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Research Article

The Distribution of Acme Gene and MRSA Related Virulence Genes in *Staphylococcus Aureus* Strains from Nigeria

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Abstract

Background: Arginine Catabolic Mobile Element (acme) is a Staphylococcal genomic island that enhances fitness and ability of bacteria cells to colonize on mucous membrane and skin. It is stongly associated with the epidemic and virulent S. aureus USA 300. This study determined the distribution of acme and Methicillin Resistant *Staphyllococus Aureus* (MRSA)-related genes in *S. aureus* isolated from patients in five tertiary hospitals in Nigeria.

Methods: A total of 51 S. aureus isolates from the clinical specimens submitted to laboratories in five tertiary hospitals in Nigeria were used in this study. Phenotypic and genotypic identifications of the S. aureus were performed. Antibiotic Susceptibility Test (AST) was carried out to determine the susceptibility pattern of the isolates using various antibiotics discs. Minimum Inhibitory Concentration (MIC) was used to determine the degree of resistance of the isolates to methicillin and vancomycin. Polymerase Chain Reaction (PCR) was used to screen for the presence of mecA, acme sae, sarA, PVL, α-psm and norB genes using specific primers. The SCCmec type was determined for all the MRSA isolates using polymerase chain reaction.

Results: The MIC for mecA negative strains was ≤4 µg/ml, while the MIC for mecA positive was 8 µg/ml. mecA gene was detected in 35 (68.6%) of 51 strains of *S. aureus*. The prevalence of sae, sarA, mecA, acme, PVL, α-psm and norB gene were estimated to be 70.6%, 68.6%, 68.6%, 29.4%, 5.1%, 39.2% and 33.3% respectively. There was an association between the distribution of mecA+ and norB+ strains (P = 0.034) and the hospitals (P =0.008) where the isolates were obtained from, whereas there was no association between acme positive strains and the hospitals (P = 0.669) from which the isolates were obtained from. Also, there was no association between mecA, norB, acme with the sex, age and hospital admission status (P>0.05). Furthermore, there was an association between PVL gene and the two geographical regions (South-Western and North-Western, Nigeria) ($\chi 2 = 7.77$; $p < 0.05$). The SCCmec typing showed that 21 (60.0%) out of the 35 MRSA used in this study carried the SCCmec type elements such as type I, II, III and VIII which were all the characteristic of HA-MRSA while the remaining 14 (40%) carried the SCCmec type elements such as type IV, V, VI and VII which were all characteristic of CA-MRSA. Thus the overall prevalence of HA-MRSA and CA-MRSA in this study was 60.0% and 40.0%, respectively.

Conclusion: The prevalence of acme and α-psm genes in *S. aureus* are high and this is a novel discovery in Nigeria which has opened a new era in the transmission and fitness of circulating MRSA in causing infection in the community at large. The study concluded that there is high prevalence of HA-MRSA in South-Western, Nigeria and high prevalence of CA-MRSA in the North-Western, Nigeria.

Keywords: Acme; Norb; Meca; *Staphylococcus Aureus*; Genome; Methicillin Resistance

Introduction

Staphylococcus aureus has been well documented as one of the most important human opportunistic pathogens in the hospital and community at large [1]. Methicillin resistant *S. aureus*, which is a sub-group of *S. aureus* has gained much publicity for over 3 decades because of the multi-resistant nature of the organism to the frequently used antibiotics such as tetracycline, macrolide group of antibiotics and penicillinase resistant penicillin such as cephalosporins making the infection caused by this organism difficult to treat. In addition to this, MRSA is known to be a nosocomial pathogen, which in the last few years has shown propensity to spread in the community; a situation that makes the control of the infection very difficult. The ability of *S. aureus* to cause various infections has been linked to various virulence genes located on the plasmid or bacterial genome. There are about 40 virulence genes/factors that are known to be involved in almost all processes from colonization of the host to nutrition and dissemination [2].

The most remarkable feature of MRSA is the discovery in recent times that there are some virulence genes that have been found to be associated with this particular clone [3]. For example, the Panton-Valentine Leukocidin (PVL) otherwise known as lukF-PV/lukS-PV gene (a virulence gene) has been associated with community acquired MRSA (CA-MRSA) in many parts of the world [4]. A number of investigations have provided evidence that the prevalence of the PVL gene is high among methicillin-susceptible *S. aureus* (MSSA) in Nigeria and data emerging from our laboratory and other laboratories have seen the increase in prevalence of PVL positive MRSA in recent times [5-8].

Arginine Catabolic Mobile Element (ACME) gene, a gene that is thought to play important role in growth and survival of sub-clone of Community acquired MRSA (CA-MRSA) by aiding the spread has been well studied in various countries [9]. However, there is paucity of data on the prevalence of acme gene in *S. aureus* isolates from Nigeria. Therefore this study was aimed to determine the prevalence of acme and selected virulence / regulatory genes (α-psm, norB, plc, sae, sarA and PVL) in *S. aureus* isolates from hospitals in South Western and North Western Nigeria [10].

