

Molecular Characterisation of *Staphylococcus aureus* isolated from Patients in Healthcare Facilities in Zaria Metropolis, Kaduna State, Nigeria

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Abstract

Background: *Staphylococcus aureus* is an important global bacterial pathogen causing a wide spectrum of hospital and community-acquired infections. It has been a challenge for clinicians for more than half a century because of its multidrug resistance. This study assessed the molecular characteristics of *Staphylococcus aureus* isolated from patients in health facilities in Zaria.

Methods: The 60 *Staphylococcus aureus* isolates from clinical samples were evaluated at National Veterinary Research Institute, Vom, Nigeria, and Wellcome Trust Sanger Institute, United Kingdom by Multiplex Polymerase Chain Reaction and Multilocus Sequence Typing and phylogenetic analysis.

Results: The detected genes were *mec A* (15%), *pvl* (10%), and *spa* (13.3%). Three isolates were positive for *fem B* (10%) and Multilocus Sequence Typing showed them to be Sequence Type 1, which had similar allelic profiles at all seven loci and belonged to the clonal complex 1. The *Staphylococcus aureus* genes were relatively uniform with no variable nucleotide sites at the seven loci. All the isolates (23448_1#126, 23448_1#127 and 23448_1#130) were multidrug resistant. The phylogenetic relationship established based on a subset of core genes using the 16S rRNA sequence of the typed strains revealed 100% identity with the available *Staphylococcus aureus* (BX571857, BA000033, AP015012, CP017115, and CP01780) genome in the database. The ST1 (CC1) clones are known to be community-acquired human biotypes.

Conclusion: *mec A*, *pvl* genes were detected from the isolated *Staphylococcus aureus*. There is need for rational use of antibiotics through antimicrobial stewardship programme, periodic clinical auditing using molecular analysis and effective hospital infection control measures.

Keywords: *Staphylococcus aureus*, molecular characteristics, *mec A* gene, *fem B* gene, *pvl* gene, *Spa*, health institutions, Zaria

Introduction

Staphylococcus aureus is one of the species of the genus *Staphylococcus* which can elaborate numerous virulence factors.¹ The success of the organism as a pathogen is partly due to its ability to express a variety of virulence factors that mediate host colonization, tissue invasion, and dissemination.² Colonization and transmission of *S. aureus* strains in hospitals and communities have been attributed to social risk factors such as high population density, urbanization, inadequate infection control policies, inappropriate antibiotic use, and lack of appropriate healthcare delivery.³

The virulence of *Staphylococcus aureus* depends on a variety of components, such as capsule and protein A, *mec A*, the clumping factor, and coagulase, hemolysins, enterotoxins, toxic shock syndrome (TSS) toxins, exfoliatins, and Panton-Valentine leukocidin (PVL).⁴ Panton-Valentine leukocidin is a cytotoxin, one of the β pore-forming toxins; its presence is associated with the increased virulence of certain strains of *S. aureus*. It is present in the majority of community-associated methicillin-resistant *S. aureus* (CA-MRSA) and is the cause of necrotic lesions, an aggressive condition that often kills patients within 72 hours.⁵

Staphylococcus aureus develops resistance to commonly used antibiotics which results in therapeutic failure and is one of the major pathogens causing a wide range of minor and major infections leading to morbidity and mortality.⁶ The presence of *mec A* gene and either an oxacillin Minimum Inhibitory Concentration (MIC) of >2mg/L and methicillin MIC of >4mg/L or a ceftaxime MIC of >4mg/L and production of altered penicillin binding protein (PBP2a), are accepted criteria for methicillin resistance.⁷

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a leading cause of hospital-associated infections. The evolution of methicillin-resistant *Staphylococcus aureus* (MRSA) against beta-lactam antibiotics is a challenging issue for clinicians in the management of infections.⁸ Widespread use of antibiotics results in the evolution of more resistant strains. The resistance is a result of genetic alterations or the transfer of resistant genes.⁹ *Staphylococcus aureus* acquired resistance to beta-lactam antibiotics by harboring a gene called *mec A* which usually results in poor clinical response. This restricts the use of beta-lactam antibiotics against MRSA infections.¹⁰ *mec A* encodes an altered penicillin-binding protein (PBP) called PBP2' or MRSA-PBP, which executes low affinity to almost all beta-lactam antibiotics.¹¹

