-tested by growth and fermentation on mannitol salt agar, Gram stain, and positive results for catalase and tube coagulase tests (Lyophilized Rabbit plasma; Becton, Dickinson and company Sparks, USA) at the Gram-Positive Bacteria Research Laboratory, Department of Microbiology, Faculty of Medicine, Kuwait University. Kuwait.

Antimicrobial sensitivity testing

Antibiotic susceptibility testing was performed by the disc diffusion method according to the guidelines of the Clinical Laboratory Standards Institute[24] with the following antimicrobial discs (Oxoid): benzyl penicillin (10U), cefoxitin (30 µg), kanamycin (30 µg), mupirocin (200 µg and 5 µg), gentamicin (10 µg), erythromycin (15 µg), clindamycin (2 µg), chloramphenicol (30 µg), tetracycline (10 µg), trimethoprim (2.5 µg), fusidic acid (10 µg), rifampicin (5 µg), ciprofloxacin (5 µg). Minimum inhibitory concentration (MIC) for oxacillin, cefoxitin, mupirocin, vancomycin and teicoplanin were determined with E-test strips (AB BioMerieux, Marcy l'Etoile, France) according to the manufacturer's instructions. The interpretation of the MIC values was based on the antibiotic breakpoint concentration recommended by the CLSI,[24] *S. aureus* strains ATCC25923 and ATCC29213 were used as a quality control strain for disc diffusion and MIC determination respectively. The D-test was used to test for inducible resistance to clindamycin. Methicillin resistance was confirmed by *mecA* PCR.[25]

Sensitivity to fusidic acid by disc diffusion method was interpreted according Skov *et al.*,[26], Sensitive ≥24, resistance ≤20. Also, sensitivity to three non-antibiotic agents cadmium acetate, mercuric chloride and ethidium bromide were tested. The heavy metals and dye impregnated disc were prepared in the laboratory with the indicated concentrations: cadmium acetate (50µg), mercuric chloride, (109µg) and ethidium bromide (5µg). *S. aureus* WBG 248[27] was used as a control strain to compare its inhibition zone to the test organism.

Molecular techniques

DNA isolation

DNA from *S. aureus* isolates was prepared as follows: 3-5 colonies of overnight culture were mixed with 50 µl of lysostaphin (150 µg/ml) and 10 µl of RNase (10 µg/ml) solution in a sterile microfuge tube and incubated at 37°C in the heating block (Thermo Mixer, Eppendorf, Hamburg, Germany) for 20 min. After incubation, 50 µl of proteinase K (20 mg/ml) and 150 µl of Tris buffer (0.1 M) were added to each sample and incubated at 60°C in the heating block (Thermomixer, Eppendorf, Hamburg, Germany) for 10 min. To inactivate the enzymes, the tubes were incubated at 95°C for 10 min in the heating block (Thermomixer, Eppendorf, Hamburg, Germany).[28] The tubes were then centrifuged at 13,000 rpm for 5 minutes and the supernatant were transferred to a fresh sterile microfuge tube and stored at 4°C till used for PCR.

Detection of *mecA* gene by PCR

Methicillin resistance was confirmed by *mecA* PCR as described by.[25] Amplification of *mecA* gene was performed on all isolates resistant to cefoxitin in order to confirm MRSA. The total reaction volume 25 µL was

used for PCR using the set of primers described in table 1. This volume contained 2µl of genomic DNA, 12.5µl of Hot Star Red Taq Master mix, 8.5µl PCR H_2O and 1µl each of *mecA* primers (Qiagen, Hilden, Germany). The *mecA* DNA amplification was carried out for 40 cycles according to the following protocol: denaturation at 94° C for 30 s, annealing at 55° C for 30 s, and extension at 72° C for 1 min with a final extension at 72° C for 5 min. The amplicon was placed in agarose gel, the set-up was maintained at 120V for 30minutes. The DNA bands were visualized using UV in a transilluminator (SynGene Bioimaging System).

Staphylococcal cassette chromosome mec (SCCmec) typing

Staphylococcal cassette chromosome *mec* (SCC*mec*) typing was performed on all MRSA strains using the multiplex PCR technique described by.[17] A multiplex PCR was performed in a 25 µl reaction volume using the set of primers described in table 1. The 25 µl volume contains the following: 2µl of template DNA, 12.5µl of Hot Star Red Taq Master mix, PCR water 8.5µl, 2 µl of primer mix. PCR amplifications was performed in a thermal cycler for 30 cycles under the following conditions: denaturation for 4 min at 94° C; denaturation at 94° C for 30s, annealing at 53° C for 30s, and extension at 72° C for 1 min; post extension for 4 min at 72° C. The amplicon was placed in agarose gel, the set-up was maintained at 120V for 30minutes. The DNA bands were visualized using UV in a transilluminator (SynGene Bioimaging System) (Figure 3a).

Table 1: Primer sequences used for mecA PCR and SCC*mec* typing.

Target genes	Primer sequences (5'→ 3')	Amplicon size (bp)
MecA	Forward GTGAAGATATACCAAGTGATT Reverse ATGCGCTATAGATTGAAAGGAT	147
SCCmec I	Forward GCTTTAAAGAGTGTCGTTACAGG Reverse GTTCTCTCATAGTATGACGTCC	613
SCCmec II	Forward CGTTGAAGATGATGAAGCG Reverse CGAAATCAATGGTTAATGGACC	398
SCCmec III	Forward CCATATTGTGTACGATGCG Reverse CCTTAGTTGTCGTAACAGATCG	280
SCCmec Iva	Forward GCCTTATTCGAAGAAACCG Reverse CTACTCTTCTGAAAAGCGTCG	776
SCCmec IVb	Forward TCTGGAATTACTTCAGCTGC Reverse AAACAATATTGCTCTCCCTC	493
SCCmec IVc	Forward ACAATATTTGTATTATCGGAGAGC Reverse TTGGTATGAGGTATTGCTGG	200
SCCmec IVd	Forward CTCAAAATACGGACCCCAATACA Reverse TGCTCCAGTAATTGCTAAA	881
SCCmec V	Forward GAACATTGTTACTTAAATGAGCG Reverse TGAAAGTTGTACCCTTGACACC	325

Source:[25]

Staphylococcal protein A (*Spa*) typing

Spa typing was performed by amplification and sequencing of *spa* gene as previously described by.[29] Spa gene was evaluated using a 3130x1 genetic analyzer (Applied Bio systems, Forster City, CA. USA) in accordance with the manufacturer protocol. Isolates were assigned to spa types using the spa typing website (http://www.spaserver.ridom.de).

Multilocus sequence typing

The MLST is a nucleotide-based typing method performed on isolates using the method previously described by.[30] MLST was performed by initial amplification and sequencing of seven housekeeping genes in each isolate. The MLST technique directly measures the DNA sequence variations (alleles) in a set of housekeeping genes and characterized strains by their unique allelic profile. Sequences from MLST were submitted to a large database (http://saureus.mlst.net/) where the sequences of each locus are compared with all the previously identified sequences and numbers are assigned to alleles (gene variants) at each seven loci. Isolates were assigned a sequence type (ST) according to the MLST website (http://www.pubmlst.net).

DNA microarray analysis

All isolates were analyzed using DNA microarray technique. DNA microarray technique detects various genetic determinants including species markers, genes encoding antibiotic resistance and virulence, toxins, immune evasion complex (IEC), the arginine catabolic mobile element (ACME). Others include adhesion and biofilm genes, microbial surface components recognizing adhesive matrix molecules (MSCRAMMs), accessory gene regulator (agr), capsule and SCC*mec* types. The assay also delineates *S. aureus* to clonal complexes (CCs). The analysis was performed using the Identibac *S. aureus* genotyping Kit 2.0 (Alere Technology, Jena,

Germany as described.[31, 16, 32] Data generated were analyzed using the ArrayMate software in the ArrayMate Reader (Alere Technology) as reported previously.[16]

Statistical analysis

Data obtained from this study were analyzed using Statistical Package for the Social Sciences (SPSS) version 26.0. The MRSA, gender, age-group, and hospitals were compared using Pearson chi-square tests. Results were presented in tables, bar chart and percentages. P-values of <0.05 were considered statistically significant.

RESULTS

The 214 isolates were susceptible to vancomycin (MIC: ≤ 2 µg /ml), teicoplanin (MIC: ≤ 2 µg /ml), tigecycline (MIC: ≤ 2 µg /ml) and spectinomycin. Forty-seven (21.9%) isolates were resistant to cefoxitin and oxacillin (MIC: ≥16 µg /ml; ≥8 µg/ml respectively). One isolate expressed low-level resistance to mupirocin (MIC=125 µg /ml). The 47 cefoxitin-resistant isolates were characterized further in this study. All 47 cefoxitin-resistant isolates were positive for *mecA* (Figure 1) confirming them as methicillin-resistant *S. aureus* (MRSA) yielding an MRSA prevalence of 21.9% (47/214).