Methodology

Bacterial Strains

A total of 51 non-duplicate *S. aureus* isolates obtained from clinical specimens submitted to four diagnostic laboratories of tertiary health care institutions in Nigeria including Zamfara State in the North-Western Nigeria, Oyo and Osun States in the South Western Nigeria for routine laboratory diagnosis between June and August, 2014 were investigated in a cross-sectional / observational study. Demographic data such as sex, age and site of isolation of specimen were obtained from the patients' laboratory request forms forwarded to the diagnostic microbiology laboratories. Confirmation as *S. aureus* was based on the identification profile of the API 20 Staph kit (Biomerieux, France) and tube coagulase test. All the isolates were stored in glycerol broth at -20ºC until ready for use. Ethical approval was sought and obtained from the Ethical Research Committee of LAUTECH Teaching Hospitals, Osogbo, Nigeria prior to the commencement of the study. All the organisms analyzed in this study were culture collections from various hospitals with adequate histories and no active solicitation of clinical samples from patients was involved. This study had no bearing on clinical diagnosis and treatment of patients.

Antibiotic Susceptibility Testing

The isolates were sub-cultured onto mannitol salt agar and incubated at 35°C for 24 h to ensure that the isolates were pure isolates of *S. aureus*. The antimicrobial disc diffusion susceptibility testing was performed on MH agar. The following antibiotics: penicillin G (10 units), cefoxitin (30 μg), erythromycin (15 μg), tetracycline (30 μg), gentamicin (10 μg), fusidic acid (10 μg) and trimethoprim-sulfamethaxazole (1.25/23.75 μg) were used to determine the susceptibility pattern of the isolates to antibiotics according to the guidelines of CLSI (10).

DNA Extraction

The genomic DNA of the *S. aureus* isolates was extracted by boiling method [11]. This was done by picking about 20 colonies suspended in 500 μl of sterile distilled water at 100oC for 15 min. The DNA lysate was stored at -20ºC for the duration of this work.

Polymerase Chain Reaction for Detection of the Meca and Other Virulence Genes

Polymerase chain reaction (PCR) for detection of the mecA gene was carried out on all the isolates as previously described using PCR kit from New England Biolab (NEB, USA). Sequencing of the PCR products and BLAST analysis were performed on representative positive isolates to confirm the identity of the mecA gene [12]. In this study, MRSA would be referred to as mecA positive, while MSSA would be regarded as mecA negative. PCR was also carried out to detect six different virulence genes: PVL, norB, α-psm, sae, sarA and acme genes using the primers and cycling parameters listed in Table 1. The PCR was carried out for each gene singly.

SCCmec Typing

SCCmec typing was performed on the mecA positive isolates as described previously using the sets of primers as shown in Table 1 [13]. Each primer had a concentration of 1μm while the Taq mix made up of the following: 10 mM of $MgCl_2$, 0.2 mM of dNTP mix and 1 U of Taq polymerase (NEB, USA).

RAPD Typing of S. aureus Isolates

A total of 26 *S. aureus* isolates were randomly selected for the experiment. Genomic DNA was extracted using Quiagen DNA extraction kit. Random Amplified DNA Polymorphic (RAPD) PCR assay was carried in a reaction of 25ul reaction mixture containing 0.5ul each primers (GCGATTGATGGTGATACGGTT, AGCCAAGCCTTGACGAACTAA). Amplified products were separated by electrophoresis in a 1.5% agarose gel stained with ethidium-bromide (0.5mg/ml) and photographed with Sygene UV imaging illuminated system. A 100 bp DNA marker (Promega) was used as a DNA molecular size standard. Genetic relationships were established by scoring the presence or absence of each RAPD polymorphic band, a dendrogram was constructed by using the gel picture results of the RAPD assay performed.

Statistical Analysis

Data were analyzed using statistical packages within the Microsoft Excel and Epi-info software from Centre for Disease Control and Prevention, USA. The Chi-square was used to determine if there was association between various socio demographic data (sex, age, hospital and specimen type/clinical diagnosis) and virulence and mecA genes. Analysis of Variance (ANOVA) and Student's t-test were calculated for selected data using Microsoft Excel software. The $p < 0.05$ was considered to be statistically significant.

Gene	Role	Primer Sequence 5′→ 3′	Bp	Cycling Parameters	References
Nuc	Thermonuclease	GCGATTGATGGTGATACGGTT AGCCAAGCCTTGACGAACTAAAGC	280	94°C–30 s; 55°C–30 s; 72°C–1 min; 40 cycles	Brakstad et al., 1992
mecA	Methicillin resistance	AGT TCT GCA GTA CCG GAT TG AAA ATC GAT GGT AAG GTT CGC	533	94°C–30 s; 55°C–30 s; 72°C–1 min; 40 cycles	Murakami et al., 1993
PVL	Leukocidin	ATC ATT AGG TAA TAA AAT GTC TGG ACA TGA TCC A GCA TCA AAT GTA TTG GAT AGC AAA AGC	433	94°C–30 s; 55°C–30 s; 72°C–1 min; 35 cycles	Lina et al., 1999
α-psm	Phenol soluble modulin	ACAGTTAGGCAGTATTTCCCG ACAAAGCAAAGCCACCATC	617	94°C–30 s; 57°C–30 s; 72°C–1 min; 35 cycles	This study
norB	Nitrate oxido reductase	ACAGCAGAGGCATTACATCA TTCCCGTTCCGACTCATAC	560	94°C–30 s; 54°C–30 s; 72°C–1 min; 35 cycles	This study
Acme	Arginine catabolic mobile element	CGATATCATCTATACCTAGTACG GAAAATCCTCAAGTAAGAAGTG	205	94°C–30 s; 52°C–30 s; 72°C–1 min; 35 cycles	This study
Sae	Accessory element	GTCCAAGGGAACTCGT ACATTCACGGTATTAGCATC	273	95°C–15 s; 60°C–30 s; 72°C–1 min; 40 cycles	This study
	Accessory regulatory gene	AAAGCGTTGATTTCGGTAGTA AGTGCCATTAGTGCAAAACCT	375	94°C–30 s; 57°C–30 s; 72°C–1 min 30 s; 40 cycles	This study
Plc	Phospholipase	AGTTGCCAAAGCCGAATCTAAG	323	94°C–30 s; 57°C–30 s;	This study

