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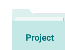

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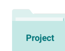

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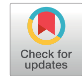

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ABSTRACT

Background: Resistant and virulent *Staphylococcus aureus* is a global public health challenge. Staphylococcal Bi-component leukotoxins are cytolytic to immune cells and evolve to disarm the innate immunity during infections, hence the severity of the disease.
Objective: We studied drug resistance profile and the occurrence of bi-component leukocidin in clinical and nasal *S. aureus* in Lagos, Nigeria.
Method: Ninety-two *S. aureus* (70 clinical and 22 nasal) strains were characterized by conventional and molecular methods.
Result: Of the resistance profiles generated, no isolate was resistant to fosfomycin, fusidic acid, teicoplanin, vancomycin, linezolid, mupirocin, nitrofurantoin and tigecycline. Twelve MRSA carrying staphylococcal cassette chromosome *mec*A gene types I, III, and IV elements were identified only in the clinical samples and type I dominated. High rates of *luk*E/D (100% among MRSA) and *luk*PV (dominated MSSA) were recorded among the nasal and clinical isolates. *Staphylococcus aureus* harboring only *luk*E/D (from clinical & colonizing MSSA) and combined *luk*E/D and *luk*PV (mostly from clinical MSSA, colonizing MSSA and clinical MRSA) toxins were found.
Conclusion: Although, *mec*A resistant genes were found only among clinical MRSA, the occurrence of other bi-component leukocidin genes in a large proportion among the isolates from both community and clinical settings is a major concern. The need for effective resistance and virulence factor surveillance, re-enforcement of antibiotic stewardship and good infection control policy, to prevent dissemination of epidemic strains is highlighted.

1. Background

Staphylococcus aureus is one of the most important and well-kown human pathogens. While some strains could be common commensals, some are leading causes of hospital and community associated infections [1]. The major factors associated with its pathogenicity are acquired antibacterial resistance and production of several virulent factors. The development of resistance is precipitated principally by indiscriminate and increasing use of antimicrobials. Initially, multidrug resistant (MDR) and methicillin resistant *S. aureus* (MRSA) strains predominate in hospitals, but, in the past few decades, several epidemics of community infections have been reported in apparently healthy populations [2]. From the available reports, about five community associated (CA)-MRSA clones causing outbreaks have been reported [3]. Universally, *S. aureus* causes a variety of infections including skin and soft tissue infections (SSTIs) and life-threatening systemic complications such as bactereamia [4].

The seriousness of the infections associated with this organism reflects its distinct abilities to escape the immune response, using multiple virulence factors including a group of pore-forming toxins known as bicomponent leucotoxins [5]. The bi-component pore forming toxins (BCPFTs) include the phage-encoded Panton–Valentine leucocidin (PVL; *lukPV*), the chromosomally encoded *luk*E/D and *luk*A/B and gamma-hemolysins (*hlgA* and *hlgCB*) genes. The BCPFTs are made up of two subunits of proteins designated as S and F. When the S subunit binds to cellular receptor, it forms heterodimer with the F component which is followed by multi-merization with subsequent pore formation on red blood cells and polymorphonuclear cells [6,7]. Additionally,

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individual subunits from different BCPFTs could form new functional toxins [6]. A report showed the interference of *lukS-PV* with the binding of three antibodies to human complement C5a receptor, an important step in sensing bacterial infection by phagocytes [8].

The binding of *luk*S-PV to C5aR causes the inhibition of neutrophils activation by displacing the natural ligand [8]; thereby signifying that *luk*S-PV alone may mediate immune evasion in *S. aureus* infections. Also, the individual subunits of these toxins could cross, combine and cause distinct lysis. Morinaga et al. [9] provided evidence that the S and F subunits between *luk*E/D and *hlg* are basically switchable and a combination of S subunit of PVL (*luk*S-PV) with *luk*D may form heamolytic toxin [6]. Thus, different toxins could be formed by cross-combination of leucotoxin subunits. A significant association has also been demonstrated between *luk*E/D expression and *S. aureus* invasive infections [10] especially those linked with cutaneous and urinary tract [5]. Similarly, studies have underscored the potential role of *luk*E/D as a critical virulence factor of *S.aureus* from Human Immunodeficiency Virus (HIV) infected persons with skin dermatitis and furuncles [11], diabetic foot ulcers [12], impetigo [13], and *S. aureus*-associated diarrhoea [14]. In Iran, more than 73% of *S. aureus* screened for *luk*E/D possessed the gene [5]. Data from Hilla/Iraq, indicated that *luk*ED-containing strains were detected among 9 and 11 of 24 CA- and HA-MRSA isolates analysed [15].

