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Prevalence and genotypic characteristics of Panton-Valentine Leukocidin-producing *Staphylococcus aureus* isolates obtained in Jos, North Central Nigeria

Unyime C. Essien [1, 2, *], Samar S. Boswihi [3], Nneka R. Agbakoba [2] and Edet E. Udo [3]

[1] *Department of Medical Laboratory Science, Faculty of Health Sciences and Technology, University of Jos, Nigeria.*
[2] *Department of Medical Laboratory Science, Nnamdi Azikiwe University, Nnewi Campus, Anambra State, Nigeria.*
[3] *Department of Microbiology, Faculty of Medicine, Kuwait University, Kuwait.*

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Abstract

Background: *Staphylococcus aureus* is an opportunistic pathogen that colonizes and causes infections in humans. Panton Valentine Leukocidin (PVL) is a cytolytic toxin produced by some strains of *S. aureus* and are mostly associated with skin and soft tissue infections and necrotizing pneumonia.

Aim: To investigate the prevalence and genotypic characteristics of PVL-positive *S. aureus* strains cultured from patients in three tertiary hospitals in Jos, Nigeria.

Methods: Two hundred and fourteen clinical *S. aureus* isolates were obtained from three tertiary hospitals in Jos. Polymerase chain reaction was used to detect *lukSF-PV* gene that encodes PVL, and sensitivity to antimicrobial agents was performed on PVL-positive *S. aureus*. Genotypic characteristics of the PVL-positive *S. aureus* was determined by *spa* typing and multilocus sequence typing (MLST).

Results: The genes for PVL were detected in 67/214 (31.3%) of *S. aureus* isolates. Majority of the PVL-positive isolates were obtained from wound (n=37; 55.2%), blood (n=11; 16.4%) and urine (n=10; 14.9). Most of PVL-positive isolates (n=58; 34.7%) were methicillin sensitive *S. aureus* (MSSA) while nine isolates (19.1%) were methicillin resistant *S. aureus* (MRSA). *Spa* typing identified 14 different *spa* types, dominated by t355 (n=33; 49.3%), followed by t174 (n=7; 10.4%), t019 and t159 (n=5; 7.5%). MLST revealed six sequence types (ST) namely, ST152 (n=35), ST121 (n=9), ST1 (n=8), ST30 (n=8), ST772 (n=6) and ST15 (N=1).

Conclusion: This study revealed that 31.3% of *S. aureus* isolated in Jos hospitals carried genes for PVL, belonged to six sequence types and 14 *spa* types with t355-ST152-MSSA as the dominant genotype.

Keywords: *Staphylococcus aureus*; Panton Valentine Leukocidin; Polymerase chain reaction; *Spa* typing; Multilocus sequence typing

1. Introduction

Staphylococcus aureus remains an important opportunistic pathogen that cause a wide range of infections in humans and animals. In humans, infections caused by *S. aureus* vary from skin lesions to severe invasive infections including

* Corresponding author: Unyime C Essien
Department of Medical Laboratory Science, Faculty of Health Sciences and Technology, University of Jos, Nigeria.

bloodstream infections, necrotizing pneumonia and endocarditis [1]. The capacity of *S. aureus* to cause severe infections is due to their ability to express a wide variety of virulence factors that facilitate adhesion, invasion, and evasion of the host's immune system [2, 3].

Panton Valentine leukocidin (PVL) is a pore forming cytotoxin that causes destruction of leucocytes and tissue necrosis [4, 5]. PVL is encoded in *S. aureus* strains by two co-transcribed genes, *lukS-PV* and *lukF-PV,* which are carried by bacteriophages [6, 7]. PVL producing *S. aureus* was originally associated with pus formation, carbuncles, furuncles severe skin and soft tissue infection (SSTI), necrotizing pneumonia and sepsis [4, 8].

The prevalence of PVL-producing *S. aureus* varies in different countries including African countries [9, 10, 11]. The proportion of *S. aureus* that carry the genes for PVL gene in Nigeria have been investigated in isolates obtained in the South West [12] and North East [13, 14] of the country. Data on the prevalence of PVL-producing *S. aureus* in the North Central region of Nigeria is currently unavailable.

In this study we investigated *S. aureus* isolates obtained in tertiary hospitals in Jos, North Central Nigeria for the carriage of genes for PVL and their genetic background.

2. Material and methods

2.1. Ethical approval

This study was approved by the Ethical Committees of Jos University Teaching Hospital, Jos (JUTH) (Approval No. JUTH/DCS/ADM/127/XXV/314), Plateau State Specialist Hospital, Jos (PSSH) (Approval No. PSSH/ADM/ETH.CO/2017/006) and Bingham University Teaching Hospital, Jos, Plateau State (BUTH) (Approval No. NHREC/21/05/2005/00495).