	C	TACAATCGCTACGCCACCA		72°C–40 s; 35 cycles	
RAPD-PCR	Random amplification	GCGATTGATGGTGATACGGTT AGCCAAGCCTTGACGAACTAAAGC	100-800	94°C–5min; 35°C–30 s; 76°C– 45s; 35 cycles	This study

Table 1: List of primers used for detection of virulence genes, mecA gene and RAPD-PCR.

Results

Distribution of Sample Population and S. Aureus Isolates from Clinical Samples

Cultures collection made of 51 non-duplicate strains of *S. aureus* were confirmed to be *S. aureus* following standard microbiological tests and PCR amplification of thermonuclease gene (nuc gene) (Table 2). Table 3 show sex, type of specimen, admission status and hospital distributions of the isolates collected from 3 different states in Nigeria. Overall, 15 (29.4%), 19 (33.3%) and 17 (37.3%) isolates were collected from Osun, Zamfara and Oyo States, respectively. Of the total collections of *S. aureus*, 26 (51.0%) and 25 (49.0%) were from male and female individuals, respectively.

Antibiotic Susceptibility Testing Results and Minimum Inhibitory Concentration

Isolates had highest degree (100%) of antibiotic resistance for penicillin whereas low resistance of 1 (2.0%) was observed in mupirocin. The MIC result of all the *S. aureus* strains with vancomycin showed that all were sensitive to vancomycin (MIC value ≤ 2 μg/ml) as the MIC result ranged between 0.5 and 2 μg/ml. Based on the result obtained from the susceptibility pattern of the isolates to cefoxitin (Table 4), a surrogate antibiotic used to determine susceptibility/resistance of *S. aureus* to methicillin, 29 (56.9%) out of the 51 isolates were resistant to cefoxitin. Thus, MRSA prevalence based on cefoxitin result was 56.9%. Furthermore, the antibiotics susceptibility pattern of the *S. aureus* isolates with respect to the distribution of the mecA gene carriage of the isolates was examined. The MIC50 and MIC90 of mecA negative strains were 2 μg/ml and 4 μg/ml, respectively while the MIC50 and MIC90 for mecA positive strains of *S. aureus* were 64 μg/ml and 256 μg/ml, respectively.

Polymerase Chain Reaction Detection mecA Gene

The result showed that 35 (68.6%) isolates were mecA MRSA positives showing 100% correlation with the phenotypic cefoxitin susceptibility test result. Zamfara was found with highest prevalence 15 (88.2%) of mecA gene (MRSA) while Osun state had lowest prevalence of 6 (40.0%). There was a significant association between the selected states and mecA gene distribution (χ^2 = 8.971; p = 0.011). It was found that 19 (54.3%) out of the 35 mecA positive strains were from the In-patient whereas 16 (45.7%) were from the Out-patient but there was no significant association between the distribution of mecA gene and hospital admission status of the patients (χ2= 0.948; p = 0.330). Furthermore, the antibiotics susceptibility pattern of the *S. aureus* isolates with respect to the distribution of the mecA gene carriage of the isolates was examined. There was no association between the resistance pattern of either mecA positive or mecA negative strains and any of the antibiotics used (χ^2= 2.26; p = 0.22).

Detection and Distribution of Acme Gene

Using PCR amplification techniques, 16 (31.4%) of 51 isolates were acme positive. Isolates from urine samples had the highest prevalence of acme gene and there was equal distribution of the genes among sterile and non-sterile sites. Table 5 shows the distribution of acme gene in different samples and sample types. All acme positive isolates were all MRSA, thus the 16 of 35 (45.7%) MRSA were positives for acme. There was a significant association between MRSA and acme positive isolates (χ^2 = 5.24; p=0.022). Table 6 shows the relationship between MecA Positive and acme genes. Comparing acme positive strains with SCCmec typing, 11 (45.7%) of 24 of both SCCmectype 111 and SCCmectype VI were acme positive while SCCmectype 11, V, V11 and VIII did not have acme gene. Table 7 shows distribution of acme gene amongst different SCCmec types.

Detection of Other Virulence Genes

The prevalence of other virulence genes (PVL, norB, sarE, sarA, α-psm and plc) in all the 51 *S. aureus* tested is described in Table 8, Fig. 1,2. Plc gene had the highest prevalence of 82.4% (42 out of 51) while sarA had lowest prevalence of 5.9% (3 out of 51). There was an association between mecA gene and other virulence genes as shown in Table 9. Fig. 2 show the distribution of virulence genes and Table 10 shows the distribution of virulence genes in relation to hospitals. Table 11 shows the distribution of virulence genes amongst different SCCmec types. SCCmec typing results for the 51 isolates showed that SCCmectype VIII was not detected in this study Eleven (11) of the acme positive MRSA strains were SCCmec types II and V while the remaining

four were nontypeable. Based on Huang et al, (2007) which state that isolates positive for both HA-MRSA (SCCmec I-III) and CA-MRSA (SCCmec IV and V) should be taken to be HA-MRSA because SCCmec IV and V are both small and presumably motile. Thirteen (38%) out of the 35 MRSA isolates were community- acquired MRSA (CA-MRSA) while 21 (62%) were hospital-acquired MRSA (HA-MRSA). High prevalence of CA-MRSA (73.0%) was recorded for Northwestern, Nigeria, while high prevalence of HA-MRSA (75.0%) was documented for Southwestern Nigeria. Table 7,12 shows the relationship between SCCmec types and acme gene. We identified the emergence of 1, 11, 1 and 11 acme positive MRSA strains that carried the SCCmec type I, SCCmec type III, SCCmec type IV and SCCmec type VI, respectively.