Several investigators have, however, denoted that PVL is the core virulence factor for CA-MRSA [16] and indeed, it can be found in both MRSA and MSSA strains [17,18]. It has been shown that PVL produces cytolytic activity specifically, on human and rabbit cells and induce pro-inflammatory cytokines in human and murine macrophages [19]. Furthermore, it acts to target prophylaxis and immunotherapy. Panton–Valentine leucocidin (PVL) - related infections or colonization have been reported among healthy individuals [15], travelers [20], rural settlers [21] and hospital staff/students [18]. Most *pvl* positive - *S. aureus* are associated with furunculosis and severe pneumonia [22]. The West and Central African countries have particularly shown high prevalence of PVL-positive MSSA isolates [23,24].

Some authors have expressed concerns about *S. aureus* strains possessing unique combinations of antibiotic resistance determinants and toxins. For instance, the genetic analysis of CA-MRSA in France described the co-occurrence of *pvl* and *luk*E/D genes in all the isolates [22]. Vandenesch et al. [25] also revealed the presence of both *pvl* and *hlg* sequences in MRSA from Oceania. In Nigeria, however, particularly the Southwest region, high occurrence of *pvl*-positive *S. aureus* has been described in both clinical and nasal isolates of *S.aureus* [26–28]. Most of these investigators have also insisted that the increasing resistance of our isolates to antibiotics remains a challenge [27,28]. Even though, the results of a study in 2009 showed the occurrence of *luk*E/D genes in CA-MRSA [28], information on Nigerian *S. aureus* for their bi-component leucotoxin contents other than PVL is extremely inadequate. Thus, the significance of these toxins necessitated the design and their investigation amongst invasive and colonizing methicillin sensitive and resistant strains. Efforts was also directed at determining the co-occurrence of the toxins in the isolates screened and the resistance status of the isolates to enhance the implementation of drug restriction/control policy needed for effective management of staphylococcal infections.

2. Materials and methods

2.1. Ethics and study site

The study approval was obtained from the Institutional Review Board (IRB) of the College of Medicine, University of Lagos, Nigeria (ref. No: CM/COM/8/VOL.XIX). Between June 2007 and April 2009, clinical samples were collected from Lagos University Teaching Hospital (LUTH), Idi-araba and National Orthopaedic Hospital, Igbobi (NOHI). Nasal swabs were also obtained from non-hospitalised and apparently healthy individuals in six local council areas of Lagos state (Mushin, Surulere, Mainland, Alimosho, Ikeja and Agege). All participants consented and filled the informed consent form.

2.2. Study population

The cohort comprised of 200 adults (male and female of 18 years and above) within hospital settings that had various clinical conditions (septicemia, urinary tract infection, wound infection, urogenital infection and respiratory infection). One hundred apparently healthy volunteers were screened for carriage of *S. aureus* in the anterior nares using sterile swabs.

2.3. Inclusion and exclusion criteria

Patients included in this study were those hospitalised and specimens were collected for bacterial aetiology after admission (clinical). Patients not on admission and those on the first day of admission were also excluded (clinical). For determination carriage status, apparently healthy volunteers with no history of recent hospitalization In addition, samples were not collected from individuals with any form of facial wounds, rhinitis, catarrh and skin conditions.or antibacterial consumption (Community/colonizing) were screened. Children and all those who refused consent were excluded.