2.2. Sources and identification of bacterial isolates

S. aureus (n=214; MRSA=47, MSSA=167) used in the study were cultured from different clinical specimens collected from three tertiary hospitals (JUTH=126; PSSH=66; BUTH=22) within the period of December 2017 to July 2019 in Jos, North Central Nigeria. These isolates were collected as part of routine bacteriological diagnostic investigations in the hospitals. The isolates were preserved in semi-solid agar medium (0.3% agar in brain heart infusion broth). They were re-tested and confirmed for growth and fermentation on mannitol salt agar, Gram stain, and positive results for catalase and tube coagulase tests at the Gram-Positive Bacteria Research Laboratory, Department of Microbiology, Faculty of Medicine, and Kuwait University, where the molecular studies were performed.

2.3. Antibiotic susceptibility testing

Antibiotic susceptibility testing was performed by the disc diffusion method according to the Clinical Laboratory Standard Institute [15]. Pure colonies of an overnight culture were suspended in normal saline and adjusted to a turbidity equivalent to 0.5 X MacFarland standard (1.5×10^8 CFU/ml). The suspension was poured on to Mueller-Hinton agar (MHA) (Oxoid, UK) plates and excess fluid was discarded and plates left to dry. Antibiotic discs were placed on the dried surface and the plates were incubated at 37°C for 24 hours. *S. aureus* strain, ATCC 25923, was used as control for sensitivity testing. The inhibition zone around each disc was measured and interpreted according to the Clinical Laboratory Standards Institute [15]. Sensitivity to fusidic acid by disc diffusion method was interpreted according [16], Sensitive ≥24, and resistance ≤20. The following antibiotic impregnated discs were used for sensitivity testing; cefoxitin (30µg), penicillin (2µg), gentamicin (10µg), kanamycin (30µg), erythromycin (15µg), clindamycin (2µg), chloramphenicol (30µg), tetracycline (30µg), trimethoprim (5µg), rifampicin (15µg), spectinomycin (25µg), fusidic acid (10µg), ciprofloxacin (5µg), and mupirocin (200µg) (OXOID, UK, Ltd.). Susceptibility to three non-antibiotic compounds, cadmium acetate, mercuric chloride and ethidium bromide, were also tested. The heavy metals and ethidium bromide impregnated discs were prepared in the laboratory with the indicated concentrations: cadmium acetate (50µg), mercuric chloride, (109µg) and ethidium bromide (50µg). *S. aureus* WBG 248 [17] was used as quality control strain to compare its inhibition zone to the test organism. Minimum inhibitory concentrations of cefoxitin, vancomycin, teicoplanin, and mupirocin were determined using Etest strips (BioMerieux, Marcy" Etoile, France). Methicillin resistance was confirmed by the detection of PBP2a with a rapid latex agglutination test (Denka Seiken, Tokyo, Japan) following instructions of the manufacturer.

2.4. Molecular analysis of S. aureus isolates

2.4.1. Extraction of S. aureus DNA for PCR

DNA isolation was carried out according to the method described by [18]. Three to five identical colonies of an overnight culture were picked using a sterile loop and suspended in a microfuge tube containing 50 µl of lysostaphin (150 µg/ml) and 10 µl of RNase (10 µg/ml) solution. The tube was incubated at 37 °C in the heating block (Thermo Mixer, Eppendorf, Hamburg, Germany) for 20 min. To each sample, 50 µl of proteinase K (20 mg/ml) and 150 µl of Tris buffer (0.1 M) were added and mixed by pipetting. The tube was then incubated at 60 °C in the water bath (VWR Scientific Co., Shellware Lab, United States) for 10 min. The tube was transferred to a heating block at 95^{o}C for 10 min to inactivate proteinase K activity. Finally, the tube was centrifuged, and the extracted DNA was stored at 4 °C till used for PCR.

2.4.2. Preparation of agarose gel

Gel electrophoresis was used to separate DNA on the basis of their sizes by applying an electric field to move the DNA through an agarose matrix. Two 2% (w/v) concentration of agarose gel used in the study was prepared by weighing and dissolving 4 grams of agarose powder (Promega, Madison, USA) in 200 ml Tris-Borate-EDTA (1XTBE buffer) (Gibco, UK) using microwaved oven. The mixture was heated in a microwave oven until it became clear and transparent. The molten agarose was allowed to cool to about 45^{o}C and 300 µl (1mg/ml) of ethidium bromide was added to the gel. The molten gel was gently poured in a mould (casting), and was allowed to solidify after which the comb was removed to create wells for DNA sample application. The gel was transferred from the mould into an electrophoretic chamber (Bio-Rad, USA) filled with (1x TBE) buffer.