Detection of specific genes and variations in the genome or within genes of a pathogen is used for molecular typing and the choice of method is dependent on the biology of the organism and the epidemiological questions. Phage typing was originally used for the formal typing of *S. aureus* isolates, but it was gradually replaced by pulsed field gel electrophoresis (PFGE), the most recent gold standard method for the typing of *S. aureus* isolates.¹² However, due to its laborious nature and difficulties in exchanging data between laboratories, and the requirement for inter-laboratory standardization, PFGE was replaced by multi-locus sequence typing (MLST) and staphylococcal protein A (*spa*) typing.¹³ *Staphylococcus aureus* Protein A typing are widely used to establish clonal relationships between strains and to compare the geographical locations of *S. aureus* clones.¹⁴ MLST is used to detect whether the strains originate from a hospital or community, and it is a great tool for evolutionary investigations and

differentiates isolates according to nucleotide variations in 7 housekeeping genes. This study assessed the molecular characteristics of *S. aureus* isolated from patients in healthcare facilities in Zaria metropolis, Kaduna State, Nigeria.

Methodology

Study Area and the healthcare facilities

The five selected hospitals for the study were Ahmadu University Teaching Hospital (ABUTH), Zaria, Sickbay, Ahmadu Bello University (Medical centre ABU), Samaru, St Luke's Hospital (SLH), Wusasa, Major Ibrahim Bello Abubakar Hospital (MIBA), Sabon Gari and Gambo Sawaba Hospital (GSH), Zaria City. All the hospitals provide secondary health services except ABUTH, Zaria. The study was carried out between November 2013 and December 2016.

Storage and Transportation of *S. aureus* isolates

Staphylococcus aureus isolates were inoculated onto Mannitol Salt agar and Tryptic Soy agar slants in cryo vials and also spotted on Whatman filter Paper placed in a container with ice and shipped to Welcome Trust, Sanger Institute, the United Kingdom for the molecular characterization of the isolates.

Molecular characterisation of *S. aureus* isolates

A) Detection of *mec A*, *pvl*, and *spa* genes were carried out at National Veterinary Research Institute, Vom, Plateau State.

Staphylococcal DNA extraction

This was carried out as described by Stegger *et al.*¹⁵ Two or three colonies of a fresh overnight culture of *S. aureus* cells was suspended in 100µl lysis buffer (InstaGene Matrix, Biorad) in a 1.5 ml eppendorf tube, vortexed for 15 min and incubated for 56°C for 1 hr. It was mixed well by vortexing and incubated at 95°C for 1 hr, mixed well again by vortexing, and centrifuged at 13,200 rpm for 5 min. The DNA suspension was vortexed and centrifuged (13,200 rpm for 5 min) before use. The eluted DNA was stored at -20°C.

PCR Amplification of Staphylococcal Genes

Multiplex Polymerase Chain Reaction to amplify *mec A*, *pvl*, and *spa* genes was carried out using DNA template as described by Stegger *et al.*¹⁵ with the following amplification mixture- dNTPs, magnesium chloride, forward and reverse primers of each gene and 5µl of template in a total volume of 25µl. The sequence of the primers, the thermocycler program, and their corresponding references are summarized in Table I.

Table I: Primers used in the study to detect and characterize *mecA*, *spa* and *pvl* genes

Primer name	Gene	Sequence (5'-3')	Amplicon size (bp)	References
MecA P4	<i>mec A</i>	TCCAGATTACAACCTTCACCAGG CCACTTCATATCTTGTAACG	162	16
MecA P7				
Pvl-FP	<i>lukF-PV</i>	GCTGGACAAAACCTTCTTGGAATAT	83	17
Pvl-RP		GATAGGACACCAATAAATTCTGGATTG		
Spa-113f	<i>spa</i> -250bp	TAAAGACGATCCTTCGGTGAGC	Variable (180-600bp)	13
Spa-1514r	repeat, 350bp,	CAGCAGTAGTGCCGTTTGCTT		
	400bp, 450bp			

Thermal Cycling Procedure for various programme

DNA amplification was carried out in a Thermocycler (Techneprogene Thermodux, Wertheim, Germany): An initial denaturing step of 15 min at 94°C, followed by 30 cycles of 94°C for 30 seconds, 59°C for 1 min, and 72°C for 1 min and then a final extension at 72°C for 10 min.

PCR Controls: spa- *S. aureus* ATCC 29213; *mec A*- *mec A* positive *S. aureus* 50A247; *pvl*- PVL positive *S. aureus*.

PCR Master Mix

This included distilled water (6.5µl), reaction buffer, deoxynucleoside triphosphate (dNTPs), MgCl₂, forward and reverse primers, template DNA, and Taq DNA polymerase to add up to 25 µl. Each PCR reaction mixture contained 6.5µl PCR water, 12.5µl 2xGreen PCR Master Mix, 0.5µl of each primer 1, 0.5µl of each primer 2, and 2µl of the DNA template preparation.