2.4. Sample processing and identification of Staphylococcus aureus

The primary laboratory isolation of bacterial organisms was carried out at the Nigerian Institute of Medical Research, Yaba. Isolation of *Staphylococcus* was achieved using appropriate media [30]. Suspected *S. aureus* isolates were identified based on standard bacteriological procedures including Gram reaction, catalase test, tube coagulase test, DNase test and confirmed with VITEK 2 system ID-GP card (BioMérieux, Marcy Etoile, France). A single isolate was selected per sample. The molecular characterization of *S. aureus* was carried out at Microbiology laboratory of Otto-von-Guericke Universitat, Magdeburg, Germany.

2.5. Antimicrobial susceptibility testing

Susceptibility to twenty (20) different antimicrobial agents (penicillin, cefoxitin, oxacillin, clindamycin, erythromycin, fosfomycin, Fusidic acid, gentamycin, levofloxacin, linezolid, moxifloxacin, mupirocin, vancomycin, nitrofurantoin, rifampicin, teicoplanin, tetracycline, tigecycline, tobramycin and trimethoprim/sulfamethoxazole) was performed using VITEK 2 system, AST P580 card (BioMérieux, Marcy l'Etoile, France). *S. aureus* ATCC 29213 was used as control strain. The VITEK 2 Minimum Inhibitory Concentration (MIC) results were interpreted using the Advanced Expert System of the VITEK 2 system. Multi-Drug Resistance (MDR) was defined as resistance to one or more antibiotics in three or more categories of drugs [31].

2.6. DNA extraction

Two to three colonies of overnight blood agar culture of *S. aureus* was pre-treated with lysostaphin (QIAgen, Hilden, Germany) (20 μg/ml) in 160 μl of TE buffer (10 mmol of Tris HCl/liter, 1 mmol of EDTA/liter, pH 8.0) at 37 °C for 30 min. The cells were harvested and DNA was extracted using a DNeasy tissue kit as recommended by the manufacturer (Qiagen, Hilden, Germany). The concentration of DNA was estimated spectrophotometrically.

2.7. Determination of SCCmec type

Staphylococcal Cassette Chromosome *mec* (SCC*mec*) types were determined using multiplex PCR as previously described [32]. The eight

different loci (A through H) of SCC*mec* were amplified along with a *mec*A gene which serves as internal control. The profiles obtained were characterized and defined according to MRSA nomenclature [33].

2.8. PCR detection of PVL genes

Panton-Valentine leucocidin (PVL) gene (433bp) using primers and conditions as described by Lina et al. [34]. The PCR products were analyzed by electrophoresis using 2% agarose gel (Sigma) stained with 2 μl of 1% ethidium bromide and visualized by UV illumination (Gel logic Imaging system (Kodak, Germany)). *S. aureus* ATCC 13300 served as the reference strain for *luk*PV, *luk*E/D and γ-hemolysin gene.

2.9. PCR detection of γ-hemolysin and lukE/D genes

Sequences specific for γ-hemolysin and *luk*E/D genes were detected by PCR using previously described primers [35]. Amplification was carried out in the following conditions: an initial 1 min denaturation step at 95 °C followed by 30 stringent cycles (1 min of denaturation at 95 °C, 1 min of annealing at 55 °C and 2 min of extension at 72 °C and finally extension step at 72 °C for 3 min). PCR products were analyzed by electrophoresis using 2% agarose gel (Sigma) stained with 2 μl of 1% ethidium bromide and visualized by UV illumination (Gel Logic Imaging System, Kodak, Germany).

3. Results

From the 300 samples analyzed, 92 *S. aureus* comprising 70 clinical isolates and 22 nasal isolates were identified. Twelve (17%) of the *S. aureus* from clinical (**CL**) sources possessed *mec*A gene (MRSA), while 58 (83%) were identified as MSSA. All the nasal (**NL**) isolates were MSSA strain. Antibiotic susceptibility profile showed that the MRSA were resistant to 12 antibiotics. The clinical MSSA were resistant to 8 and the nasal MSSA were resistant to 5 antibiotics. In all, no resistance was detected against eight antibiotics tested (fosfomycin, fusidic acid, teicoplanin, vancomycin, linezolid, mupirocin, nitrofurantoin and tigecycline). The antibiotic susceptibility and resistance patterns were presented in Fig. 1. Amongst the CL-MSSA isolates, resistance to penicillin, tetracycline and trimethoprim/sulfamethoxazole were common phenotypes (Table 1), while resistance to penicillin, cefoxitin, oxacillin, tetracycline and trimethoprim/sulfamethoxazole were the common phenotypes among the MRSA (Table 2). The penicillin-trimethoprim/sulfamethoxazole phenotypes predominate among the isolates from nasal carriers (Table 3).