2.4.3. Detection of lukS-PV and lukF-PV by PCR

The presence of lukS-PV and lukF-PV genes encoding Panton-Valentine leukocidin (PVL) was detected by PCR according to the method described by [4]. The 25µl volume of PCR reaction mixture consisted of 1.5µl of genomic DNA, 12.5µl of Hot Star Red Taq Master Mix and 10µl PCR H_2O, 0.5µl each of lukS-PV/ lukF-PV primers (Qiagen, Hilden, Germany). PVL gene was amplified using the following primers: luk-PV-1 (5'-ATC ATT AGG TAA AAT GTC TGG ACA TGA TCC A-3'); luk-PV-2 (5'-GCA TCA AST GTA TTG GAT AGC AAA AGC -3'). In each batch of the PCR reaction, PVL positive DNA sample and PCR water were added as PCR positive and negative control respectively. DNA amplification was carried out for 30 cycles according to the following protocol: 30 s of denaturation at 94^{o} C, 30 s of annealing at 55^{o} C, and 1 min of extension at 72^{o} C. Ten microlitres (10µl) of each PCR product and the controls were mixed each with 3 µl of tracking dye (bromophenol blue) in a PCR tube and transferred by pipetting into the wells of 2% (w/v) agarose gels. The electrophoretic chamber was connected to the power source and maintained at 90V for 30minutes. The gel was transferred to transilluminator and visualized by illumination with UV light. The DNA bands were viewed and recorded by photography using computer system (SynGene Bioimaging System) (Figure 3).

2.4.4. Spa typing

Spa typing was performed as described previously by [19]. The PCR protocol consisted of an initial denaturation at 94 °C for 4 min, followed by 25 cycles of denaturation at 94 °C for 1 min, annealing at 56 °C for 1 min, and extension for 3 min at 72 °C, and a final cycle with a single extension for 5 min at 72 °C. Five µl of the PCR product was analyzed by 1.5% agarose gel electrophoresis to confirm amplification. The amplified PCR product was purified using Micro Elute Cycle-Pure Spin kit (Omega Bio-tek, Inc. USA) and the purified DNA was then used for sequencing PCR. The sequencing PCR product was then purified using Ultra-Sep Dye Terminator Removal kit (Omega Bio-tek, Inc. USA). The Purified DNA was sequenced in an automated 3130x1 genetic analyzer (Applied Biosystem, USA). The sequenced *spa* gene was analyzed using the Ridom Staph Type software available on Ridom SpaServer at https//spaserver.ridom.de.

2.4.5. Multilocus sequence typing

The MLST was performed on isolates using the method previously described by [20]. MLST was performed by initial amplification and sequencing of seven housekeeping genes in each isolate. The sequencing PCR protocol consisted of initial denaturation for 1 min at 94°C, followed by 25 cycles of denaturation for 10s at 96°C, annealing at 55°C for 5s, and extension for 4 min at 66°C. DNA sequencing was performed using a 3130x1 genetic analyzer (Applied Biosystems, Foster City, CA, USA) in accordance with the manufacturer's protocol. The sequences were submitted to http://www.pubmlst.net/ where an allelic profile was generated and the sequence type (ST) assigned.

2.4.6. Statistical analysis

Data obtained from this study were analyzed using Statistical Package for the Social Sciences (SPSS) version 26.0. The presence of PVL gene MSSA, MRSA, sources of isolate 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.

3. Results

3.1. Prevalence of PVL-positive *S. aureus* according to clinical sources

A total of 214 *S. aureus* isolates were investigated by PCR to detect the carriage of *lukF-PV- lukS-PV* that codes for PVL. The results revealed that 67/214 (31.3%) *S. aureus* isolates were positive for *lukS-PV /lukF-PV* (Figure 1). The 67 PVL-gene positive isolates were analyzed further in this paper. *LukS-PV* and *lukF-PV* was detected in *S. aureus* isolates obtained from 10 of 14 clinical samples. The distribution of *lukS-PV and lukF-PV* by clinical samples is illustrated in Figure 1. Most of the PVL-positive *S. aureus* isolates were cultured from wounds (n=37; 55.2%), blood (n=11; 16.4%) and urines (n=10; 14.9). The remaining PVL-positive *S. aureus* isolates were recovered from catheter tip (n=2; 2.9%), HVS (N=2; 2.9%), and in single isolates each obtained from aspirate, CSF, ear swab, throat swab and umbilical cord specimens (Figure 2).