Agarose Gel Electrophoresis of PCR products

Polymerase chain reaction products were visualized on electrophoresis gels, parallel with a 100bp ladder molecular weight marker on a 2% agarose gel in TBE (40mM Tris, 20mM Acetate, and 2mM EDTA pH8.1) 1x ran for 1hr at about 130V. The gel was stained in Ethidium bromide (10mg/ml) for 20-30 min and de-stained briefly. Photographs were taken in the transilluminator under UV light, bands observed and results interpreted accordingly.

B) Detection of *mecA*, *mec C* and *fem B* genes by multiplex polymerase chain reaction carried out at Wellcome Trust Sanger Institute, United Kingdom

DNA extraction

One or two pure colonies of bacterial cultures were picked from overnight

growth cultures on blood agar and suspended in 40µl water (DNase free water). This was then heated for 5 minutes at 95° C in a thermocycler.¹⁸ Cured lysate mixture (2.5 µl) was used as a DNA template for Multiplex PCR procedures.

Polymerase Chain Reaction Procedures

Multiplex Polymerase Chain Reaction to amplify *mec A*, *mec C* and *fem B* genes were performed using DNA template, with the following amplification mixture: 200 µM of dNTPs, 1.5 mM magnesium chloride (Promega Corporations, USA), 0.25 µM of both forward and reverse primers of each gene and 5 µL of template in a total volume of 50 µL. The amplification was carried out in MyCycler Thermal Cycler (Bio-Rad) with an initial denaturation at 94 °C for 30 s, followed by 30 cycles of 94 °C for 45 s, annealing at 55 °C for 1 min, and extension at 72 °C for 2 min, and final extension at 72 °C – 2 min. *S. aureus* ATCC43300 and ATCC25932 were used as positive and negative controls respectively.

Agarose Gel Electrophoresis

Molecular biology grade Agarose (2% w/v) was dissolved by heating in a microwave in 1×TAE buffer (40mM Tris, 20mM Acetate, and 2mM EDTA pH 8.1). Sybr® Safe DNA gel stain (Thermo Fisher, UK) was added to the melted and cooled agarose solution. Each 100ml of 2% agarose contained 2.5µL of Sybr® Safe which was poured into the gel tray (with 1mm 25 well comb from PeqLab) to set. A 10L of PCR reaction mixture was loaded onto each well. The molecular marker (5µ L) used was HyperLadder™ 100bp, consisting of 100-1000bp ladder from Bioline, United Kingdom. Electrophoresis was performed in a Sigma-Aldrich electrophoresis tank with 1 ×TAE at 80V for 75 minutes. Electrophoresed gels were visualized under blue light and their images were captured using the GelDoc™ XR System Imager (BioRad).

Table II: Oligonucleotide primers used for the detection of *mec A*, *mec C* and *fem B* genes

Name	Sequence (5'-3')	Size (bp)	gene	Reference
<i>MecCA-F</i>	CAT TAA AAT CAG AGC GAG GC	188	<i>mec C</i>	19
<i>MecCA-R</i>	TGG CTG AAC CCA TTT TTG AT			
<i>MecA-F</i>	TGG TAT GTG GAA GTT AGA TTG GGA T	155	<i>mec A</i>	20
<i>MecA-R</i>	CTA ATC TCA TAT GTG TTC CTG TAT TGG C			
<i>FemB-F</i>	CAT GGT TAC GAG CAT CAT GG	531	<i>fem B</i>	19
<i>FemB-R</i>	AAC GCC AGA AGC AAG GTT TA			

Multilocus Sequence Typing

Primers were purchased commercially and the DNA sequences of 7 housekeeping genes were supplied by Sanger Institute, United Kingdom. Each primer pair

amplified an internal fragment of the housekeeping gene (about 500 bp) and was allowed accurate sequencing of 450-bp fragments of each gene on both strands.²¹