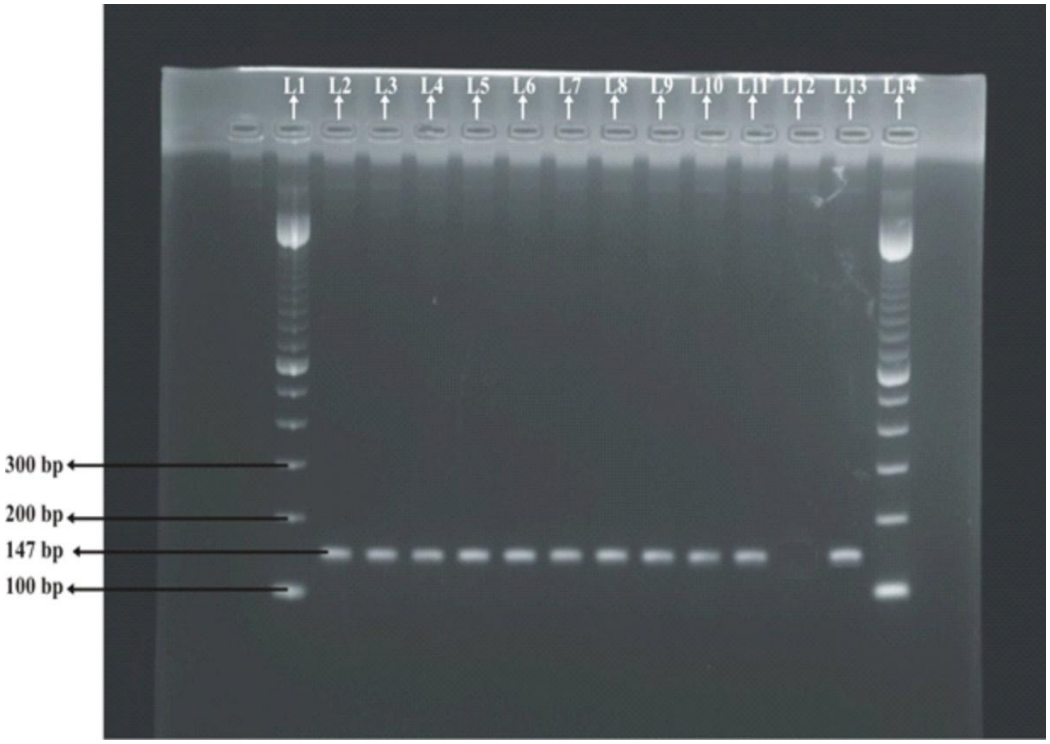

Figure I. Agarose gel electrophoresis of representative *mecA* positive *S. aureus* isolates.

Lanes 1 and 14 shows 100bp DNA molecular size ladder used for sizing DNA bands of test samples. The ladder consists of 100bp DNA bands ranging from 100 to 1500bp. Lanes 2, 3, 4, 5, 6, 7, 8, 9, 10 and 11 represent DNA bands from test samples that were positive for *mecA*. Lane 12 and 13 represents negative and positive mecA control samples respectively. The amplified *mecA* gene is 147 bp in size, therefore it was located between 100bp and 200bp.

Figure 2 shows the antimicrobial resistance of the 47 MRSA isolates. In addition to cefoxitin resistance, all isolates were resistant to benzyl penicillin and trimethoprim. In addition, the isolates were resistant to ciprofloxacin (n=39; 82.9%), cadmium acetate (n=38; 80.9%), tetracycline (n=34; 72.3%), mercuric chloride (n=28; 59.6%), gentamicin (n=18; 38.3%), kanamycin (n=18; 38.3%), ethidium bromide (n=16; 34.0%), chloramphenicol (n=11; 23.4%), erythromycin (n=8; 17.0%) clindamycin (n=6; 12.8%), fusidic acid (n=2; 4.3%), and rifampicin (n=1; 2.1%). One isolate expressed low-level resistance to mupirocin (MIC=125 µg /ml).

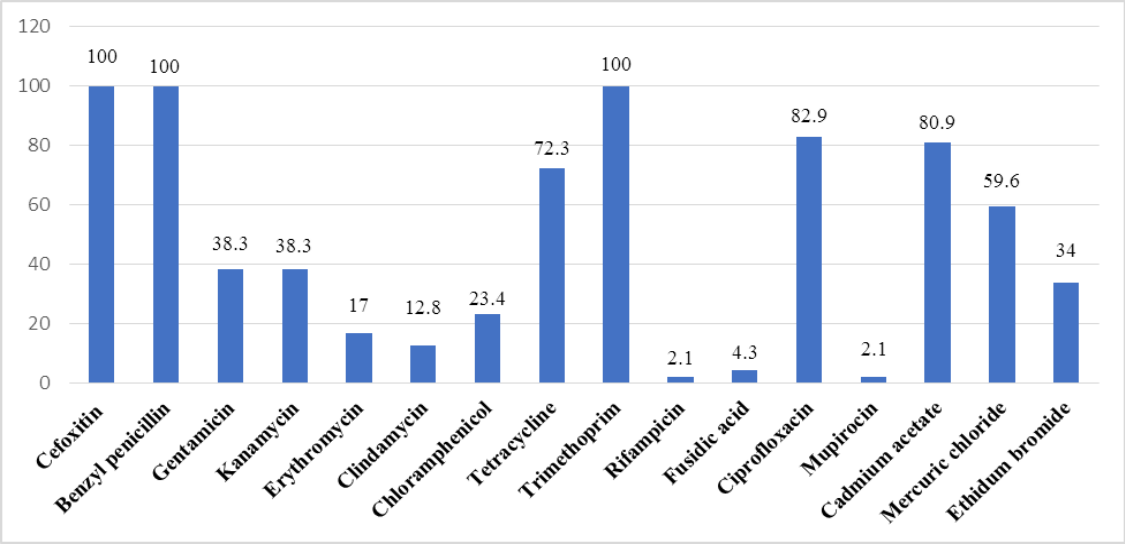

Figure 2. Antimicrobial resistance pattern of MRSA

Table 2 shows the distribution of MRSA isolates according to patients' age groups, gender, hospitals, and clinical sources of the isolates. Most of the MRSA isolates were recovered from patients within the age group of 11-29 years (63.8%) followed by patients in the age group of 30-48 years (31.9%). The proportion of MRSA isolates was higher among male patients (MRSA=27; 57.4%) than female patients (MRSA=20; 42.6%) although the difference was not statistically significant (P>0.05; P=0.160).

The distribution of MRSA isolates according to hospitals shows that JUTH provided most of MRSA (N=28; 59.6%), followed by PSSH (N=16; 34.0%)) and BUTH (N=3; 6.4%). The MRSA isolates were recovered from seven clinical sites with most of the MRSA isolates (27; 57.4%) recovered from wound swabs and urine samples (11; 23.4%). The other clinical samples comprising blood, HVS, nasal swab, urethral swab, and semen, yielded fewer MRSA isolates.

Table 2: Demographic characteristics of *S. aureus* isolates.

Variables	No. of isolates N=214	No. of MRSA N=47 (%)	P-value
Age group			0.030
≤10	10	0 (0.0)	
11-29	100	30 (63.8)	
30-48	90	15 (31.9)	
49-67	14	2 (4.3)	
Gender			0.160
Female	72	20 (42.6)	
Male	142	27 (57.4)	
Hospital			0.570
BUTH	22	3 (6.4)	
JUTH	126	28 (59.6)	
PSSH	66	16 (34.0)	
Specimens			0.391
Blood	34	3 (6.4)	
HVS	10	3 (6.4)	
Nasal swab	1	1 (2.1)	
Semen	3	1 (2.1)	
Urethral swab	7	1 (2.1)	
Urine	44	11 (23.4)	
Wound swab	100	27 (57.4)	

BUTH=Bingham University Teaching Hospital
JUTH=Jos University Teaching Hospital
PSSH=Plateau State Specialist Hospital

Molecular characterization of MRSA isolates

The isolates were positive for SCC*mec* types IV and V (Figure 3a). Most of the isolates belonged to SCC*mec* type V detected in 43 (91.5%) isolates, while SCC*mec* type IV was detected in 4 (8.5%) isolates (Figure 3b). Based on the SCC*mec* typing results all 47 MRSA identified in this study belonged to the community - associated MRSA (CA-MRSA) genotype.