Results of RAPD Typing

Twenty six (26) *S. aureus* isolates comprises of twenty (21) and five (5) isolates from south- west and northern region of Nigeria respectively were subjected to RAPD-PCR. Fig. 3 shows a gel picture of the RAPD-PCR which has many bands ranges from three (3) to eight (8) and a base sizes ranges from 100 to 800bp with mixture of a few deeper intensity band and many light intensity band. Dendrogram constructed with each gel electrophoresis gel shows that several isolates could be grouped into five (5) different profiles based on their molecular weight and degree of relatedness. The dendogram is represented by figure 5.0 and 6.0. Approximately, Profile A has the highest molecular weight of 6.0 while E has the least molecular weight of 1.0., Profile A, B, C, D and E has 7.7%, 15.4%, 15.4%, 42.3% and 19.2% respectively. From the profile, it was discovered that south-west isolates fall into all profiles whereas northern isolates fall within Profile 4 and 5 which has molecular weight of 2.0 and 1.0 respectively. In addition, all the isolates (except one) from LAUTECH Teaching Hospital, Ogbomoso which share boundary with northern part also has profile 4 with molecular weight of 2.0.

Primers	***Oligonucleotide sequence (5′→3′)***	**Amplicon size bp)**	**Specificity**	**References**
Type I-F Type I-R	GCTTTAAAGAGTGTCGTTACAGG GTTCTCTCATAGTATGACGTCC	613	SCC*mec* I	Zhang et al., 2005
Type II-F TypeII-R	CGTTGAAGATGATGAAGCG CGAAATCAATGGTTAATGGACC	398	SCC*mec* II	Zhang et al., 2005
Type III-F Type III-R	CCATATTGTGTACGATGCG CCTTAGTTGTCGTAACAGATCG	280	SCC*mec* III	Zhang et al., 2005
Type IVa-F Type Iva-R	GCCTTATTCGAAGAAACCG CTACTCTTCTGAAAAGCGTCG	776	SCC*mec* Iva	Zhang et al., 2005
Type IVb-F Type IVb-R	TCTGGAATTACTTCAGCTGC AAACAATATTGCTCTCCCTC	493	SCC*mec* IVb	Zhang et al., 2005
Type IVc-F Type IVc-R	ACAATATTTGTATTATCGGAGAGC TTGGTATGAGGTATTGCTGG	200	SCC*mec* IVc	Zhang et al., 2005
Type IVd-F Type IVd-R	CTCAAAATACGGACCCCAATACA TGCTCCAGTAATTGCTAAAG	881	SCC*mec* IVd	Zhang et al., 2005
Type V-F Type V-R	GAACATTGTTACTTAAATGAGCG TGAAAGTTGTACCCTTGACACC	325	SCC*mec* V	Zhang et al., 2005
Type VI-F	CTCAAAATACGGACCCCAATACA	881	SCC*mec* VI	Zhang et al., 2005
Type VI-R	TGCTCCAGTAATTGCTAAAG			Zhang et al., 2005
Type VII-F	CTGGAATTCGTAAAAGCCG	532	SCC*mec* VII	Zhang et al., 2005
Type VII-R	ATACTAGGGCTAGTAG			Zhang et al., 2005
Type VIII-F	TAGGGTGAAATGCCTGGT	243	SCC*mec* VIII	Zhang et al., 2005
Type VIII-R	TAGAAAGAGTTTCCCTGTGA			Zhang et al., 2005

Table 2: List of primers used for SCCmec typing of mecA +*Staphylococcus aureus* isolates.

Specimen	**UCH**	**LTHS**	**LTHO**	**OAUTH**	**FMCG**	**Total**	**Sterile**	**Non-Sterile**
Aspirate	0	1	0	2	0	3	3	0
Blood	1	1	0	0	3	5	5	0
Ear swab	2	1	1	0	2	6	0	6

Eye swab	0	O	2	0	0	2	2	0
HVS	1	O	0	0	1	2	0	2
Semen	0	O	0	0	2	2	2	0
Sputum	0	1	1	1	0	3	0	3
Urethrals	1	0	0	0	0	1	0	1
Urine	1	4	4	0	4	13	13	0
Wounds	2	3	3	1	5	14	0	14
Total	8 (15.7%)	11 (21.6%)	11(21.6%)	4 (7.84%)	17(33.3%)	51	25(49.0%)	26 (51.0%)

Table 3: The distribution of the *S. aureus* isolates among hospitals.

Sex	Male	26(51.0%)	Female	25 (49.0%)	
Adm Status	Inpatient	30 (58.8%)	Outpatient	21 (41.2%)	
State	Zamfara	Oyo		Osun	
Hospitals	FMC, Gusau	LTHOG	UCHIB	LTHOS	OAUTCH
	17 (33.3%)	11(21.6%)	8(15.7%)	11 (21.6%)	4(7.8%)
Total	17(33.3%)		19(37.3%)		15(29.4%)

Table 4: It showed sex and hospital distributions of the isolate collected from 3 different states in Nigeria.