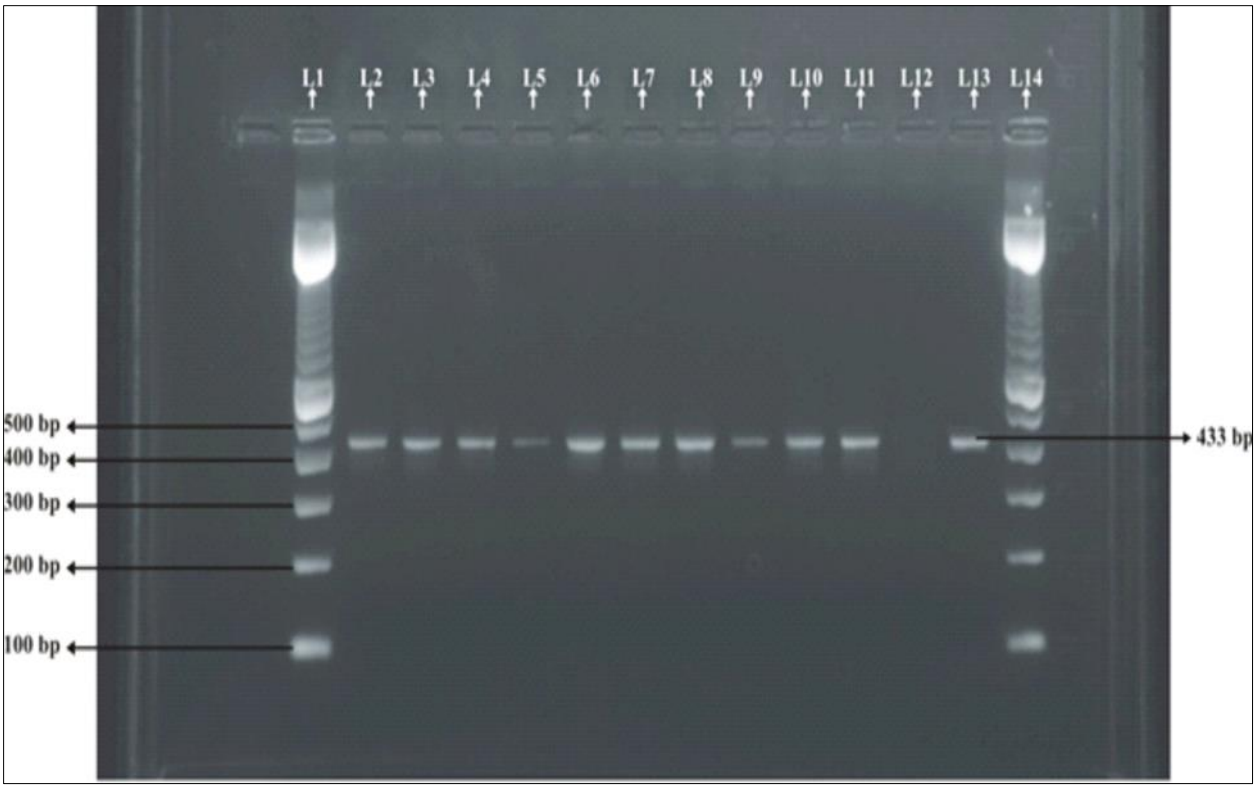

Figure 1 Agarose gel electrophoresis used for detection of amplified *lukSF-PV* gene by PCR. Lane 2, 3, 4, 5, 6, 7, 8, 9, 10 and 11 represents DNA bands from test samples positive for *lukSF-PV*. Lane 12 and 13 represents negative and positive *lukSF-PV* control sample respectively. The lane 1 and 14 represents 100bp DNA molecular ladder which was used for sizing DNA bands. However, the *lukSF-PV* gene is a 433 bp (amplicon size) DNA, therefore it was located between 400bp and 500bp.