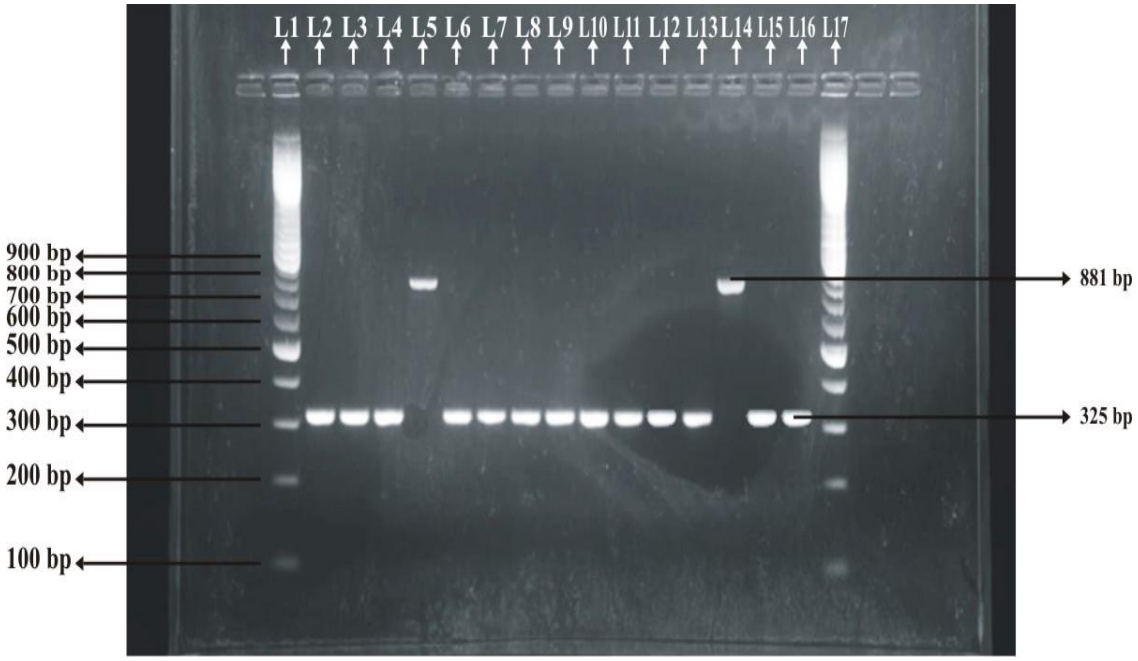

Figure 3a. Agarose gel electrophoresis of representative SCC*mec* types.

Lanes 1 and 17 show 100bp DNA molecular size ladder (control) used for sizing DNA bands of test samples. Lanes 2, 3, 4, 6, 7, 8, 9, 10, 11, 12, 13, 15 and 16 show DNA bands from test samples that were positive for SCC*mecV*. Lanes 5 and 14 represent DNA bands from test samples that were positive for SCC*mecIV*. The SCC*mecV* element is 325 bp in size, therefore it was located between 300bp and 400bp, whereas the SCC*mecIV* is 881 bp in size and was located between 800 bp and 900 bp.

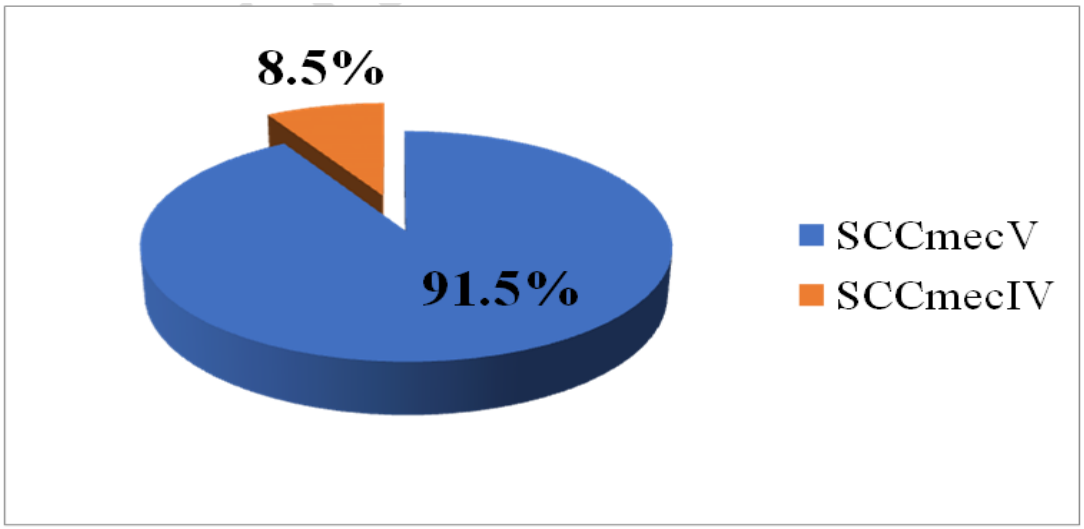

Figure 3b. Distribution of SCC*mec* types among MRSA isolates.

Staphylococcal protein (*spa*) typing of the MRSA isolates revealed 10 *spa* types with t008 (n=14; 29.8%) identified as the dominant *spa* type, followed by *spa* types, t064 and t2658 (n=7; 14.9%). The other common *spa* types were t091, t345 and t355 detected in five (10.6%), four (8.5%) and three (6.4%) isolates respectively. Two (4.3%) isolates each were associated with *spa* types, t032, t4690 and t657, while t786 was detected in one isolate.

Multilocus sequence typing of MRSA isolates identified six sequence types (STs) namely, ST8 (n=28; 59.6%), ST772 (n=6; 12.8%), ST7 and ST152 (n=5; 10.6%), ST22 (n=2; 4.3%) and ST88 (n=1; 2.1%).

Results of DNA microarray analysis classified the isolates into six clonal complexes (CC) namely, CC8 (n=28), CC1 (n=6), CC7 and CC152 (n=5), CC22 (n=2) and CC88 (n=1). Most of the isolates belonged to CC8-ST8-MRSA-V-t008 genotype (n=14) followed by CC-ST8-MRSA-V-t2658 (n=7), CC8-MRSA-V-t064 (n=7), CC7-ST7-MRSA-V-t091 (n=5), CC1-ST772-MRSA-V-t345 (n=4), CC152-ST152-MRSA-V [PVL+]-t355 (n=3), CC152-ST152-MRSA-V-t4690(n=2), CC1-ST772-MRSA-V-t657 (n=2), CC22-ST22-MRSA-IV, [tst^{+}]-t032 (n=2) and CC88-ST88-MRSA-IV-t786 (n=1).

Distribution of virulence and antibiotic resistance genes

The isolates were positive for a range of virulence and antibiotic resistance determinants as summarized in Table 3. The MRSA isolates showed similarities in the carriage of the following virulence genes; biofilm-associated genes (*icaA, icaC, icaD*), microbial surface components recognizing adhesion matrix molecules (MSCRAMMs) such as clumping factor A/B (*clfA,clfB*), fibronectin-binding protein A/B (*fnbA, fnbB*), collagen-binding adhesin (*cna*), enolase (*eno*), van Willebrand factor binding protein (*vwb*) and serine rich fibrinogen (*sdrC, sdrD*), haemolysins (*hla, hlb, hld*), genes that codes of proteases such as serine protease (*spIA/B/E*), aureolysin (*aur*) and immune evasion genes such as staphylokinase (*sak*), staphylococcal complement inhibitor (*scn*) and chemotaxis-inhibiting protein (*chp*). In addition, all the isolates were positive for *blaZ* that mediates penicillin G resistance and, *dfrS1* that confers trimethoprim resistance.

The characteristics of isolates belonging to the different sequence types are described below.

CCI

The CC1 consisted of six isolates that belonged to ST772-MRSA-V, [PVL^{+}] clone. The isolates were cultured from wound (n=4); blood (n=1) and urine (n=1), and were associated with two *spa* types, t345 (n=4) and t657 (n=2). The isolates harbored accessory gene regulator type II (*agrII*), capsular polysaccharide type 5 (*cap5*) genes, and were homogenous in their carriage of staphylococcal enterotoxin genes. One isolate lacked *seu*. The isolates were resistant to multiple antibacterial agents including resistance to ciprofloxacin and cadmium acetate.

CC7

The ST7-MRSA-V, WA-131 isolates were associated with of a single *spa* type, t091 (n=5). All isolates carried the following resistance genes; *mecA* (cefoxitin), *blaZ* (penicillin), *aacA-aphD* (gentamicin), *aphA3* (kanamycin), *dfrS1*(trimethoprim), *tet(K)*(tetracycline), and *sat* (streptothricin). In addition, two isolates were positive for *erm(C)* (erythromycin) and *qacC* (ethidium bromide), while *cat* (chloramphenicol) was detected in a single isolate. All five isolates were resistant to ciprofloxacin. Four of the isolates were positive for a single staphylococcal enterotoxin gene (*sea*). One isolate was negative for staphylococcal enterotoxin genes.

CC8

The CC8 constituted the majority (28/47) of MRSA isolates. The 28 CC8 isolates were classified into four clones; ST8-MRSA-V-WA-53/120 (n=14), ST8-MRSA-V (n=10), ST8-MRSA-V, WA-115/132 (n=3), ST8-MRSA-IV-SCCfus (n=1) and were associated with three different *spa* types, t2658 (n=7), t064 (n=7) and t008 (n=14), The ST8-MRSA-V-WA-53/120 and ST8-MRSA-V, WA-115/132 were positive for *agrI* and *cap5*. Twenty-three of the isolates were positive for staphylococcal enterotoxin genes (*sea, seb, sek, seq*). Four of the isolates were positive for staphylococcal enterotoxin genes (*sej, ser*) and toxic shock syndrome toxin gene (*tst1)*. The isolates were enriched with the following antimicrobial resistance genes namely; erythromycin (*erm(C)*), clindamycin (*erm(C)*), tetracycline (*tetK*), chloramphenicol (*cat*), gentamicin (*aacA-aphD*), kanamycin (*aphA3*), fosfomycin (*fosB*), mercuric chloride (*merA/merB*) and ethidium bromide (*qacC*). The single ST8-MRSA-IV-SCCfus isolate was unique because it carried the gene for fusidic acid resistance within its SCC*mec* element and possesses numerous staphylococcal enterotoxin genes (*seb, sed, seg, sei, sej, sem, sen, seo, ser, seu*).