Antibiotics	Number of Sensitive Strain	Number of Resistant Strains
Erythromycin (15 μg)	36 (70.6)	15 (29.4)
Teicoplanin (30 μg)	49 (96.1)	2 (3.9)
Clindamycin (2 μg)	35 (68.6)	16 (31.4)
Tetracycline (30 μg)	24 (47.1)	27 (52.9)
Gentamicin (30 μg)	30 (58.5)	21 (41.2)
Cefoxitin (30 μg)	22 (43.1)	29 (56.9)
Penicillin G (10 units)		51 (100%)
Linezolid (30 μg)	45 (88.2)	6 (11.8)
Sulphonamides (300 μg)	9 (17.6)	42 (82.4)
Co-trimoxazole (25 μg)	13 (25.5)	38 (74.5)
Mupirocin (200 μg)	50 (98.0)	1 (2.0)

Table 5: The antibiotic susceptibility pattern of *S. aureus* isolates used in this study.

Gene	Blood	Breast asp	Ear sw	Eye sw	HVS	Semen	Sputum	Urethral	Urine sw	Wound Swab	Sterile	Non-sterile
mecA	3/5	0/1	4/6	½	2/2	2/2	2/3	1/1	10/13	10/16	15/21	20/30
Acme	1/5	0/1	2/6	0/2	1/2	½	0/3	1/1	6/13	4/16	8/21	8/30
Psm	1/5	1/1	3/6	0/2	1/2	0/2	1/3	1/1	8/13	4/16	8/21	10/30
Pvl	5/5	1/1	3/6	0/2	2/2	2/2	2/3	1/1	10/13	9/16	18/21	17/30
norB	1/5	0/1	3/6	½	1/2	½	0/3	1/1	6/13	4/16	8/21	10/30
sarE	3/5	1/1	5/6	½	2/2	2/2	2/3	1/1	9/13	10/16	15/21	21/30
sarA	1/5	0/1	1/6	0/2	0/2	½	0/3	0/1	0/13	0/16	2/21	1/30
Plc	5/5	1/1	5/6	½	2/2	2/2	2/3	1/1	12/13	11/16	11/16	22/30
Lecithinas	3/5	1/1	5/6	2/2	1/2	½	1/3	0/1	8/13	9/16	13/21	18/31

Table 6: The distribution of the gene with different sample types.

	mecA		
Acme	**Negative**	**Positive**	**Total**
Negative	15(42.9%)	20(57.1%)	35
Positive	1(6.3%)	15(93.8%)	16
Total	16(31.4%)	35(68.6%)	51

Table 7: The relationship between mecA and acme genes.

	SCCmectype Positive	Acme -	Acme +	P-value
SCCmectype1	1	0	1	
SCCmectype11	3	3	0	
SCCmectype111	24	13	11	
SCCmectype1V	2	1	1	
SCCmectype V	0	0	0	
SCCmectypeV1	24	13	11	
SCCmectype V11	1	1	0	
SCCmectypeV111	0	0	0	

Table 8: Association of acme gene with SCCmectyping.

Gene	Prevalence	MecA +ve	MecA-ve	X^2	P value
Acme	16	16	0	5.24	0.022
Psm	20	18	2	5.44	0.0196
Pvl	35	30	5	12.7	0.0003
sarE	36	30	6	10.1	0.001
SarA	3	2	1	0.32	0.57
NorB	18	16	2	3.95	0.046
Plc	37 (72.5%)	32	5	13.7	0.071
Pbp4	31 (60.8%)	25	6	4.37	< 0.05
dfrA	8 (21.1%)	8	0	5.66	0.002
Lecithinase	31 (60.8%)	20	11		

Table 9: Association of mecA gene in relation to other virulence genes.

Hospital	mecA	acme	Psm	Pvl	norB	sarE	sarA	Type III	Type V	Type II	Type VII	Type 1	Plc	plc
LTH	11/22	5/22	7/22	10/22	3/22	13/22	0/22	9/22	9/22	2/22	1/22	0/22	14/22	14/22
OAUTHC	2/4	1/4	2/4	¾	0/4	2/4	0/4	2/4	2/4	0/4	0/4	0/4	3/4	3/4
UCH	7/8	5/8	4/8	6/8	7/8	7/8	1/8	5/8	5/8	1/8	0/8	0/8	8/8	8/8
FMC	15/17	5/17	7/17	16/17	8/17	14/17	2/17	8/17	8/17	0/17	0/17	0/17		
Total	35	16	20	35	18	36	3	24	24	3	1	1/17	17/17	17/17

Table 10: Association of different virulent genes in relation to hospitals.

	SCCmec I	SCCmec II	SCCmec III	SCCmec IV	SCCmec V	SCCmec VI	SCCmec VII
mecA	0	3	24	0	24	2	0
Acme	0	0	11	0	11	1	0
Psm	0	1	14	0	14	1	0
Pvl	0	2	20	0	20	2	0

norB	0	0	12	0	12	1	0
sarE	0	2	19	0	19	2	0
sarA	0	0	1	0	1	0	0
Plc	0	3	23	0	23	2	0
lecithinase	0	1	17	0	17	2	1

Table 11: Distribution of virulence genes amongst different SCCmec types.