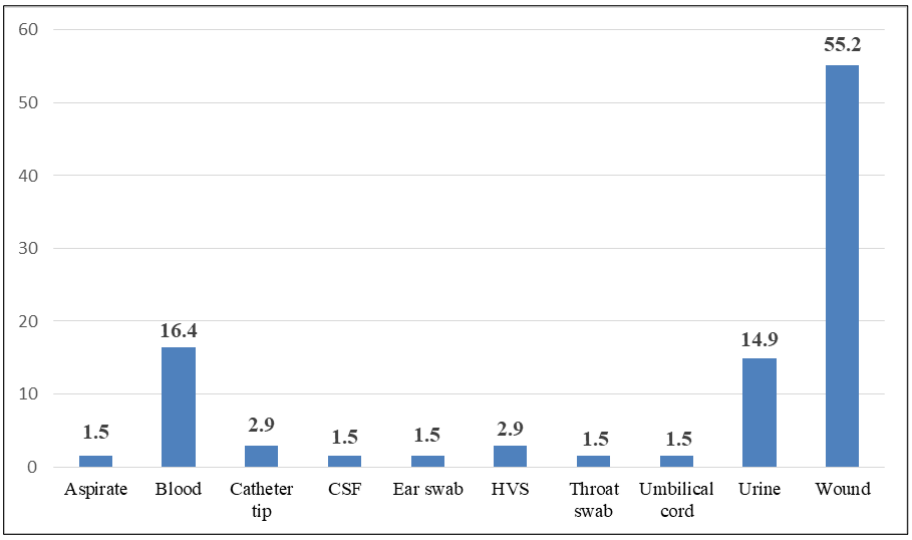

Figure 2 Prevalence of PVL-positive *S. aureus* according to clinical sources

3.2. Prevalence of PVL-positive *S. aureus* according to hospitals, MSSA and MRSA

The PVL gene was detected in *S. aureus* isolates obtained in all three hospitals, although the proportion of PVL-positive isolates varied among the hospitals. The proportion of PVL-positive isolates was lower in isolates obtained from PSSH (N=12; 18.2%) compared to those obtained from BUTH (N=7; 31.8%), and JUTH (N=48; 38.1%). However, the differences in the proportion of isolates carrying PVL genes according to hospitals were not statistically significant (P=0.600; P>0.05). Also, most of PVL-positive isolates (n=58; 34.7%) were MSSA (Table 1).

Table 1. Prevalence of PVL-positive *S. aureus* according to hospitals, MSSA and MRSA

Variables	No. of isolates	PVL positive isolates N (%)	P-value
Hospital			0.600
BUTH	22	7 (31.8)	
JUTH	126	48 (38.1)	
PSSH	66	12 (18.2)	
Strains			0.010
MRSA	47	9 (19.1)	
MSSA	167	58 (34.7)	

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

3.3. Antimicrobial resistance of *lukS-PV* and *lukF-PV* -positive isolates

The antimicrobial resistance of the PVL-positive *S. aureus* isolates is presented in Figure 3. All 67 isolates were sensitive to vancomycin (MIC ≤ 2µg/ml), teicoplanin (MIC ≤ 2µg/ml), rifampicin, mupirocin, spectinomycin and chloramphenicol but were resistant to penicillin 60/67 (89.6%) and trimethoprim 50/67 (74.6%). Fifteen isolates (22.4%) were resistant to ciprofloxacin. Tetracycline resistance was found in 13 (19.4%) isolates. Nine (13.4%) isolates each were resistant to cefoxitin, gentamicin and kanamycin. Erythromycin resistance was detected in two (2.9%) isolates, while fusidic acid resistance was found in a single (1.5%) isolate. Resistance to non-antibiotic agents were as follows; cadmium acetate 44 (65.7%), mercuric chloride 19 (28.4%) and ethidium bromide 8 (11.9%). The cefoxitin-resistant isolates were positive for PBP 2a and were therefore confirmed as methicillin-resistant.

Nine MRSA isolates (19.1%) were positive for PVL gene, while 58 (34.7%) of MSSA isolates were PVL-positive.

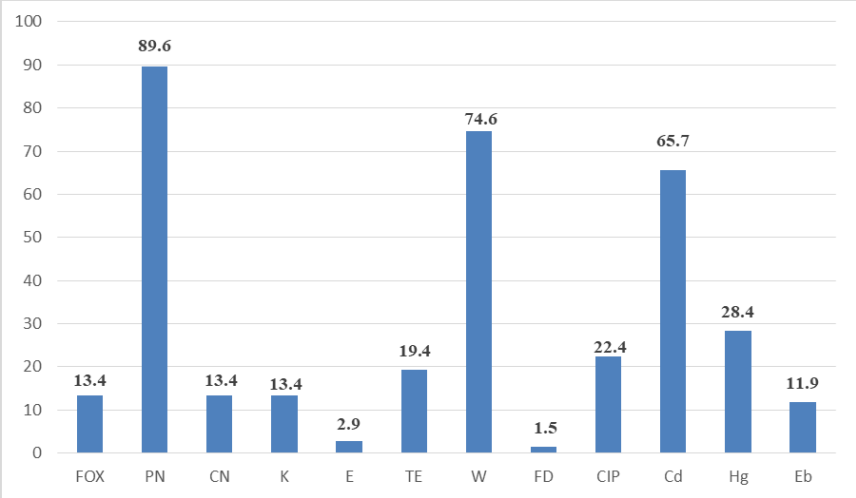

Figure 3 Antimicrobial resistance profile of PVL-positive isolates

FOX, Cefoxitin, PN, Penicillin G, CN, Gentamicin, K, Kanamycin, E, Erythromycin, TE, Tetracycline, W, Trimethoprim, FD, Fusidic acid, CIP, Ciprofloxacin, Cd, Cadmium acetate, Hg, Mercuric chloride, Eb, Ethidium bromide.