CC22

The two CC2 isolates belonged to ST22-MRSA-IV [tst1^{+}] and resembled UK-MRSA-15/ Middle Eastern variant. These isolates were cultured from wound specimens obtained from JUTH and were associated with *spa* type, t032. Both isolates possessed *agrI, cap5* and *tst1*. One of the isolates was positive for epidermal cell differentiation inhibitor (*edinB*) gene. In addition, one isolate was resistant to erythromycin mediated by *erm(C)* and chloramphenicol mediated by *cat*.

CC88

The single ST88-MRSA-IV, WA-MRSA-2 isolate was associated with *spa* type, t786. This isolate was positive for *agrIII* and *cap8*. Additionally, staphylococcal enterotoxin gene, sed, was detected in the isolate.

CC152

The five CC152 isolates were identified in two clones namely, ST152-MRSA-V [PVL^{+}] (n=3) and ST152-MRSA-V (n=2) and consisted of *spa* types, t355 (n=3) and t4690 (n=2). They were all cultured from wound specimens and were positive for *agrI* and *cap5* but lacked staphylococcal enterotoxin genes. Four of the isolates possessed epidermal cell differentiation inhibitor (*edinB*) gene, while the three isolates of ST152-MRSA-V [PVL^{+}] harbored the PVL gene. The isolates carried genes that mediate resistance to gentamicin (*aacA-aphD*), kanamycin (*aphA3*), tetracycline (*tet(K)*), mercuric chloride (*merA/merB*) and ethidium bromide (*qacC*).

Table 3a. Characterization of MRSA isolates.

Strain ID	Clone	Spa types	Source	Hospital	Agr	Cap	Enterotoxins	Other toxins	Antibiotic resistance genes
	CC1								
427	ST772-MRSA-V, [PVL$^+$] Bengal Bay clone	t345	Urine	JUTH	II	5	*sea,sec,seg,sei,sel,sem,sen, seo,seu.*		*msr(A),aacA-aphD,aphA3,sat,fosB.*
209	ST772-MRSA-V, [PVL$^+$] Bengal Bay clone	t657	Wound	JUTH	II	5	*sea,sec,seg,sei,sel,sem,sen, seo*		*msr(A), mph(C), aacA-aphD aphA3,sat,fosB, merA,merB.*
425	ST772-MRSA-V, [PVL$^+$] Bengal Bay clone	t345	Blood	JUTH	II	5	*sea,sec,seg,sei,sel,sem,sen, seo,seu,*		*msr(A), aacA-aphD, aphA3,sat,fosB.*
493	ST772-MRSA-V, [PVL$^+$] Bengal Bay clone	t345	Wound	JUTH	II	5	*sea,sec,seg,sei,sel,sem,sen, seo,seu.*		*msr(A), aacA-aphD, aphA3,sat,fosB*
500	ST772-MRSA-V, [PVL$^+$] Bengal Bay clone	t345	Wound	JUTH	II	5	*sea,sec,seg,sei,sel,sem,sen, seo,seu.*		*msr(A), aacA-aphD, aphA3, sat,fosB*
171	ST772-MRSA-V, [PVL$^+$] Bengal Bay clone	t657	Wound	JUTH	II	5	*sea,sec,seg,sei,sel,sem,sen, seo,seu.*		*tet(K) merA, merB ,msr(A),aacA-aphD,aphA3,sat,fosB*
	CC7								
46	ST7-MRSA-V-WA-131	t091	Urine	PSSH	I	8	*Sea*		*,erm(C), aacA-aphD,aphA3,sat merA,merB, qacC tet(K)*
98	ST7-MRSA-V-WA-131	t091	HVS	PSSH	I	8	-		*aacA-aphD,aphA3,sat, tet(K) merA,merB*
108	ST7-MRSA-V-WA-131	t091	Urethral swab	PSSH	I	8	*Sea*		*erm(C), aaA-aphD, aphA3, sat, tet(K), cat, dfrS, merA,merB, qacC*
153	ST7-MRSA-V-WA-131	t091	Semen	JUTH	I	8	*Sea*		*aacA-aphD, aphA3,sat tet(K) merA,merB.*
160	ST7-MRSA-V-WA-131	t091	Urine	JUTH	I	8	*Sea*		*aacA-aphD, aphA3,sat tet(K), merA,merB.*
	CC8								
7	ST8-MRSA-V-WA-MRSA-53/120	t2658	Urine	PSSH	I	5	*sea,seb,sek,seq*		*merA, merB, cat tet(K),qacC, fosB,*
41	ST8-MRSA-V-WA-MRSA-53/120	t2658	Urine	PSSH	I	5	*sea,seb,sek,seq*		*merA, merB, cat tet(K),qacC, fosB,*
42	ST8-MRSA-V-WA-MRSA-53/120	t2658	Urine	PSSH	I	5	*sea,seb,sed,sek,seo,seq.*		*merA, merB, tet(K), qacC fosB,*
93	ST8-MRSA-V-WA-MRSA-53/120	t2658	HVS	PSSH	I	5	*sea,seb,sek,seq*		*merA, merB, cat tet(K) qacC, fosB*

Table 3b. Characterization of MRSA isolates.

Strain ID	Clone	Spa types	Source	Hospital	Agr	Cap	Enterotoxins	Other toxins	Antibiotic resistance genes
	CC8								
97	ST8-MRSA-V-WA-MRSA-53/120	t064	Urine	PSSH	I	5	*sea,seb,sek,seq*		*merA, merB, erm(C), tet(K) qacC, fosB,*
106	ST8-MRSA-V-WA-MRSA-53/120	t064	Wound	PSSH	I	5	*sea,seb,sek,seq*		*merA, merB, tet(K),qacC, fosB,*
117	ST8-MRSA-V-WA-MRSA-53/120	t2658	Urine	PSSH	I	5	*sea,seb,sek,seq*		*merA, merB, cat tet(K),qacC, fosB,*
118	ST8-MRSA-V-WA-MRSA-53/120	t2658	Wound	PSSH	I	5	*sea,seb,sek,seq*		*merA, merB, cat, tet(K),qacC, fosB,*
137	ST8-MRSA-V-WA-MRSA-53/120	t064	Blood	JUTH	I	5	*sea,seb,sek,seq*		*merA, merB, tet(K),qacC, fosB,*
318	ST8-MRSA-V-WA-MRSA-53/120	t064	Urine	JUTH	I	5	*sea,seb,sek,seq*		*merA, merB tet(K), fosB.*
341	ST8-MRSA-V-WA-MRSA-53/120	t064	Wound	BUTH	I	5	*seb,sek,seq*		*merA, merB, cat, tet(K), fosB.*
397	ST8-MRSA-V-WA-MRSA-53/120	t064	Nasal swab	BUTH	I	5	*sea,seb,sek,seq*		*merA, merB, cat, tet(K),qacC.*
400	ST8-MRSA-V-WA-MRSA-53/120	t064	Urine	PSSH	I	5	*sea,seb,sek,seq*		*merA, merB tet(K) cat, qacC,fosB.*
163	ST8-MRSA-V-WA-MRSA-53/120	t2658	Urine	JUTH	I	5	*sea,seb,sek,seq*		*merA, merB, cat tet(K), qacC, fosB.*
410	ST8-MRSA-V	t008	Blood	JUTH	I	5	*sej,ser.*	*tsst*	*merA, merB tet(K), aacA-aphD, aphA3, qacC, fosB*
422	ST8-MRSA-V	t008	Wound	JUTH	I	5	*sea,seb,sek,seq*		*tet(K), fosB*
449	ST8-MRSA-V	t008	Wound	JUTH	I	5	*sea,seb,sek,seq*		*merA, merB, erm(C), tet(K) qacC, fosB.*
468	ST8-MRSA-V	t008	Wound	JUTH	I	5	*sea,seb,sek,seq*		*tet(K), fosB,*
469	ST8-MRSA-V	t008	Wound	JUTH	I	5	*sea,seb,sek,seq*		*tet(K), fosB,*
482	ST8-MRSA-V	t008	Wound	PSSH	I	5	*sea,seb,sek,seq*		*tet(K), fosB,*
486	ST8-MRSA-V	t008	Wound	JUTH	I	5	*sea,seb,sek,seq*		*erm(C),aacA-aphD, aphA3, fosB,*

MecA (methicillin resistance gene), merA, merB, (Mercury resistance gene), blaZ (Beta-lactamase gene), tetK (tetracycline resistance gene), fosB (fosfomycin resistance gene), msr(A) (macrolide efflux), aacA-aphD(aminoglycoside adenyl/phosphotranferase), aphA3(aminoglycoside phosphotransferase), qacC(multidrug efflux protein), sat (streptothricin transferase), cat(chloramphenicol acetyltransfer), dfrS(trimethoprim resistance), erm(C) (macrolide/ lincosamide resistance), tsst(toxic shock syndrome toxin), edin(epidermal cell differention inhibitor)

Table 3c. Characterization of MRSA isolates.