Table III: Sequences of primers used in the

Gene	Primer	Sequence (5'-3')
Carbamate kinase (<i>arcC</i>)	<i>arcC</i> -Up	TTGATTCACCAGCGCGTATTGTC
	<i>arcC</i> -Dn	AGGTATCTGCTTCAATCAGCG
Shikimate dehydrogenase (<i>aroE</i>)	<i>aroE</i> -Up	ATCGGAAATCCTATTTACATTC
	<i>aroE</i> -Dn	GGTGTGTATTAATAACGATATC
Glycerol kinase (<i>glpF</i>)	<i>glpF</i> -Up	CTAGGAACTGCAATCTTAATCC
	<i>glpF</i> -Dn	TGGTAAAATCGCATGTCCAATTC
Guanylate kinase (<i>gmk</i>)	<i>gmk</i> -Up	ATCGTTTTATCGGGACCATC
	<i>gmk</i> -Dn	TCATTA ACTACAACGTAATCGTA
Phosphate acetyltransferase (<i>pta</i>)	<i>pta</i> -Up	GTAAAATCGTATTACCTGAAGG
	<i>pta</i> -Dn	GACCCTTTTGTGAAAAGCTTAA
Triosephosphateisomerase (<i>tpi</i>)	<i>tpi</i> -Up	TCGTTCAATTCTGAACGTCGTGAA
	<i>tpi</i> -Dn	TTTGACCTTCTAACAATTGTAC
Acetyl coenzyme A acetyltransferase (<i>yqiL</i>)	<i>yqiL</i> -Up	CAGCATA CAGGACACCTATTGGC
	<i>yqiL</i> -Dn	CGTTGAGGAATCGATACTGGAAC

Polymerase Chain Reaction was carried out with a 50-µl reaction volume containing 0.5 µl of chromosomal DNA (approximately 0.5 µg), 0.5 M of each primer, 1 U of *Taq* DNA polymerase (Qiagen, Crawley, United Kingdom), 5 µl of 10× buffer (supplied with the *Taq* polymerase), and 0.2 mM deoxynucleoside triphosphates (Perkin-Elmer Applied Biosystems; Foster City, Calif.). The PCR was performed in a PTC-

200 DNA engine (MJ Research, Boston, Mass.) with an initial 5-min denaturation at 95°C, followed by 30 cycles of annealing at 55°C for 1 min, extension at 72°C for 1 min, followed by a final extension step of 72°C for 5 min. The amplified products were precipitated with 20% polyethylene glycol–2.5 M NaCl, re-suspended in cold 70% ethanol, and re-precipitated; and the sequences of both strands were determined with an ABI Prism 377 DNA sequencer

with Big Dye fluorescent terminators and the primers used in the initial PCR amplification.²¹

For each locus, the sequences obtained from all the isolates were compared and the different sequences were assigned allele numbers. For each isolate, the alleles at each of the seven loci define the allelic profile which corresponded to its ST. The clustering of isolates was achieved by the Unweighted Pair Group Method with Arithmetic Averages (UPGMA) from the matrix of the percentage of pair-wise differences between the allelic profiles of the isolates by using Statistica (StatSoft, Tulsa, Okla). The non-randomness in the distribution of variable sites along the sequence of each gene fragment was examined by the method of Sawyer.²² Polymorphic sites were displayed by using Sequence Output, a Macintosh program available from the MLST website (<http://mlst.zoo.ox.ac.uk>).

The evolutionary origins were examined by the algorithm eBURST, to group the received allelic profiles into clonal complexes. Grouping was based on the similarity between sequence types in six of seven loci and singleton STs defined as not sharing six out of seven loci with any other STs in the data set. Four groups of related genotypes were identified and in addition 10 singletons. Comparison with all *S. aureus* isolates in the database at mlst.net revealed the presence of only one clonal complex (CC).

Phylogenetic Studies and Analysis of *S. aureus*

The phylogenetic relationship was established using the 16 rRNA sequences of the type strains defining the genus *Staphylococcus* (BX571857, BA000033, AP015012, CP017115 and CP01780). The BLAST search for previously reported sequences that are identical to the three (3) local isolates was done using NCBI GenBank (<http://www.ncbi.nlm.nih.gov/>).

Multiple alignments were carried out using cluster algorithm. Neighbor-joining trees²³ were constructed on the basis of genetic distances, estimated by Kimura's (1980) two-parameter method, using MEGA 5.²⁴ The reliability of the trees was estimated by bootstrap confidence values²⁵ and 1000 bootstrap replications were used. A bootstrap value of 70% was considered significant evidence for phylogenetic grouping.

The nucleotides of the *Staphylococcus aureus* 23448_1#126, 23448_1#127 and 23448_1#130 genes were used for the final construction of both Neighbor-joining (NJ) phylogenetic trees. The methods produced a topology revealing species clustering of *Staphylococcus aureus* obtained in this study and those obtained from the Genbank. This further consolidated the molecular identities of the *S. aureus* that were found in this study. *Escherichia coli* was used as an outgroup in the tree.