Hospital	Freq	SCCmec1 (1%)	SCCmec 11(1%)	SCCmec111 (1%)	SCCmec1Vb (1%)	SCCmec1Vd (1%)	SCCmecV (1%)	SCCmecV11 (1%)
LTH	22	0	2	9	1	37.5%	9	1
OAUTCH	4	0	0	2	0	8.3%	2	0
UCH	8	0	1	5	0	20.8%	5	0
FMCG	17	1	0	8	1	25.0	8	0
TOTAL	51	1	3	24	2		24	0

Table 12: Distribution of SCCmec types amongst the various hospitals.

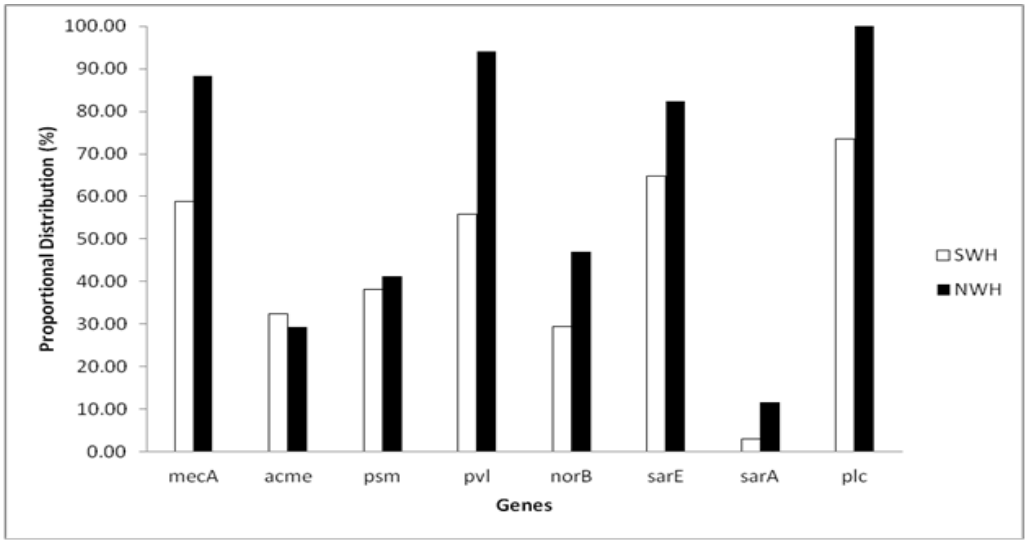

Figure 1: Prevalence of acme and other virulence genes in Southwest and Northwest hospitals in Nigeria.

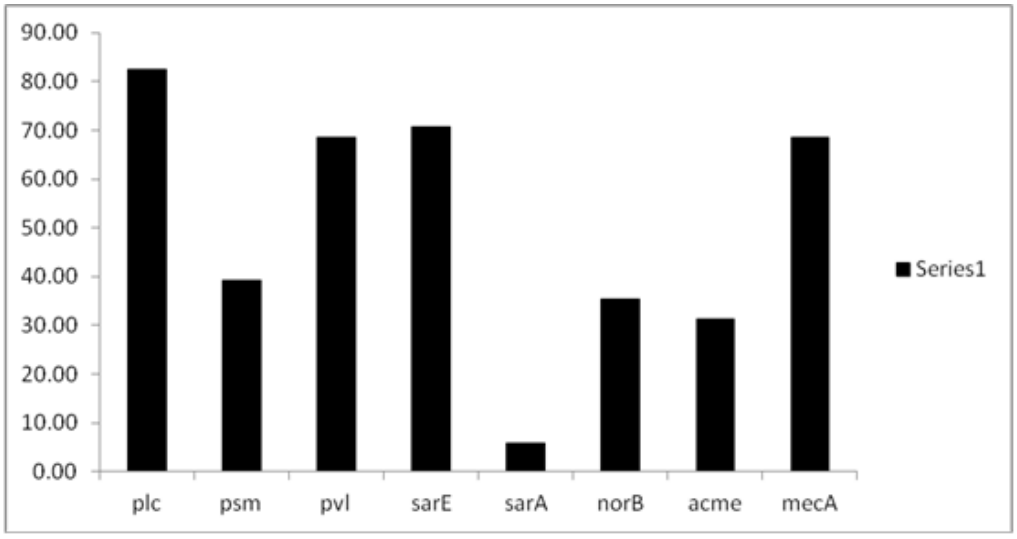

Figure 2: Prevalence of acme and other virulence genes in *Staphylococcus aureus* isolated.

Discussion

Antibiotic susceptibility testing results showed that all the 51 *S. aureus* isolates used in this study were resistant to penicillin G, which connotes that treatment of *S. aureus* infections in the 3 selected states using this antibiotic is of no clinical usefulness to patient health. The knowledge of the local antimicrobial resistance patterns of bacterial pathogens is essential to guide empirical and pathogen specific therapy. About 47 (92.2%) of 51 isolates used in this study exhibited resistance to more than two classes of antibiotics, a situation known as Multidrug Resistance (MDR). This prevalence is very high and this could have been as a result of indiscriminate use of antibiotics. It can therefore be inferred from this study that aside avoidance of indiscriminate prescription of antibiotics by many clinicians these days, to curb this menace, clinical microbiologists should brace up and ensure quality control of their susceptibility test procedures before releasing patients result to their clinician counterparts. All the 32 *S. aureus* isolates tested against vancomycin by determination of their MIC were all sensitive (MIC = ≤ 2 μg/ml) to the antibiotic. This shows that vancomycin still reserves the prerogative of being a potent antibiotic for treatment of staphylococcal infections [7-14].