Strain ID	Clone	Spa types	Source	Hospital	Agr	Cap	Enterotoxins	Other toxins	Antibiotic resistance genes
	CC8								
487	ST8-MRSA-V	t008	Wound	JUTH	I	5	*sea,seb,sek,seq*		*tet(K), fosB,*
503	ST8-MRSA-V	t008	Wound	JUTH	I	5	*sea,seb,sek,seq*		*tet(K) fosB, qacC.*
505	ST8-MRSA-V	t008	Wound	JUTH	I	5	*sea,seb,sek,seq*		*tet(K), fosB,*
460	ST8-MRSA-IV-SCCfus	t008	Wound	JUTH	I	5	*seb,sed,seg,sei,sej,sem,sen, seo,ser,seu.*		*erm(C),fusC, fosB,*
411	ST8-MRSA-V-WA-MRSA-115/132	t008	Wound	BUTH	I	5	*sej, ser.*	*Tsst*	*merA, merB, tet(K) aphA3, aacA-aphD, qacC, fosB,*
472	ST8-MRSA-V-WA-MRSA-115/132	t008	Wound	JUTH	I	5	*sej, ser.*	*Tsst*	*merA, merB, tet(K), aphA3, aacA-aphD, qacC, fosB, tet(K)*
478	ST8-MRSA-V-WA-MRSA-115/132	t008	Wound	JUTH	I	5	*sej, ser.*	*Tsst*	*merA, merB, tet(K), aphA3, aacA-aphD, qacC, fosB,*
	CC22								
219	ST22-MRSA-IV, [tst], UK-MRSA-15/ Middle Eastern variant	t032	Wound	JUTH	1	5	*sea,seg,sei,sem,sen,seo,seu.*	*Tsst*	*mecA, blaZ, erm(C),dfrS, cat*
506-1	ST22-MRSA-IV, [tst], UK-MRSA-15/ Middle Eastern variant	t032	Wound	JUTH	1	5	*sec,seg,sei,sel,sem,sen,seo,seu.*	*tsst, edinB*	*mecA, blaZ, dfrS.*
	CC88								
123	ST88-MRSA-IV, WA-MRSA-2	t786	HVS	PSSH	III	8	*sed*		
	CC152								
492	ST152-MRSA-V [PVL$^+$]	t355	Wound	JUTH	I	5		*edinB*	*aacA-aphD, aphA3,*
504	ST152-MRSA-V [PVL$^+$]	t355	Wound	JUTH	I	5		*edinB*	*aacA-aphD, aphA3,*
507	ST152-MRSA-V [PVL$^+$]	t355	Wound	JUTH	I	5		*edinB*	*tet(K)*
483	ST152-MRSA-V	t4690	Wound	PSSH	I	5		*edinB*	*tet(K)*
236	ST152-MRSA-V	t4690	Wound	PSSH	I	5			*merA, merB, qacC.*

DISCUSSION

Methicillin resistant *S. aureus* are clinically important pathogens because of their ability to resist all beta-lactam and other antibacterial agents thereby limiting treatment options for MRSA infections.[1] As MRSA are associated with myriads of infections in healthcare facilities as well as in the community, knowledge of the local antibiotic resistance patterns and carriage of virulence genes by these strains can enhance better treatment outcomes, and the control and/or prevention of infections.[2]

The results of our study revealed that MRSA constituted 21.9% MRSA of *S. aureus* isolates obtained from human patients attending three hospitals in Jos, North Central Nigeria. Results of previous studies conducted at other centres in Nigeria revealed MRSA prevalence of 20.3% and 22.1% at the University College Hospital Ibadan[20, 33] respectively. Similarly, MRSA constituted 26.9% of *S. aureus* isolated from patients at the National Hospital in Abuja.[34] The MRSA prevalence in our study was lower than the 42.7% reported among *S. aureus* isolates collected in University of Benin Teaching Hospital[35] and the 42.3% prevalence of MRSA reported in a multicentre study on *S. aureus* conducted in the South West Nigeria.[36] Also, an MRSA prevalence of 38.5% of *S. aureus* was reported at Abia State University Teaching Hospital, Aba, South East Nigeria were MRSA.[37] In contrast, lower prevalence of 13.1%, 12.5%, 8% and 15.5% were reported in Abuja, North Central[9], North East[22, 10] and in South West Nigeria.[12] These reports highlight differences in the prevalence of MRSA in different health facilities in Nigeria which may reflect differences in antibiotic use or infection control practices. Overall, the prevalence of MRSA isolates in Nigerian healthcare facilities is much lower than the 47.0% obtained in Ethiopia[38] and in other countries such as Iraq [39; 42.5%] and Saudi Arabia [40; 38%].

The MRSA isolates in this study were susceptible to vancomycin, teicoplanin, tigecycline, and linezolid. Similarly, other studies on antibiotic susceptibility of *S. aureus* to antibacterial agents in Nigeria[8, 22, 41, 34], South Africa[42], India[43, 44], Cameroon[45] and Nepal[46] all revealed 100% susceptibility of MRSA isolates to vancomycin and teicoplanin indicating that these antibiotics remain useful options for treating infections caused by *S. aureus* including infections caused by MRSA. In contrast, a high proportion (82.9%) of our isolates were resistant to ciprofloxacin. Similarly, high prevalence of ciprofloxacin resistance was reported in MRSA studied in the North Central[34, 35] and South Eastern[47] Nigeria. Furthermore, studies conducted in Uganda also observed a higher prevalence of ciprofloxacin resistance.[48] The high rate of resistance to ciprofloxacin observed in this and other studies implies that ciprofloxacin should not be used in the treatment of MRSA infections without the results of antibiotic susceptibility testing.

Our results also showed that all the MRSA isolates were resistant to trimethoprim. This is probably due to the easy availability of co-trimoxazole (trimethoprim/sulfamethoxazole) over the counter and its abuse in Nigeria. Surprisingly, trimethoprim resistance was also detected in 100% of MRSA isolates investigated in a hospital in Ethiopia[49], in 95% of MRSA isolates investigated in Pakistan[50] and in 88.2% of MRSA isolates in Uganda.[48] The high rate of resistance to trimethoprim is cause for concern, since it can no longer be used for treating infections caused by MRSA.

All 47 MRSA isolates in this study carried SCCmec types IV or V making them genotypically community associated MRSA (CA-MRSA). The MRSA isolates in this study belonged to six clonal complexes, and 11 genotypes dominated by CC8-MRSA-V detected in 28 of the 47 MRSA isolates.

The CC8-MRSA-V isolates were associated with three *spa* types t064, t008 and t2658 (ST8-MRSA-V-t064/t008/t2658), with t008 as the dominant *spa* type. The isolates were resistant to erythromycin, gentamicin, tetracycline and trimethoprim mediated by *erm(C), aacA-aphD, tet(K)* and *dfrS1*, respectively, and carried genes for enterotoxin *sea, seb, sek* and *seq*. ST8-MRSA-V isolates belonging to *spa* type, t064, and with the same resistance and virulence gene profile as the isolates in this study have previously been reported in other studies from other regions of Nigeria[8, 21], Ghana[51] and South Africa[52] indicating that the clone is widespread in African countries. In addition, ST8-MRSA-V-t064 isolates that were resistant to erythromycin and gentamicin, carried gene for *agrI*, *cap5* and enterotoxin *sea*, *seb*, *sek* and *seq* were reported in Kuwait hospitals[53] suggesting that ST8-MRSA-V-t064 is not restricted to the African continent.

We detected a rare CC8-MRSA isolate belonging to ST8-MRSA-IV, SCCfus/t008. The isolate carries a unique combination of fusidic acid resistance gene (*fusC*) in the SCC*mec* region. It was isolated from a wound specimen and was resistant to fusidic acid, erythromycin, clindamycin, trimethoprim, and ciprofloxacin. This lineage is rarely reported in studies conducted on *S. aureus*, but[53], in a study conducted in Kuwait, reported a variant of this strain harboring SCCmec V in six MRSA isolates (ST8-MRSA-V-SCCfus). These isolates were also associated with *spa* type, t008, and were resistant to fusidic acid and ciprofloxacin and harbored *sea* and *seb*.

The ST7-MRSA-V-t091 clone, detected in five isolates was the next common clone in this study. Their detection in this study is significant because, although CC7-MSSA is commonly reported, CC7-MRSA are rare[16] and were previously reported sporadically in human patients in Australia[16] and Netherlands[54] but has also recently been isolated from livestock[55] suggesting that the CC7-

MRSA clone can cause infections in humans as well as in livestock. However, ST7-MRSA-V [PVL+]-t091 isolates have recently been reported in a study conducted in Southwestern Nigeria.[56] Although our isolates lacked gene for PVL, our report together with the report of[56] signify the emergence of this clone in the human population in Nigeria.