Three different SCCmecA types were identified. Sixty-six (66) percent of the isolates belong to SCC*mec* type I, and 17% were SCC*mec* type III and IV respectively (Fig. 2a and b) below.

Lane M contained 100 kb molecular weight marker. Lane C was a negative *S. aureus* control. Lane 1–10 had isolates within the loci specific to SCCmec types: (A) SCCmec types I; (B) SCCmec type III; and (C) SCCmec type IV.

The PVL-positive isolates were detected in all the 3 groups of *S. aureus* studied. Eleven of the 12 (92%) MRSA isolates, 57/58 (98%) clinical MSSA isolates and 20/22 (90%) carrier MSSA were positive for *luk*PV gene. All (100%) MRSA harboured the *luk*E/D gene and 53 (91%) clinical MSSA had *luk*E/D gene and 18 (82%) carrier MSSA possessed *luk*E/D gene. A combination *luk*PV - *luk*E/D was recognized in all clinical-MRSA except one (11/12; 92%). Likewise, 51 (%) of clinical-MSSA and 16 (%) colonizing-MSSA possessed this combination of *luk*PV-*luk*E/D genes. The prevalence of the virulence genes among the three groups of *S. aureus* is shown in Fig. 3. However, the hemolysin (*hlg* A/B) genes were not detected in all the 92 *S. aureus* analyzed.

4. Discussion

The present study delineated three groups of *S. aureus* (CL-MRSA, CL-MSSA, NL-MSSA) which showed high resistance to some antimicrobial agents of different classes. Staphylococcal studies have identified increasing rates of resistance to β-lactam and non β-lactam antibiotics [17,26,29,36,37]. In the past decade, there has been an increase in fusidic acid resistance in a number of countries [3]. Also, the prevalence of mupirocin resistance in *S.aureus* has increased in settings where this agent is used extensively [3]. Fortunately, resistance to this drug is not detected in this study. Where resistance is not recorded, both fusidic acid and mupirocin are effective topical antibacterial agents for the management of skin infections and *S. aureus* colonization.

In this study, we report resistance rates of 23% and 17% for levofloxacin and moxifloxacin respectively. Previous Nigerian literature has shown high rates (100%) of *S. aureus* susceptibility to levofloxacin and moxifloxacin [26,36]. However, our result is consistent with an earlier finding on moxifloxacin resistance among staphylococcal isolates from burn subjects in Lagos [37]. In recent time, fluoroquinolones have been one of the most prescribed drugs in Nigeria for major infections [38]. However, misuse of these agents may have conferred sufficient selective pressure for *S. aureus* resistance to levofloxacin and moxifloxacin. This remains the bane of current multi-drug resistance in developing countries and globally. The extent of drug resistance and presence of virulence genes makes the treatment of staphylococcal infections difficult