MRSA is growing throughout the world with prevalence ranging from 23.3% to 73% across the globe and Nigeria is not an exception [15]. The prevalence of MRSA in this study was 68.6% which is similar to 60.7% reported by Chibuike, et al., in Southeast Nigeria and this further establish the fact that there is changing epidemiology of *S. aureus* as circulating strains of MSSA are being replaced with strains with acquisition of a resistant gene (mecA gene) mediating resistance to methicillin, a beta-lactamase insensitive antibiotic [16]. This prevalence is high compared to a prevalence of 44.2%, 12.5% and 3% reported recently by Alli, et al., in Southwest Nigeria, Okon, et al., in Northeast Nigeria and Egyir, et al., in Ghana, respectively [8,17,18]. The prevalence of MRSA in Zamfara, Oyo and Osun States were 15 (88.2%), 14 (73.7%) and 6 (40.0%), respectively. These differences may be as a result of different rate of indiscriminate use of beta-lactamase insensitive penicillins. Twenty-nine (56.8%) out of 51 *S. aureus* isolates used were resistant to cefoxtin whereas 35 (68.2%) were mecA positive which implies that molecular detection of MRSA by PCR amplification of mecA gene is more accurate and reliable than the phenotypic detection confirming previous studies [19,20]. The mecA negative strains of *S. aureus* (MSSA) were found to be sensitive to cefoxitin as indicated by MIC50 and MIC90 of 2 μg/ml and 4 μg/ml respectively, while the MIC50 and MIC90 of mecA positive strains of *S. aureus* were 64 μg/ml and 256 μg/ml respectively indicating high level of resistance to methicillin being coded for by mecA gene.

The acme may play an important role in the growth, transmission and pathogenesis of CA-MRSA. In *S. aureus*, acme positive isolates cluster in major MRSA clonal lineage [21]. This study found a high prevalence (42.9%) of acme amongst MRSA isolates. This prevalence is notable and high compared to a study carried out in England and Wales in which 17 (8.4%) of 203 MRSA isolates were found to be acme positive. In the same vein, Shore, et al., reported 9.7% (23 of 238) MRSA to be acme positive strains [22]. This study is consistent with the fact that acme gene is highly consevered among clones of MRSA as evident by the fact that all *S. aureus* strains positive for acme in this study were MRSA [23-25]. In this study, the proportional distribution of acme gene in *S. aureus* is higher in UCH isolates where 50% was recorded, compared to LTH Oshogbo isolates where the lowest acme positive strains of 18.2% was recorded. This study showed that there was an association between acme and MRSA positive strains. SCCmec typing in *S. aureus* showed acme is often integrated in the bacteria chromosome adjacent to a SCCmec IV element. Furthermore, it has been suggested that the physical linkage between acme and SCCmec type IV leads to enhanced fitness by co- selecting for beta-lactam resistance [1,23]. In line with Miragaia, et al., we found no association between acme and SCCmec type IV [24,25].

PVL is a bi-component exotoxin transmitted by bacteriophages that is encoded by two genes; lukF-PV and lukSPV. PVL genes are carried by nearly every CA-MRSA strain as well as a small proportion of clinical MSSA strains. This suggests that PVL has an important role in fitness, transmissibility and virulence, but the role of PVL in the pathogenesis of CA-MRSA infections is controversial. We found 35 (68.6%) of 51 *S. aureus* isolates to have PVL gene, this value is high compared with 51.2 % and 60% by Okon, et al., in Northeast Nigeria and Beverly, et al., in Ghana [17,26]. It was found that 30 (85.7%) out of the 35 MRSA obtained in this study were positive for PVL gene carriage, whereas on the contrary 5 (31.2%) out of the I6 MSSA recorded in this study were positive for PVL gene. This shows that the prevalence of PVL gene is more in MRSA compared to MSSA and there was a significant association between the carriage of PVL amongst the isolates and the distribution of mecA gene. This finding is in contrast with Alli, et al., where it was reported that 28 (24.1%) of 116 isolates were positive for PVL gene but none of the 28 PVL positive strains were mecA positive [19]. This further re-instates the fact that there is changing epidemiology of the circulating strains of *S. aureus* here in Nigeria as there is now emergence of MRSA with consistent carriage of virulence genes such as the PVL gene. The state with the leading population of *S. aureus* positive for PVL as far as this study is concerned was

Zamfara State, with prevalence of 45.7% (16 out of 35) and there was a significant association between the distributions of PVL gene carriage across the selected states. The implication of this is that we have a "superbug" amongst the circulating strains of S. aureus in this part of the country; the occurrence of *S. aureus* strains with dual arsenals viz-a-viz antibiotic-resistant gene and virulence genes. The high prevalence of PVL gene in Zamfara State highly suggested that the circulating clones of MRSA in the state were community associated MRSA (CA-MRSA) because CA-MRSA had been shown to be special virulent strains of MRSA with consistent carriage of PVL gene [27,28].