We detected isolates belonging to the ST772-MRSA-V [PVL$^+$], also known as the Bengal Bay Clone. The Bengal Bay MRSA clone is a multi-resistant PVL-positive CA-MRSA that was initially isolated in Bangladesh and India between 2004 and 2005.[57] The six ST772-MRSA-V [PVL+] isolates in this study were associated with t657 (n=2) and t345 (n=4). Spa type, t657 is the classical *spa* type of the Bengal Bay clone. The t345 isolates probably represent a local variant since previous reports of the Bengal Bay MRSA clone in different countries have been associated with t657.[58, 16, 59, 60, 61, 21, 62, 63]

We detected ST152-MRSA-V, a common MRSA genotype, in Nigeria[8, 64], in five isolates in this study. Our ST152-MRSA-V isolates were associated with two *spa* types, t355 [PVL$^+$], the classical *spa* type in the clone[8, 64] and t4690 representing a new variant in Nigeria. Significantly, the ST152-MRSA-V lacked genes for staphylococcal enterotoxins, a characteristic reported previously in PVL-positive ST152-MRSA-V isolates in Kuwait hospitals.[53]

The two CC22-MRSA-IV MRSA isolates in this study were positive for *tst1*, a characteristic of UK-EMRSA-15/Middle Eastern variant clone.[65, 66, 67] The ST22-MRSA-IV-[tst1$^+$] clone which is dominant in the Middle East is usually associated with *spa* type t223.[65, 66, 67] In contrast, the two isolates in this study had t032, the *spa* type usually associated with the UK EMRSA-15/Barnim MRSA.[68, 67, 16] This suggests that our isolates represent the UK EMRSA-15/ Barnim MRSA that has recently acquired genes encoding toxic shock syndrome toxin (*tst1*).

We detected a single CC88-MRSA-IV isolate that was associated with spa type t786 and was resistant to trimethoprim and was positive for *agrIII*, *cap8* and enterotoxin D (*sed*) genes as was previously reported in Nigeria.[20, 21, 69] Our isolates lacked genes for exfoliative toxin A that was identified in isolates recovered from pediatric patients and healthy children in Japan[70], and PVL that was found in ST88-MRSA-IV isolates in UK[71] and from burn patients in Iran.[72]

A weakness of this study is that, although the isolates were obtained from three tertiary hospitals in Jos, North Central Nigeria, the number studied was small and may not reflect the situation throughout the region. Nevertheless, the study has provided an initial data on the molecular characteristics of MRSA obtained in Jos hospitals that was not previously available. This study will serve as platform for further studies.

CONCLUSION

The study has documented the prevalence of MRSA in *S. aureus* isolated in tertiary hospitals in Jos and provided data on the clonal distribution and their antibiotic resistance and virulence gene profiles. Most of the isolates belonged to ST8-MRSA-V-t008/t064 previously isolated in other regions in Nigeria. Other clones including the Bengal Bay MRSA clone and CC22-MRSA-IV-[tst1$^+$] were also detected. The study has provided an initial data on the molecular characteristics of MRSA obtained in Jos hospitals that will serve as a platform for further studies.

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Disclosure of conflict of interest

We have no conflict of interest to disclose

REFERENCES

1. Tong SY, Davis JS, Eichenberger E, Holland TL, Fowler VG Jr. *Staphylococcus aureus* infections: epidemiology, pathophysiology, clinical manifestations, and management. Clinical Microbiology Reviews, 2015; 28(3): 603-61. Doi: 10.1128/CMR.00134-14. PMID: 26016486; PMCID: PMC4451395.
2. Udo EE, Boswihi SS. Antibiotic Resistance Trends in Methicillin-Resistant *Staphylococcus aureus* Isolated in Kuwait Hospitals: 2011–2015. Medical Principles and Practice, 2017; 1-6, DOI: 10.1159/000481944.
3. Jevons MP. Clebenin-resistant *Staphylococci.* British Medical Journal, 1961; 1(5219): 124-125. https://www.ncbi.nlm.nih.gov.
4. Sit PS, Teh CSJ, Idris N, Sam C, Omar SFS, Sulaiman H, Thong KL, Kamarulzaman A, Ponnampalavanar S. Prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) infection and the molecular characteristics of MRSA bacteraemia over a two-year period in a tertiary teaching hospital in Malaysia. *BMC Infectious*

Diseases, 2017; 17(1): 1-14. Doi: 10.1186/s12879-017-2384-y.
5. Lakhundi S, Zhang K. Methicillin resistant *Staphylococcus aureus*: molecular characterization evolution and epidemiology. Clinical Microbiology Reviews, 2018; 31(4): 1-103. Doi: 10.1128/CMR.00020-18.
6. Wu M, Tong X, Liu S, Wang D, Wang L, Fan H. Prevalence of methicillin resistant *Staphylococcus aureus* in healthy Chinese population: a system review and meta-analysis. *PLoS One*, 2019; 14(10): e0223599, 1-22. Doi:10.1371/journal.pone.0223599.
7. Ikeh EI. Methicillin Resistant *Staphylococcus aureus* (MRSA) at Jos University Teaching Hospital. African Journal of Clinical and Experimental Microbiology, 2003; 4(1): 52-55. Doi: 10.4314/ajcem.v4i1.7324
8. Shittu AO, Okon K, Adesida S, Oyedara O, Witte W, Strommenger B, Layer F, Nubel U. Antibiotic resistance and molecular epidemiology of *Staphylococcus aureus* in Nigeria. BioMed Central Microbiology, 2011; 11(92): 5-12. Doi:10.1186/1471-2180-11-92.
9. Akanbi BO, Mbe J.U. Occurrence of methicillin and vancomycin resistant *Staphylococcus aureus* in University of Abuja Teaching Hospital, Abuja, Nigeria. African Journal of Clinical and Experimental Microbiology, 2012; 14(1): 1-8. https://agris.fao.org/agris.
10. Okon KO, Shittu AO, Kudi AA, Umar H, Becker K, Schaumburg F. Population dynamics of *Staphylococcus aureus* from Northeastern Nigeria in 2007 and 2012. Epidemiology and Infection, 2014; 142(8): 1737–1740. Doi:10.1017/S0950268813003117.
11. Okwu MU, Okorie TG, Mitsan O, Osakue EO. Prevalence and comparison of three methods for detection of methicillin-resistant *Staphylococcus aureus* (MRSA) isolates in tertiary health institutions in Nigeria. Canadian Open Biological Sciences Journal, 2014; 1(1): 1-12. Available online at http://crpub.com/Journals.php.
12. Ayepola OO, Olasupo NA, Egwari LO, Becker K, Schaumburg F. Molecular characterization and antimicrobial susceptibility of *Staphylococcus aureus* isolates from clinical infection and asymptomatic carriers in Southwest Nigeria. PLoS One, 2015; 10(9): 1-8. Doi:10.1371/journal.pone.0137531.
13. Udo EE, Pearman J W, Grubb W. B. Genetic analysis of community isolates of methicillin-resistant *Staphylococcus aureus* in Western Australia. Journal of Hospital Infection, 1993; 25(2): 97-108. Doi:10.1016/0195-6701(93)90100-e
14. Udo EE. Community-acquired methicillin resistant *Staphylococcus aureus*: the new face of an old foe? Medical. Principles and Practice, 2013; 22(1): 20-29. Doi:10.1159/000354201.
15. ALFouzan W, Boswihi SS, Dhar R, Udo E. Evaluating the antibacterial activity of ceftaroline against clinical isolates of methicillin-susceptible and- resistant *Staphylococcus aureus* in Kuwait hospitals. Journal of Infection Public Health, 2020; (10): 1589-1591. Doi: 10.1016/j.jiph.2020.07.018. Epub 2020 Aug 26. PMID: 32859552.
16. Monecke S, Coombs G, Shore AC, Coleman DC, Akpaka P, Borg M, Chow H, Ip M, Jatzwauk L, Jonas D, Kadlec K, Kearns A, Laurent F, O'Brien FG, Pearson J, Ruppelt A, Schwarz S, Scicluna E, Slickers P, Tan H, Weber S, Ehricht R. A Field Guide to Pandemic, Epidemic and Sporadic Clones of Methicillin-Resistant *Staphylococcus aureus*. PLoS One, 2011; 6(4): 1-24. Doi: 10.1371/journal.pone.0017936.
17. Strommenger B, Kehrenberg C, Kettlitz C, Cuny C, Verspohl J, Witte W, Schwarz S. Molecular characterization of methicillin-resistant *Staphylococcus aureus* strains from pet animals and their relationship to human isolates. Journal of Antimicrobial Chemotherapy, 2006; 57(3): 461-465. Doi:10.1093/jac/dki471.
18. Ito T, Kuwahara-Arai K, Katayama Y, Uehara Y, Han X, Kondo Y, Hiramatsu K. Staphylococcal Cassette Chromosome mec (SCC mec) Analysis of MRSA. Methods in Molecular Biology, 2014; 1085: 131-48. Doi: 10.1007/978-1-62703-664-1-8.
19. Chambers HF, Deleo FR. Waves of resistance: Staphylococcus aureus in the antibiotic era. Nature Reviews Microbiology, 2009; 7(9): 629 – 641. 2009. DOI: 10.1038/nrmicro2200.
20. Ghebremedhin B, Olugbosi MO, Raji AM, Layer F, Bakare RA, Konig B, Konig W. Emergence of a Community-Associated Methicillin-Resistant *Staphylococcus aureus* Strain with a Unique Resistance Profile in Southwest Nigeria. Journal of Clinical Microbiology, 2009; 47(9): 2975-2980. Doi:10.1128/JCM.00648-09.
21. Raji A, Ojemhen O, Umejiburu U, Ogunleye A, Blanc DS, Basset P. High genetic diversity of *Staphylococcus aureus* in a tertiary care hospital in Southwest Nigeria. Diagnostic Microbiology and Infectious Disease, 2013; 77(4): 367-369. Doi.org/10.1016/j.diagmicrobio.2013.08.030
22. Okon K, Uba ABP, Oyawoye OM, Yusuf IZ, Shittu AO, Blanc DLJ. Epidemiology and characteristic pattern of methicillin-resistant *Staphylococcus aureus* recovered from tertiary hospitals in Northeastern Nigeria. International Journal of Tropical Medicine, 2011; 6(5): 106-12. http://www.medwelljournals.com.
23. Nwankwo EOK, Abdulhadi S, Magagi A, Ihesiulor G. Methicillin Resistant *S. aureus* (MRSA) and their Antibiotic Sensitivity Pattern in Kano, Nigeria. African Journal of Clinical and Experimental Microbiology, 2010; 11(1): 129-136. Doi:10.4314/ajcem.v11i1.44088
24. Clinical and Laboratory Standards Institute Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Fourth Informational