Data analysis

The collected data was manually cleaned, then keyed into Microsoft excel and SPSS version 21 (Chicago, USA). Descriptive statistics were generated from the quantitative data and Chi-square used to test for association. A p value of less or equal to 0.05 ($p < 0.05$) was considered statistically significant. Associations between *S. aureus*, MRSA, antimicrobial susceptibility, MDR, *mec A*, *pvl*, *spa* and MLST were assessed. Sequence alignments, translations and comparisons were carried out using BIOEDIT (version 7.0.9.0).¹⁸ The BLAST algorithm was used to search the NCBI GenBank (<http://www.ncbi.nlm.nih.gov/>) database for homologous sequences. Neighbor-joining trees²³ were constructed on the basis of genetic distances, estimated by Kimura's (1980) two-parameter method, using MEGA 5.²⁴ The reliability of the trees was estimated by bootstrap confidence

values and 500 bootstrap replications were used.

The MLST genomic gene sequences used to construct the neighbor-joining tree were by NCBI GenBank accession numbers, and the *S. aureus* MLST genomic genes sequences are *S. aureus* (23448_1#126, 23448_1#127 and 23448_1#130).

Ethical Consideration

Ethical clearance was sought and obtained from Health Research Ethics Committee

of Ahmadu Bello University Teaching Hospital (ABUTH) Zaria (ABUTH/HREC/A22/2012). Informed consent was obtained from each patient after thoroughly explaining the aim of the study. In the case of children, assent was obtained from the parent or guardian. All information collected was treated as highly confidential.

Results

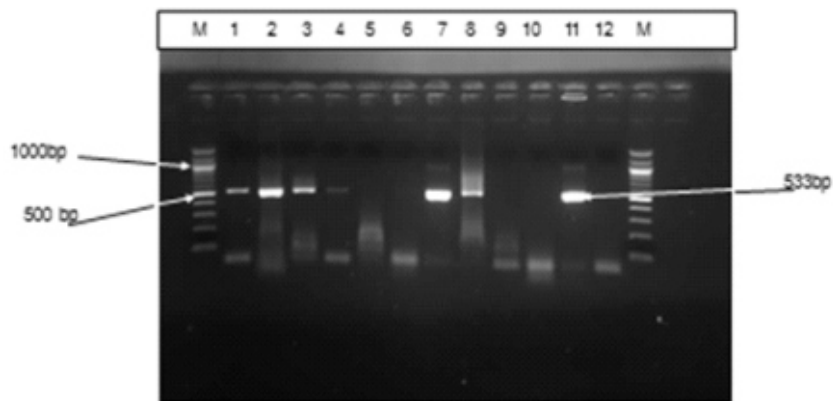


Plate 1: Monoplex PCR amplification of *mec A* gene from *S. aureus*. Lane 1-10: Clinical isolates; lane 11: Positive control; Lane 12: negative control; Lane 1,2,3,4,6,7,8, positive (533bp); Lane 9 & 10: negative; Lane M: 100bp DNA Ladder (New England Biolab Inc).

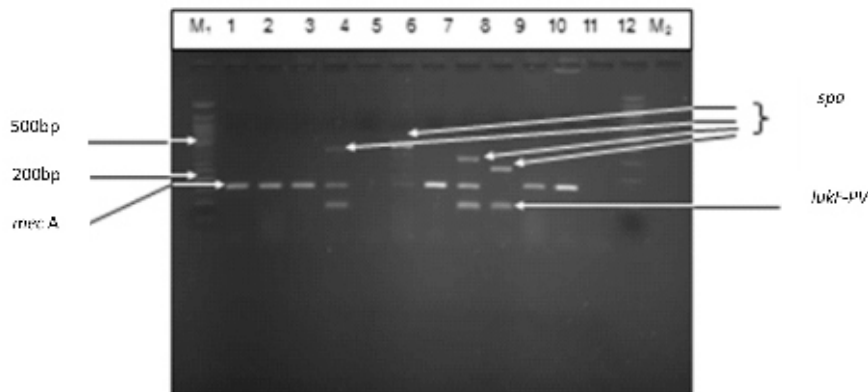


Plate 2: Triplex PCR for *mec A*, *spa*, LukF-PV genes from *S. aureus*; Lane M1:50bp ladder; Lane 1-10; Clinical isolates; Lane 11: *mec A* positive control; Lane 12; negative control; M2; 100bp ladder Lane 1: *mec A* positive (162bp); Lane 2; *mec A* positive (162bp); Lane 3; *mec A* positive (162bp); Lane: *pvl* (83bp), *mec A* (162bp); *spa* (400bp) positive; Lane 6; *mec A* (162bp), *spa* (450p) positive; Lane 7; *mec A* (162bp); Lane 8; *pvl* (83bp), *mec A* (162bp), *spa* (350bp) positive; Lane 9; *pvl* (83bp), *spa* (250bp) positive; Lane 10; *mec A* (162bp) positive.