3.4. Molecular characterization of PVL-positive *S. aureus* (PPSA) isolates

The molecular characteristics of the isolates are summarized in Table 2. Multilocus sequence typing of the 67 PPSA isolates identified six sequence types (ST). These were ST152 (n=35), ST121 (n=9), ST1 (n=8), and ST30 (n=8), ST772 (n=6) and ST15 (n=1).

Spa typing revealed 14 *spa* types comprising t355 (n=33; 49.3%), t174 (n=7; 10.4%), t019 (n=57; 5%), t159 (n=5; 7.5%) and t345 (N=4; 5.9%) as the common *spa* types. *Spa* types, t657, t037, t1991 and t18034 were each detected in 2 (2.9%) isolates, and *spa* types, t127, t346, t318, t3488, and t2304 were each detected in single isolates.

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

3.4.1. ST772-MRSA, [PVL⁺]

The ST772-MRSA [PVL⁺] clone consisted of six isolates that were associated with two *spa* types, t345 (n=4) and t657 (n=2). All six isolates were resistant to cefoxitin, penicillin, trimethoprim, gentamicin, kanamycin, erythromycin, and ciprofloxacin and cadmium acetate. In addition, two isolates belonging to ST772-MRSA-V [PVL⁺] + t657, were resistant to mercuric chloride, and one of the isolates was resistant to tetracycline.

3.4.2. ST1-MSSA, [PVL⁺]

The ST1-MSSA, [PVL⁺] clone consisted of eight MSSA isolates that belonged to *spa* types, t174 (n=7) and t127 (n=1). Six of the isolates were resistant to trimethoprim. Five and three isolates were resistant to penicillin and tetracycline respectively.

3.4.3. ST15-MSSA [PVL⁺]

A single isolate was identified as ST15-MSSA [PVL⁺] clone. This isolate was cultured from a urine specimen. It belonged to *spa* type t346, and was resistant to trimethoprim, mercury chloride and ethidium bromide.

3.4.4. ST30-MSSA, [PVL⁺]

Eight isolates were identified as ST30-MSSA, [PVL⁺]. The isolates were associated with *spa* types t019 (n=5), t037 (n=2) and t318 (n=1). All eight isolates were resistant to penicillin. In addition, two isolates were resistant to ciprofloxacin.

3.4.5. ST121-MSSA [PVL⁺]

The ST121-MSSA [PVL⁺] clone consisted of nine isolates that were associated with *spa* types, t159 (n=5), t1991 (n=2), t3488 (n=1) and t2304 (n=1). All nine isolates were resistant to penicillin. Six isolates were resistant to trimethoprim.

3.4.6. ST152 -MRSA [PVL+]/ST152-MSSA [PVL+]

A total of 35 PVL-positive *S. aureus* isolates belonged to sequence type, ST152 with 32 of the isolates being ST152-MSSA and only three were ST152-MRSA. The 35 isolates belonged to *spa* types, t355 (n=33) and a novel *spa* type, t18034 (n=2). Apart from cefoxitin resistance observed in the three ST152-MRSA, [PVL+] isolates, all 35 isolates had the same resistance profiles and were resistant to penicillin, gentamicin, kanamycin, tetracycline, and trimethoprim. All three ST152-MRSA and 30 ST152-MSSA belonged to t355. Two ST152-MSSA isolates were associated with the novel *spa* type, t18034.