Supplement. Wayne, PA, USA: CLSI; 2017. M100-S24.
25. Zhang K, McClure J, Elsayed S, Louie T, Conly JM. Novel Multiplex PCR Assay for Characterization and Concomitant Subtyping of Staphylococcal Cassette Chromosome *mec* Types I to V in Methicillin-Resistant *Staphylococcus aureus*. Journal of Clinical Microbiology, 2005; 43(10): 5026–5033. Doi:10.1128/JCM.43.10.5026-5033.2005.
26. Skov R, Frimodt-Moller N, Espersen F. Tentative interpretative zone diameters for fusidic acid Neosensitabs on Mueller Hinton agar and three blood containing media. International Journal of Antimicrobial agents, 2003; 22(5): 502-507. Doi: 10.1016/s0924-8579(03)00123-7.
27. Townsend DE, Ashdown N, Greed LC, Grubb WB. (1984). Transposition of gentamicin resistance to Staphylococcal plasmids encoding resistance to cationic agents. Journal of Antimicrobial and Chemotherapy, 1984; 14(2): 115-124. Doi:10.1093/jac/14.2.115.
28. Udo EE, Farook VS, Mokaddas EM, Jacob LE, Sanyal SC. Molecular fingerprinting of mupirocin-resistant *Staphylococcus aureus* from a burn unit. International Journal of Infectious Diseases, 1999; 3(2): 82-87. Doi:10.1016/s1201-9712(99)90014-0.
29. Harmsen D, Claus H, Witte W, Rothganger J, Turnwald D, Vogel U. Typing of methicillin-resistant *Staphylococcus aureus* in a university hospital setting by using novel software for spa repeat determination and database management. Journal of Clinical Microbiology, 2003; 41(12): 5442-5448. Doi: 10.1128/JCM.41.12.5442-5448.2003.
30. Enright MC, Day NPJ, Davies CE, Peacock SJ, Spratt BG. Multilocus sequence typing for characterization of methicillin-Resistant and methicillin susceptible clones of *Staphylococcus aureus.* Journal of Clinical Microbiology, 2000; 38(3): 1008-1015. Doi:10.1128/JCM.38.3.1008-1015.2000
31. Monecke S, Slickers P, Ehricht R. Assignment of Staphylococcus aureus isolates to clonal complexes based on microarray analysis and pattern recognition. Federation of the European Biochemical Societies Immunology and Medical Microbiology, 2008; 53(2): 237-251. Doi:10.1111/j.1469-0691.2008.01986.x.
32. Monecke S, Jatzwauk L, Muller E, Nitschke H, Pfohl K, Slickers P, et al. Diversity of SCCmec elements in staphylococcus aureus as observed in south-eastern Germany. PLoS One. 2016. https://doi.org/10. 1371/journal.pone.0162654 PMID: 27648947.
33. Alli OT, Ogbolu DO, Akorede E, Onemu OM, Okanlawon BM. Distribution of mecA gene amongst *Staphylococcus aureus* isolates from Southwestern Nigeria. African Journal Biomedical Research, 2011; 14(1): 9-16. https://www.ajol.info/index.php/ajbr.
34. Abdullahi N, Iregbu KC. Methicillin-Resistant *Staphylococcus aureus* in a Central Nigeria Tertiary Hospital. Annals of Tropical Pathology, 2018; 9: 6‑10. https://www.atpjournal.org/text.asp?2018/9/1/6/234154.
35. Yusuf E, Airauhi L. Prevalence and pattern of methicillin resistant *Staphylococcus aureus* in a tertiary healthcare facility in Nigeria. Medical Journal of Zambia, 2015; 42(1): 7-11. https://www.ajol.info/index.php.
36. Alli OA, Ogbolu DO, Shittu AO, Okorie AN, Akinola JO, Daniel JB. (2015). Association of virulence genes with mecA gene in *Staphylococcus aureus* from Tertiary Hospitals in Nigeria. Indian Journal of Pathology and Microbiology, 2015; 58(4): 464-467. Doi:10.4103/0377-4929.168875
37. Nsofor CA, Nwokenkwo VN, Ohale CU. Prevalence and Antibiotic Susceptibility Pattern of *Staphylococcus aureus* Isolated from Various Clinical Specimens in South East Nigeria. MedCrave Online Journal of Cell Science and Report, 2016; 3(2): 60-63. Doi:10.15406/mojcsr.2016.03.00054.
38. Deyno S, Fekadu S, Astatkie A. (2017). "Resistance of *Staphylococcus aureus* to antimicrobial agents in Ethiopia: a meta-analysis," Antimicrobial Resistance and Infection Control, 2017; 6(1): 85-91. Doi:10.1186/s13756-017-0243-7.
39. Pirko EY, Tektook NK, Saleh MMS, Jaffar ZA. Prevalence of methicillin resistance Staphylococcus aureus (MRSA) and methicillin sensitivity *Staphylococcus aureus* (MSSA) among hospitalized Iraqi patients. Biomedical Research, 2019; 30(4): 1-5. http://www.biomedres.info.
40. Adam KM, Abomughaid MM. Prevalence of Methicillin-resistant *Staphylococcus aureus* in Saudi Arabia Revisited: A Meta-analysis. The Open Public Health Journal, 2018; 11: 584-591. Doi:10.2174/1874944501811010584.
41. Okon KO, Shittu AO, Usman H, Adamu N, Balogun ST, Adesina OO. Epidemiology and Antibiotic Susceptibility Pattern of Methicillin-Resistant *Staphylococcus aureus* Recovered from Tertiary Hospitals in Northeastern, Nigeria. Journal of Medicine and Medical Sciences, 2013; 4(5): 214-220. http://www.interesjournals.org/JMMS.
42. Marais E, Aithma N, Perovic O, Oosthuysen WF, Musenge E, Duse AG. Antimicrobial susceptibility of methicillin-resistant *Staphylococcus aureus* isolates from South Africa. South African Medical Journal, 2009; 99(3): 170-173. http://www.samj.org.za/index.php/samj.
43. Lyall KS, Gupta V, Chhina D. (2013). Inducible clindamycin resistance among clinical isolates of *Staphylococcus aureus*. Journal of Mahatma Gandhi Institute of Medical Sciences, 2013; 18(2): 112-115. Doi: 10.4103/0971-9903.117799.