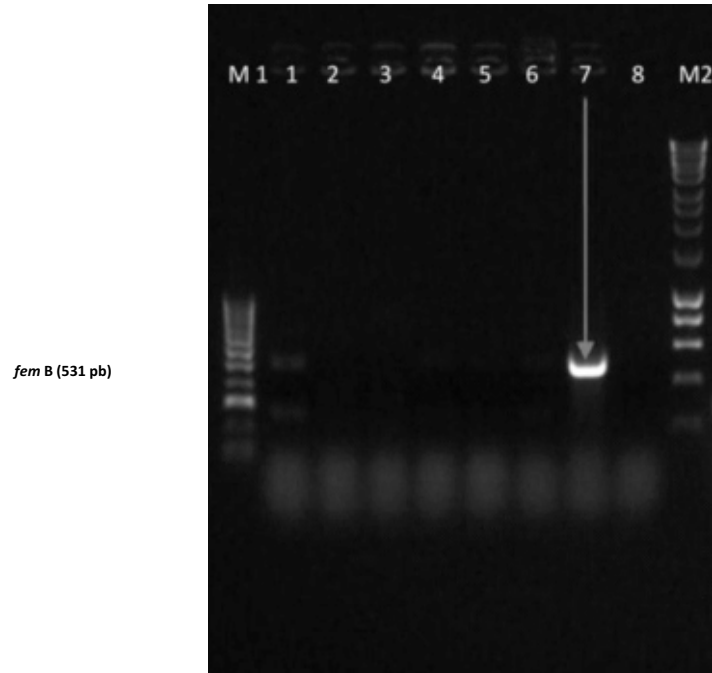


Plate 3: M1= Molecular ladder, Lane 7 = *fem B* positive (1B) M2= Molecular ladder, Lane 1-16= samples, M3= Molecular ladder, positive control N1, N2and 3 = *fem B* negative controls.

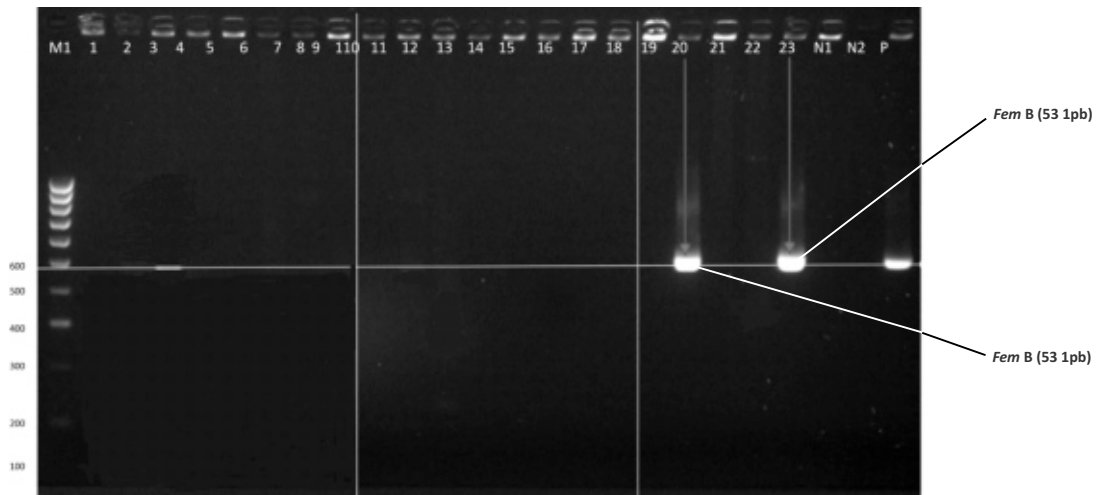


Plate 4: M1= Molecular ladder, Lane 1-23= samples, Lane 20 and 23 = poaitive for *fem*(325 and 313) B M2= Molecular ladder, N1 and N2=*fem B* negative controls, P=*fem B* positive control

The detection rates of the targeted genes were:

mec A -15%, *pvl*- 10%, *spa* -10.3% and *fem B*- 10%. No *mec C* gene was detected.