Table 2 Characteristics of PVL-positive isolates

Strain Description	Spa types (N)	Clinical samples (N)	Antimicrobial resistance
ST772-MRSA [PVL+]	t345 (4)	Urine (1), Blood (1), Wound (2)	FOX (4), P (4), CN (4), K (4), E (4), W (4), CIP (4), CD (4).
ST772-MRSA [PVL+]	t657 (2)	Wound (2)	FOX (2), P (2), CN (2), K (2), E (2), TE (1), W (2), CIP (2), CD (2), Hg (2)
ST1-MSSA [PVL+]	t174 (7)	Wound (4), Catheter (2), Umbilical cord (1)	P (4), TE (3), W (5), CD (3)
ST1-MSSA [PVL+]	t127 (1)	Urine (1)	P, W, Hg
ST15-MSSA [PVL+]	t346 (1)	Urine (1)	W, Hg, Eb
ST30-MSSA [PVL+]	t019 (5)	Wound (3), Blood (2)	P (5), CD (2)
ST30-MSSA [PVL+]	t037 (2)	Blood (1), Wound (1).	P (2), CIP (2), TE (1), CD (2), Hg (2), Eb (2)
ST30-MSSA [PVL+]	t318 (1)	Wound (1)	P, W, CD, Eb
ST121-MSSA[PVL+]	t159 (5)	Wound (2), Bood (2), Ear swab	P (5), W (4), CD (1)
ST121-MSSA[PVL+]	t1991 (2)	HVS (1), Aspirate (1)	P (2), CD (2), Hg (2)
ST121-MSSA[PVL+]	t3488 (1)	Wound (1)	P, W, CD, Hg
ST121-MSSA[PVL+]	t2304 (1)	Urine (1)	P, W, Hg
ST152-MRSA[PVL+]	t355 (3)	Wound (3)	FOX (3), P (3), CN (2), K (2), CD (3), TE (1)
ST152-MSSA [PVL+]	t355 (30)	Wound (18), Urine (5), Blood (4), HVS (1), CSF (1), Throat (1)	P (27), W (25), TE (5), CIP (7), CN (1), K (1), CD (21), Hg (7), Eb (4), FD (1).
ST152-MSSA [PVL+]	t18034 (2)	Urine (1), Blood (1)	P (2), TE (2), W (2), CD (2), Hg (2), Eb (1)

Abbreviations: FOX, Cefoxitin, PN, Penicillin G, CN, Gentamicin, K, Kanamycin, E, Erythromycin, TE, Tetracycline, W, Trimethoprim, FD, Fusidic acid, CIP, Ciprofloxacin, Cd, Cadmium acetate, Hg, Mercuric chloride, Eb, Ethidium bromide.

4. Discussion

Some *S. aureus* strains cause severe infections because of the diverse virulence factors at their disposal which enhance their invasiveness, evasion of host immune facilities and capacity to cause tissue damage [10, 21]. PVL is one of the virulence factors that enhance bacterial virulence by destroying human tissues and white blood cells [4, 5]. Knowledge of the carriage of the genes for PVL in *S. aureus* enhances our understanding of their capacity to cause infections.

Our study detected the genes for PVL in 31.3% of the *S. aureus* isolates obtained from three tertiary hospitals in Jos, North Central Nigeria. This result concurs with the 34.6% prevalence reported previously in *S. aureus* obtained in South West Nigeria [22], and the 39.3% prevalence reported among clinical *S. aureus* isolates obtained from Obafemi Awolowo Teaching Hospital, Nigeria [23]. Similarly, PVL-positive strains accounted for 36% of *S. aureus* investigated in a study in USA [24]. Our result was lower than the proportion of PVL-positive *S. aureus* reported in Maiduguri, North East Nigeria (52.1%) [13]; the 61.4% in the Gambia [25], the 58% in Sudan [26], the 73.91% in Colombia [27], the 61.5% in India [28], the 56.2% in Iran [29], the 46.3% in Kazakhstan [30] and the 62.5% in Trinidad and Tobago [31]. In contrast, our result was higher than the prevalence of PVL-positive strains reported from several other studies in Abuja, Nigeria that reported a prevalence of 10.7% among isolates obtained from Maitama District Hospital [14], the 12.7% prevalence reported in Kuwait [8], the 23% reported in Iran [32] and the 18.6% prevalence reported in Brazil [33]. These studies suggest geographical differences in the prevalence of PVL-positive *S. aureus*.

The PVL-positive *S. aureus* were grown from different clinical samples including wounds, Blood, respiratory samples, and a variety of other samples except eye swab, nasal swab, semen, and urethral swab. Previously PVL-producing *S. aureus* were associated with skin and soft tissue infections, and necrotizing pneumonia [4, 5]. Despite the small numbers of PVL-positive *S. aureus* in this study, their presence in a range of clinical samples indicates their capacity to cause a wider range of infections than were previously reported.

Most of the PVL-positive isolates in this study (58/167; 34.7%) were MSSA with only 19.1% (9/47) being MRSA. This confirmed the results of previous studies conducted in Nigeria [22] that reported more PVL-positive strains among MSSA (53.3%) compared to 9.1% in MRSA isolates. Similarly, PVL was positive in 63% of MSSA and in 37% MRSA that were investigated in a study in Iran [32]. In contrast, higher PVL-positive strains were reported in MRSA (14.6%) than in MSSA (12.0%) in Kuwait [8], and MRSA (80.92%) and MSSA (67.59%) in Colombia [27]. These results further suggest geographic differences in the distribution of PVL-positive *S. aureus* strains.

The PVL-positive isolates in this study belonged to diverse genetic backgrounds with most belonging to ST152 and t355. Similarly, studies in many countries in Africa including Nigeria [12, 34], Mali [35], Cameroon [9], Ghana [11], Senegal [36], Gabon [37] and Burkina Faso [38] have reported ST152 as the dominant sequence type in these countries.