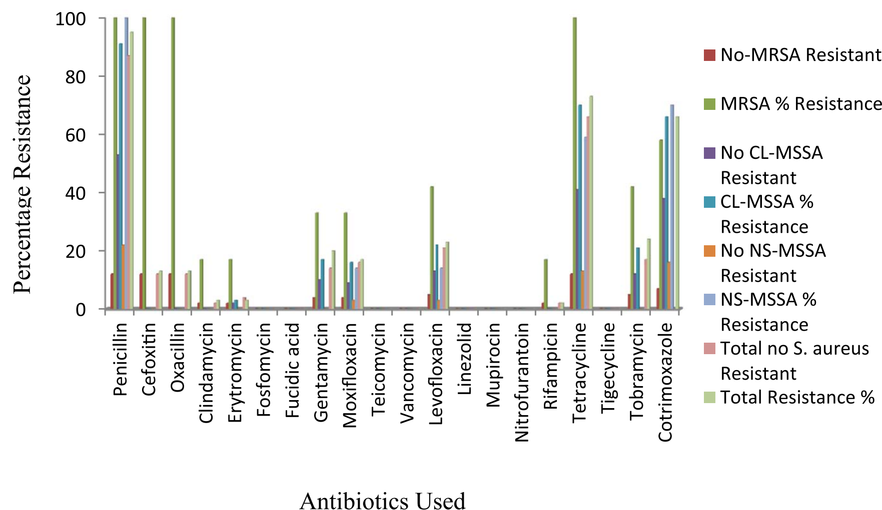


Fig. 1. Resistance Rates of the 92 *Staphylococcus aureus* to different Antibiotics.

Table 1
Gene content and resistant phenotypes of clinical methicillin susceptible *Staphylococcus aureus*.

Isolate ID	Sample Source	Genotype				Resistant phenotype
		PVL	*hlg*A	*hlg*B	*luk*E	
CL1	Wound	+	–	–	+	0
CL8	Urine	+	–	–	+	0
CL10	Blood	–	–	–	+	Pen
CL14	Wound	+	–	–	+	SXT
CL20	Ear	+	–	–	–	SXT
CL23	Urine	+	–	–	+	Pen, Tet
CL32	Pus	+	–	–	+	Pen, Tet
CL38	Wound	+	–	–	+	Pen, Tet
CL41	blood	+	–	–	+	Pen, Tet
CL47	Ear	+	–	–	+	Pen, Tet
CL53	Wound	+	–	–	+	Pen, Tet
CL54	Blood	+	–	–	+	Pen, Tet
CL62	Blood	+	–	–	+	Pen, Tet
CL67	US	+	–	–	+	Pen, Tet
CL75	Ear	+	–	–	+	Pen, Tet
CL76	Wound	+	–	–	+	Pen, Tet
CL77	Pus	+	–	–	+	Pen, Tet
CL83	Wound	+	–	–	+	Pen
CL85	Wound	+	–	–	+	Pen, SXT
CL87	Wound	+	–	–	+	Pen, SXT
CL91	Blood	+	–	–	+	Pen, SXT
CL94	Wound	+	–	–	+	Pen, SXT
CL98	Urine	+	–	–	–	Pen, SXT
CL102	HVS	+	–	–	+	Pen, SXT
CL115	Ear	+	–	–	+	Pen, SXT
CL117	Wound	+	–	–	+	Tet, SXT
CL123	Sputum	+	–	–	+	Pen, Tet, Tm
CL128	CT	+	–	–	+	Pen, Tet, SXT
CL135	Wound	+	–	–	–	Pen, Tet
CL139	Pus	+	–	–	+	Pen, Tet
CL142	Ear	+	–	–	+	Pen, Tet
CL147	Sputum	+	–	–	–	Pen, Tet
CL151	HVS	+	–	–	+	Pen, Tet
CL164	Urine	+	–	–	+	Pen, Tet
CL175	Wound	+	–	–	+	Pen, Tet
CL176	Urine	+	–	–	+	Pen, Tet
CL187	US	+	–	–	+	Pen, Tet
CL189	Wound	+	–	–	–	Pen, Tet
CL193	Sputum	+	–	–	+	Pen, Tet
CL200	Wound	+	–	–	+	Pen, Tet
CL207	Wound	+	–	–	+	Pen, SXT, E
CL218	Wound	+	–	–	+	Pen, SXT, E
CL226	Wound	+	–	–	+	Pen, GM, TM, SXT
CL239	Sputum	+	–	–	+	Pen, MXF, LEV, Tet
CL245	Wound	+	–	–	+	Pen, MXF, LEV, Tet, SXT
CL247	CT	+	–	–	+	Pen, MXF, LEV, Tet, SXT
CL251	Urine	+	–	–	+	Pen, MXF, LEV, Tet, SXT
CL262	Blood	+	–	–	+	Pen, MXF, LEV, Tet, SXT
CL279	Wound	+	–	–	+	Pen, MXF, LEV, Tet, SXT
CL282	Wound	+	–	–	+	Pen, MXF, LEV, Tet, SXT
CL293	US	+	–	–	+	Pen, GM, Tet, TM, SXT
CL297	Wound	+	–	–	+	Pen, GM, Tet, TM, SXT
CL306	Urine	+	–	–	+	Pen, GM,LEV,TM, SXT
CL311	Wound	+	–	–	+	Pen, GM, LEV, Tet, TM, SXT
CL317	Wound	+	–	–	+	Pen, GM, LEV, Tet, TM, SXT
CL324	Wound	+	–	–	+	Pen, GM, LEV, Tet, TM, SXT
CL328	CT	+	–	–	+	Pen, GM,MXF, LEV, Tet, TM, SXT
CL332	Urine	+	–	–	+	Pen, GM, MXF, LEV, Tet, TM, SXT