Table 1: Sequence Variation at the Seven Loci

Gene	Sequence length (bp)	No. of alleles	No. of polymorphic sites
<i>Arc</i>	456	17	No
<i>aroE</i>	456	17	No
<i>glpF</i>	456	11	No
<i>Gmk</i>	429	11	No
<i>Pta</i>	474	15	No
<i>Tpi</i>	402	14	No
<i>yqiL</i>	516	17	No

Table 2: Properties of the 3 Sequence types

Isolate	ST	Allelic profile (allele number) ^a							CC ^b	Origin
		<i>arc</i>	<i>aroE</i>	<i>glpF</i>	<i>Gmk</i>	<i>Pta</i>	<i>Tpi</i>	<i>yqiL</i>		
126	1	1	1	1	1	1	1	1	1	Community
127	1	1	1	1	1	1	1	1	1	Community
130	1	1	1	1	1	1	1	1	1	Community

^aAllelic profile are read from left to right and correspond to genes *arc*, *aroE*, *glpF*, *Gmk*, *Pta*, *Tpi* and *yqiL*

^bCC, Clonal complex based on BURST S, singleton

Multilocus Sequence Typing

All the 3 isolates were multilocus types 1 (ST 1) with similar allelic profiles at all seven loci and comparison with all *S. aureus* isolates in the database at mlst.net revealed the presence of only one clonal complex (CC1). The three (3) ST1 23448_1#126, 23448_1#127 and 23448_1#130 were of clonal complex (CC) 1 and the isolates (23448_1#126, 23448_1#127 and 23448_1#130) were

isolated from cases of urinary tract infection in pregnancy (MIBA), otitis externa (Sickbay) and post-surgical wound infection (ABUTH).

Phylogenetic study

The tree illustrated the close relationship of *S. aureus* 23448_1#126, 23448_1#127 and 23448_1#130 with *S. aureus* isolates from BX571857, BA000033, AP015012, CP017115 and CP01780. A blastn search of all five copies of 16S rRNA sequence revealed 99 % identity with (BX571857, BA000033, AP015012, CP017115 and CP01780) genomes in the database and they clustered together more tightly as compared to those obtained from Genbank.

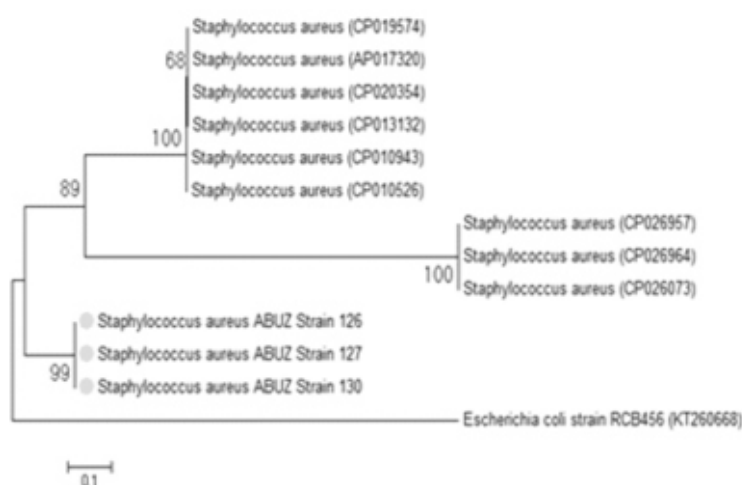


Figure. 1: Phylogenetic tree of *S. aureus* based on nucleotide residues of the ABU 126, ABU 127 and ABU 130 genes

Discussion

The virulence of *S. aureus* depends on a variety of components, such as *mec A*, protein A, and Panton Valentine Leukocidin among others. *mec A* has been suggested to be responsible for methicillin resistance. It encodes the altered PBP2a with a low affinity for beta-lactam antibiotics.²⁶ MRSA strain is the main cause of treatment failure and increases in treatment cost and also it is associated with a higher mortality rate.^{26,27}

Studies showed *mec A* prevalence of 20.7%, 38%, and 42% in Serbia, Benin, and Lagos respectively which are higher than the 15% in this study.²⁸⁻³⁰ Environmental factors could be responsible for the differences. In the Benin study, the MRSA carrying the *mec A* gene was multidrug resistant and the samples collected were both from inpatients and outpatients, though predominantly inpatients. A similar study in Iran showed a much higher prevalence of *mec A* of 53.3% detected by PCR. This could be a result of the source of the samples- specifically from blood and wounds and also from orthopedic wards of teaching hospitals in Iran. Hospital admission and wound infection are risk factors for MRSA, in addition to the fact that all the samples were from teaching hospitals (referral centres).