PSMs are a class of secreted α-helical peptides produced by several species of staphylococci. Genes for PSMs are found in all *S. aureus* and do not significantly differ among strains [29]. PSMs are able to recruit, activate and lyse human neutrophils and are generated at high concentrations by standard CA-MRSA strains [30]. Twenty (39.2%) of 51 isolates were found to be positive for α-PSM gene. This prevalence of 39.2% could be considered high in Nigeria where data as touching the prevalence α-PSM gene is unavailable currently. Eighteen (51.4%) of 35 MRSA were positive for α-PSM gene carriage whereas 2 (31.2%) out of I6 MSSA were positive for α-PSM gene. There was significant association between the carriage of α-PSM gene amongst the isolates and the distribution of mecA. The implication of this is that α-PSM gene is more conserved in MRSA than in MSSA and could contributes to the high virulence potential of the MRSA clones (30). The prevalence of norB gene in this study was 33.3% and it is lower compared to 42.6% in Korea and 49.0% by Kwak, et al. and Chan, et al., respectively. In this study, highest prevalence of norB gene was found in UCH (75.0%) and FMC Gusau (47.1%) [31,32]. This is justified as the bulk of multi-drug resistance (53.2%) incidence recorded in this study was contributed by all the norB positive strains from the two hospitals.

All genes coding for virulence is usually controlled by global virulence regulatory gene, which are agr and sarA [33]. Sae gene is a central downstream regulator that controls the expression of major virulence genes such as hla (coding for alpha-haemolysin), coa (coding for coagulase), fnbA (coding for fibronectin-binding protein A), sae prevalence in this study was 70.5%, Out of the 36 positive sae isolates, 30 (83.3%) were found in MRSA and the remaining 6 (16.7%) were MSSA. There was an association (X^2 = 10.1 $p< 0.05$) between the distribution of sae and mecA gene. This shows that the carriage of sae as a virulence genes regulator is more consistent in MRSA than MSSA strains. Hence, high prevalence of sae in MRSA compared to MSSA as found in this study can be said to contribute significantly to higher virulence potentials of MRSA. The prevalence of sarA as documented in this study was very low, 5.1% (3 out of 51 isolates). The two positive isolates were MRSA and one was MSSA. The low prevalence of sarA as found in this study can be presumably said to play little or no role in the virulence of *S. aureus* isolates used in this study.

The result of the genetic diversity of MRSA isolates used in this study showed that the overall prevalence of HA-MRSA and CA-MRSA were 60.0% and 40.0%, respectively. The prevalence of HA-MRSA obtained in this study is higher compared to a prevalence of 34.7% for HA-MRSA and that of CA-MRSA from this study is similar to a prevalence of 39.1% for CA-MRSA obtained by Alli, et al., in a study carried out to determine the distribution of genes encoding aminoglycoside modifying enzymes among MRSA and MSSA isolates from Nigerian hospitals. However, there were no non-typeable strains obtained in this study as compared to what was recorded by Alli, et al., where 8 (34.8%) of 23 MRSA isolates used in this study were non-typeable. The prevalence of HA-MRSA obtained in this study is low and that of CA-MRSA obtained is high compared to prevalence of 72.0% (for HA-MRSA) and 28.0% (for CA-MRSA) recorded 4 years ago in USA by Muhlebach, et al., in a study carried out to characterize MRSA isolated from Cystic Fibrosis (CF) patients [34,35].

In this study, HA-MRSA was more prevalent (75.0%) in Southwestern Nigeria while CA-MRSA was more prevalent (73.0%) in Northwestern Nigeria. The only inference that can be drawn from this is that, the origin of the clones of MRSA contributing to the increased morbidity and mortality rate due to *S. aureus* infections in Southwestern Nigeria as found in this study were basically hospital related as these strains infect patients who are exposed to health care facilities such as ventilators, catheters, prosthetic devices and surgical procedures while the origin of the circulating clones of MRSA causing the increasing incidence of MRSA infections in Northwestern Nigeria as obtained in this study were community associated which by speculation could be due to increased close personal contacts in households, mosques and markets, cum poor hygienic practices as common there and much contacts with livestock especially cattle.

From RAPD-PCR profiles analysis, it was discovered that the samples can be grouped into five based on degree of relatedness which implies that the isolates may be from five origins. This study shows that RAPD-PCR can be successfully applied to assess the genetic relationship of *S. aureus* isolates from different region. Several reports demonstrated that the origin of *S. aureus* isolates

can be traced with the help of a few tests. All the Isolates (except one) from LAUTECH Teaching Hospital, Ogbomoso has similar molecular weight (2.0) with northern isolates. This may be because of the shared boundary between them and it is even possible that some patients from north may be visiting LAUTECH for their clinic. This implies that northern *S. aureus* isolates are different from western isolates which is line with the previous investigators [36,37].

Conclusion

The study reported a high prevalence of acme and α-PSM gene in *S. aureus* isolates in Nigeria and this has provided new information on the transmission and fitness of circulating MRSA in causing infection in the community at large. It also discovered a rapid changing epidemiology of *S. aureus* strains which has become a matter of public health importance across the globe especially the increasing spread of MRSA carrying virulence genes with multiple antibiotic resistance which play significant roles in the pathogenesis and poor prognosis of *S. aureus* infections. Since all the isolates from North has low molecular weight and high prevalence of CA-MRSA, it can be concluded that most isolates from North has high molecular weight. Finally, there is high prevalence of HA-MRSA in South-Western, Nigeria and high prevalence of CA-MRSA in North-Western, Nigeria. A policy to curb the spread of this organism in the community needs to be put in place.

Conflict of Interest

The author declares no conflicts of interest.

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