The three PVL-positive ST152-MRSA and 30 ST152-MSSA isolates belonged *spa* type t355 and were resistant to gentamicin and tetracycline suggesting that the PVL-positive MRSA could have evolved through the acquisition of *mecA,* that confers methicillin resistance, by ST152 -MSSA isolates. A study conducted in Kuwait[39], identified PVL-positive ST152-MRSA-V isolates, that were also resistant to gentamicin, tetracycline, and trimethoprim suggesting that resistance to gentamicin and tetracycline may be a common characteristic of ST152 isolates.

ST121-MSSA [PVL+] was the second common PVL- positive *S. aureus* isolates in this study. The isolates were recovered from wound, blood culture, HVS, urine, aspirate and ear swab indicating that ST121-MSSA can cause a wide range of infections in contrast to their earlier association only with skin and soft tissue infections [40]. The nine (ST121-MSSA [PVL^+]) isolates belonged to different *spa* types: t159 (n=5), t1991 (n=2), t3488 (n=1) and t2304 (n=1) suggesting an emerging genetic diversification of the ST121-MSSA [PVL^+] strain in Jos, Nigeria. The ST121-MSSA [PVL^+] clone has also been reported from other hospitals in Nigeria [23,12], Burkina Faso [38], Cameroon [9], France [41], South Africa [42], Myanmar [43] and Italy [44] confirming the global presence of this clone [40].

The other common strain was the ST30-MSSA, [PVL^+] lineage, which has also been reported in China [45] and Lebanon [46] to be similar to MSSA clade that evolved into the Southwest Pacific MRSA clone of community-associated MRSA [47, 48, 49, 40].

The ST1-MSSA [PVL^+] isolates in this study were associated with *spa* types, t174, and t127. Whereas t127 is widely reported in association with ST1-MSSA, [PVL^+] in Malaysia [50], Lebanon [46], and Chicago [51], ST1-MSSA belonging to t174 are widespread and have been isolated in different countries including Germany, South Africa, Iceland, Austria, Ireland, Netherland and United Kingdom (www.spa.ridom.de/spa-t174.shtml).

The ST772-MRSA-V [PVL+], also known as the Bengal Bay Clone, is a multi-resistant PVL-positive MRSA that was initially isolated in Bangladesh and India between 2004 and 2005 [52]. However, the ST772-MRSA-V [PVL+] clone has been reported in other countries including Ireland [53], Saudi Arabia [54], Hong Kong [40], Nepal [55], Italy [56], New Zealand [57], and Kuwait [58]. The transmission of the Bengal Bay clone outside India has been associated with travel history to or from India [59, 60, 40]. The Bengal Bay clone described previously in Nigeria [61] among *S. aureus* isolates was recovered from patients in Lagos State University Teaching Hospital. The report of the ST772-MRSA [PVL+] in this study represents an expansion of this clone in Nigeria. The six ST772-MRSA [PVL+] isolates were resistant to cefoxitin, penicillin, gentamicin, erythromycin, kanamycin, trimethoprim and tetracycline, similar to the isolates obtained in India [62, 63] and Norway [64]. Although it is not possible to establish how this strain was introduced into Jos, this report suggests a possible importation of the Bengal Bay MRSA clone into Jos and other parts of Nigeria.

Although a single isolate of PVL-positive ST15-MSSA lineage was obtained in this study, the ST15-MSSA is widespread and was detected previously among isolates obtained from human patients [23, 12, 34] and animals [65] in Nigeria. ST15-MSSA [PVL+] isolates were also reported in *S. aureus* that were isolated from skin infection of asylum seekers in Switzerland [66] and in *S. aureus* submitted to the Staphylococcus reference center in France [41] supporting that this clone is widespread globally.

5. Conclusion

This study has revealed the rate of carriage of PVL gene among clinical *S. aureus* in Jos's hospitals. In addition, the frequency of PVL genes was higher in MSSA isolates compared to MRSA and most of the PVL-positive isolates belonged to diverse genetic backgrounds and cause a wide range infection.

Compliance with ethical standards

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Authors Contributions

Conceptualization: Edet E. Udo; Investigation: Samar S. Boswihi, Unyime C. Essien; Methodology: Samar S. Boswihi, Unyime C. Essien; Supervision: Edet E. Udo, Nneka R. Agbakoba; Writing-original draft: Unyime C. Essien; Writing-review and editing: Edet E. Udo, Unyime C. Essien, Samar S. Boswihi, Nneka R. Agbakoba.

Disclosure of conflict of interest

We have no conflict of interest to disclose.

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