44. Senthamarai S, Sivasankari S, Anitha C, Somasunder VM, Akila K, Shuba VJ, Siji Mol , S, Muthulakshmi K. Prevalence of methicillin resistant of *Staphylococcus aureus* (MRSA) its antibiotic susceptibility pattern from various clinical specimens in a tertiary care hospital. Journal of Medical Sciences and Clinical Research, 2019; 7(2): 129-132. Doi:10.18535/jmscr/v7i2.27.
45. Njoungang LL, Nwobegahay JM, Ayangma CR, Njukeng AP, Kengne M, Abeng EM, Mama EA, Tchouamo M, Goon DT. Prevalence and antibiotic resistance patterns of strains of *Staphylococcus aureus* isolated at the Yaounde Military Hospital, Cameroon. Microbiology Research International, 2015; 3(4): 56-63. http://www.netjournals.org/pdf/MRI/2015/4/15-021.pdf.
46. Bhatt CP, Karki BMS, Baral B, Gautam, Shah A, Chaudhary A. (2014). Antibiotic susceptibility pattern of *Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus* in a tertiary care hospital. Journal of Pathology of Nepal, 2014; 4(7): 548-551. Doi.org/10.3126/jpn.v4i7.10297.
47. Ugbogu OC, Nwaugo VO, Orji A, Ihuoma, N. Quinolone Resistant *Staphylococcus aureus* in Okigwe, Imo State Nigeria. Journal of Biological Sciences, 2007; 7(4): 697-700. Doi:10.3923/jbs.2007.697.700.
48. Ojulong J, Mwambu TP, Jolob M, Bwanga F, Kaddu-Mulindwa DH. Relative prevalence of methicillin resistant *Staphylococcus aureus* and its susceptibility pattern in Mulago Hospital, Kampala, Uganda. Tanzania Journal of Health Research, 2009; 11(3): 149-153. http://www.scholarsresearchlibrary.com.
49. Dilnessa T, Bitew A. Antimicrobial susceptibility pattern of methicillin resistant *Staphylococcus aureus* isolated from clinical samples at Yekatit 12 hospital medical college, Addis Ababa, Ethiopia. BioMed Central Infectious Diseases, 2016; 16(398): 1-9. Doi:10.1186/s12879-016-1742-5.
50. Akhter R, Khan KMA, Hasan, F. Isolation and antimicrobial susceptibility pattern of methicillin-resistant and methicillin sensitive *Staphylococcus aureus*. Journal of Surgery Pakistan (International), 2009; 14(4): 161-164. Doi:10.11648/j.ajcem.20160401.12.
51. Egyir B, Guardabassi L, Nielsen SS., Larsen, J, Addo KK, Newman MJ, Larsen, AR. Prevalence of nasal carriage and diversity of *Staphylococcus aureus* among inpatients and hospital staff at Korle Bu Teaching Hospital, Ghana. Elsevier. Journal of Global Antimicrobial Resistance, 2013; 1(4): 189-193. URL http://ugspace.ug.edu.gh/handle/123456789/26130.
52. Oosthuysen WF, Orth H, Lombard CJ, Sinha B, Wasserman E. Population structure analyses of *Staphylococcus aureus* at Tygerberg Hospital, South Africa, reveals a diverse population high prevalence of Panton-Valentine leukocidin genes and unique local MRSA clones. Clinical Microbiology and Infection, 2014; 20(7): 652–659. Doi:10.1111/1469-0691.12452.
53. Boswihi SS, Udo EE, Monecke S, Mathew B, Noronha B, Verghese T, Tappa SB. Emerging variants of methicillin-resistant *Staphylococcus aureus* genotypes in Kuwait hospitals. PLoS One., 2018; 13(4): 1-13. Doi:10.1371/journal.pone.0195933.
54. Donker GA, Deurenberg RH, Driessen C, Sebastian S, Nys S, Stobberingh EE. The population structure of *Staphylococcus aureus* among general practice patients from The Netherlands. Clinical Microbiology and Infection, 2009; 15: 137-143. Doi:10.1111/j.1469-0691.2008.02662.x.
55. Li SM, Zhou YF, Li L, Fang LX, Duan JH, Liu FR, Liang HQ, Wu YT, Gu WQ, Liao XP, Sun J, Xion YQ, Liu YH. (2018) Characterization of the Multi-Drug Resistance Gene cfr in Methicillin-Resistant *Staphylococcus aureus* (MRSA) Strains Isolated From Animals and Humans in China. Frontiers in Microbiology, 2018; 9(2925): 1-10. Doi: 10.3389/fmicb.2018.02925.
56. Akinduti AP, Osiyemi JA, Banjo TT, Ejilude O, El-Ashker M, Adeyemi AG, Obafemi YD, Isibor PO. Clonal diversity and spatial dissemination of multi-antibiotics resistant *Staphylococcus aureus* pathotypes in Southwest Nigeria. PLoS One, 2021 Feb 23; 16(2): e0247013. doi: 10.1371/journal.pone.0247013. PMID: 33621256; PMCID: PMC7901740.
57. Monecke S, Baier V, Coombs G, Slickers P, Ziegler A, Ehricht R. Genome sequencing and molecular characterization of *Staphylococcus aureus* ST772-MRSA-V, "Bengal Bay Clone". BioMed Central Research Notes, 2013; 6(548): 1-7. Doi:10.1186/1756-0500-6-548.
58. D'Souza N, Rodrigues C, Mehta A. (2010). Molecular characterization of methicillin-resistant *Staphylococcus aureus* with emergence of epidemic clones of sequence type (ST) 22 and ST 772 in Mumbai, India. Journal of Clinical Microbiology, 2010; 48(5): 1806-1811. Doi: 10.1128/JCM.01867-09.
59. Brennan GI, Shore AC, Corcoran S, Tecklenborg S, Coleman DC, O'Connell B. Emergence of hospital- and community-associated Panton-Valentine leukocidin-positive methicillin-resistant *Staphylococcus aureus* genotype ST772-MRSA-V in Ireland and detailed investigation of an ST772-MRSA-V cluster in a neonatal intensive care unit. Journal of Clinical Microbiology, 2012; 50(3): 841-847. Doi:10.1128/jcm.06354-11.
60. Monecke S, Skakni L, Hasan R, Ruppelt A, Ghazal SS, Hakawi A, Slickers P, Ehricht R. Characterization of MRSA strains isolated from patients in a hospital in Riyadh, Kingdom of Saudi Arabia. BioMed Central Microbiology, 2012a; 12(1): 146-510. Doi:10.1186%2F1471-2180-12-146.

61. Shambat S, Nadig S, Prabhakara S, Bes M, Etienne J, Arakere G. Clonal complexes and virulence factors of *Staphylococcus aureus* from several cities in India. BioMed Central Microbiology, 2012; 12(64): 1-9. Doi:10.1186/1471-2180-12-64.
62. Pokhrel RH, Aung MS, Thapa B, Chaudhary R, Mishra SK, Kawaguchiya M, Urushibara N, Kobayashi N. Detection of ST772 Panton-Valentine leukocidin-positive methicillin-resistant *Staphylococcus aureus* (Bengal Bay clone) and ST22 S. aureus isolates with a genetic variant of elastin binding protein in Nepal. New Microbes and New Infection, 2016; 11: 20–27. Doi:10.1016/j.nmni.2016.02.001.
63. Boswihi SS, Udo EE, Al-Sweih N. Shifts in the Clonal Distribution of Methicillin Resistant *Staphylococcus aureus* in Kuwait Hospitals: 1992-2010. PLoS One, 2016; 11(9): 1-21. Doi.org/10.1371/journal.pone.0162744.
64. Obasuyi O, McClure J, Oronsaye FE, Akerele JO, Conly J, Zhang K. Molecular Characterization and Pathogenicity of *Staphylococcus aureus* Isolated from Benin-City, Nigeria. Microorganisms, 2020; 8(912): 1-19. Doi:10.3390/microorganisms8060912
65. Biber A, Abuelaish I, Rahav G, Raz M, Cohen L, Valinsky L, et al. PICR Study Group A typical hospital-acquired methicillin-resistant Staphylococcus aureus clone is widespread in the community in the Gaza strip. PLoS One, 2012; 7(8): e42864.
66. Al Laham N, Mediavilla J, Chen L, Abdelateef N, Abu Elamreen F, Ginocchio CC, Pierard D, Becker K, Kreiswirth B.N. MRSA Clonal Complex 22 Strains Harboring Toxic Shock Syndrome Toxin (TSST-1) Are Endemic in the Primary Hospital in Gaza, Palestine. PLoS One, 2015; 10(3): 1-17. Doi.org/10.1371/journal.pone.0120008.
67. Udo EE, Boswihi SS, Al-Sweih N. High prevalence of toxic shock syndrome toxin–producing epidemic methicillin-resistant *Staphylococcus aureus* 15 (EMRSA-15) strains in Kuwait hospitals. New Microbes and New Infections, 2016; 12: 24-30. Doi:10.1016/j.nmni.2016.03.008.
68. Ghebremedhin B, Konig W, Witte W, Hardy KJ, Hawkey PM, Konig B. Subtyping of ST22-MRSA-IV (Barnim epidemic MRSA strain) at a university clinic in Germany from 2002 to 2005. Journal of Medical Microbiology, 2007; 56(3): 365-375. Doi: 10.1099/jmm.0.46883-0. PMID: 17314368
69. Shittu A, Oyedera O, Abegunrin F, Okon K, Raji A, Taiwo S, Ogunsola F, Onyedibe K, Elisha G. Characterization of methicillin susceptible and resistant Staphylococci in the clinical setting: a multicenter study in Nigeria. BioMed Central Infectious Diseases, 2012b; 12(286): 1-10. https://bmcinfectdis.biomedcentral.com.
70. Ozaki K, Takano M, Higuchi W, Takano T, Yabe S, Nitahara Y, Nishiyama A, Yamamoto T. Genotypes, intra-familial transmission, and virulence potential of nasal methicillin-resistant *Staphylococcus aureus* from children in the community. Journal of Infection and Chemotherapy, 2009; 15(2): 84-91. Doi:10.1007/s10156-009-0668-X.
71. Monecke S, Kuhnert P, Hotzel H, Slickers P, Ehricht R. Microarray based study on virulence-associated genes and resistance determinants of *Staphylococcus aureus* isolates from cattle. Veterinary Microbiology, 2007; 125(1-2): 128-140. Doi:10.1016/j.vetmic.2007.05.016.
72. Ghaznavi-Rad E, Ekrami A, (2018). Molecular Characterization of Methicillin Resistant *Staphylococcus aureus* Isolates, Isolated from a Burn Hospital in Southwest Iran in 2006 and 2014. International Journal of Microbiology, 1423939, 1-5. Doi:10.1155/2018/1423939