In a similar study in Zaria, no *mec A* was detected in all the clinical isolates.³¹ The molecular study was carried out in the USA, and the isolates had to be shipped therefore, the condition of the shipped isolates may be responsible for the loss of *mec A* gene during the process. Studies have shown *mec A* to be lost at a storage temperature of -80°C.³² Some researchers reported *mec A* prevalence of 19.2% in Ekiti State and 14% in Saudi Arabia respectively, which is slightly similar to the finding of this study.^{33,34}

The detection rate of *pvl* gene of 10% in this study disagrees with the findings of studies by some researchers with the figures of 44.4%, 40%, 30%, and 40.6% respectively.³⁵⁻³⁸ The higher prevalence recorded in 2012 could be a result of the multicentre nature of the study involving 8 tertiary hospitals.³⁵ That reported by Shittu *et al* could be because most of the samples were from inpatients; the ST was type 1. A study in Egypt reported a *pvl* prevalence of 10.3%.³⁹

Africa is now considered to be a PVL-endemic region with high rates of *pvl*-positive isolates, mainly MSSA, ranging from 17% to 74%.⁴⁰ This is in stark contrast to Europe, where the prevalence of PVL-positive isolates is low (0.9–1.4%).⁴¹ The reasons for the high prevalence of PVL are unknown but might be related to the host (i.e., altered C5a receptors, which have been identified as PVL targets), so far unidentified virulence factors of *S. aureus* that facilitate dissemination and the humid environment of tropical Africa.⁴² *pvl* gene is a cytotoxin, one of the beta pores forming toxins and its presence is associated with the increased virulence of certain strains of *S. aureus*. The Panton-Valentine Leucocidin (PVL) is a bicomponent leucotoxin composed of S-related and F-related proteins that are secreted separately but act synergistically; the cytotoxin is found to cause leukocyte destruction and tissue necrosis.⁴³ It is a marker for a community-associated MRSA, and it causes leukocyte destruction and tissue necrosis, an aggressive condition that often kills patients within 72hrs.³⁸ Contact with colonized and/or infected individuals as well as contaminated fomites with *pvl* positive *S. aureus* have been described as risk factors for the community-associated MRSA.³⁶ Colonization and transmission of *S. aureus* in hospitals and communities

have been attributed to social risk factors such as high population density, urbanization, inadequate infection control policies, antibiotic use, and lack of appropriate healthcare delivery.³

fem B genes were isolated from 3 isolates which were sequenced and clonal complex 1 with multiple drug resistance. This finding of MLST type 1 showed that the isolates were from the community. In a study in Saudi Arabia, all the MRSA isolates were positive for *fem A* and *B* genes.²⁸ Studies have reported the use of *mec A* as a marker for MRSA detection and *fem* genes for recognition of *S. aureus* species, and the detection of *fem B* and *mec A* genes may also help explain the severity of the infection. *fem B* is involved in pentaglycine side chain formation and interpeptide bridge formation, as well as the expression of methicillin resistance.²⁸ The detection of *fem A* and *fem B* together with *mec A* by PCR was considered to be a more reliable indicator to identify MRSA by differentiating it from *mec A* positive coagulase negative *S. aureus* than single *mec A*. The multiple drug resistance profile of the isolates has significant public health implications. Consequential effect of MRSA infection and multiple antibiotic resistances has resulted in, prolonged hospitalization, increased medical expenses, and difficulty in patient treatment and Management as reported in similar studies.²⁷

None of the isolates was found to be carrying the *mec C* gene, which is a homolog (70%) of *mec A* gene with a similar property of development of methicillin resistance.⁴⁴ The absence of this gene and the low detection of the *spa* genes (compared with *spa* prevalence of 88.3% as reported by some researchers⁴⁵ could be from the long storage of the isolates that might have resulted in the loss of the genes. Studies have shown bacteria genes to be

lost as a result of long or poor storage.³² The result of the phylogenetic analysis showed the 3 isolates whose genes were sequenced to be the same clade and with 99% similarity with the *S. aureus* strains established in the gene bank. The 3 sequenced genes were from isolates obtained from cases of urinary tract infection in pregnancy, otitis externa, and post-surgical wound infection.

Conclusion

The study showed the presence of *mec A*, *pvl*, *spa* and *fem B* genes in *S. aureus* isolated from the 5 selected hospitals in the Zaria metropolis. Some of the isolates carried 2 or 3 of these virulence genes. The ST 1 (CC 1) strains were indicative of community-associated MRSA. The 3 sequenced genes were from isolates obtained from cases of urinary tract infection in pregnancy, otitis media, and post-surgical wound infection. The isolates were 99% similar to BX571857, BA000033, AP015012, CP017155, and CP01780 established in the Genbank and the phylogenetic analysis showed the 3 sequenced isolates to be from the same clade. There is need for periodic clinical and laboratory auditing in hospitals to determine antibiotic resistance profiles for quality control; and regular molecular epidemiological surveys on clinical isolates to monitor changes in antimicrobial resistance over time and to detect the emergence or re-emergence of resistant clones.

Infection prevention, control and antimicrobial stewardship programmes need to be established and strengthened at healthcare facilities to mitigate the problem of antimicrobial resistance.

Conflict of Interest

There is no conflict of interest.

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