Key: PEN = Penicillin, CEF = Cefoxitin, OXA = Oxacillin, CM = Clindamycin, E = Erythromycin, FOS = Fosfomycin, FA = Fusidic acid, GEN = Gentamycin, MXF = Moxifloxacin, TEC = Teicoplanin, VAN = Vancomycin, LEV = Levofloxacin, LNZ = Linezolid, MUP = Mupirocin, NIT = Nitrofurantoin, RIF = Rifampicin, TET = Tetracycline, TGC = Tigecycline, TM = Tobramycin, and SXT = Trimethoprim/Sulfamethoxazole, CL = Clinical, US = Urethral swab, HVS = High Vaginal Swab, CT = Catheter-tip, + = Positive, − = Negative.

and a threat to public health.

For most parts of the world, the distribution of MRSA varies. The occurrence of MRSA among clinical and colonizing *S. aureus* in Nigeria between 2011 and 2016 ranges from 2.4% to 22.6% [26,27,39,40]. This frequency is low compared to those found in some Asian countries, where rates as high as 73% was reported in Korea during 2011 [41]. In the current study, we found 13.04% frequency of MRSA among seventy clinical *S. aureus* isolates screened. However, all NL-*S.aureus* isolates were *mecA* negative, which indicates that the phenotypic methicillin resistance observed was almost associated with drug misuse other than

Table 2
Molecular epidemiology and resistant phenotypes of clinical methicillin resistant *Staphylococcus aureus*.

Isolate ID	Sample Source	Genotype				Resistant Phenotype
		PVL	*hlg*A	*hlg*B	*luk*E	
CL4	Wound	+	–	–	+	Pen, CEF,OXA
CL5	Wound	+	–	–	+	Pen, CEF, OXA
CL26	Wound	+	–	–	+	Pen, CEF, OXA, Tet
CL45	Wound	+	–	–	+	Pen, CEF, OXA, Tet, SXT
CL72	Wound	+	–	–	+	Pen, CEF, OXA, GM, MXF, LEV, Tet, TM,
CL63	HVS	+	–	–	+	Pen, CEF, OXA, Tet, SXT
CL7	Blood	+	–	–	+	Pen, CEF, OXA, Tet, SXT
CL8	CT	+	–	–	+	Pen, CEF, OXA, Tet, GM, MXF,LEV, TM
CL9	Pus	+	–	–	+	Pen, CEF, OXA, CM, E,GM, MXF, LEV, RIF, Tet, TM,SXT
CL30	US	+	–	–	+	Pen, CEFF, OXA,CM,E, GM, MXF, LEV,RIF, Tet, TM, SXT
CL108	US	+	–	–	+	Pen, CEF, OXA, LEV, Tet, SXT
CL154	Sputum	–	–	–	+	Pen, CEF, OXA, Tet, SXT

Key: PEN = Penicillin, CEF = Cefoxitin, OXA = Oxacillin, CM = Clindamycin, E = Erythromycin, FOS = Fosfomycin, FA = Fusidic acid, GEN = Gentamycin, MXF = Moxifloxacin, TEC = Teicoplanin, VAN = Vancomycin, LEV = Levofloxacin, LNZ = Linezolid, MUP = Mupirocin, NIT = Nitrofurantoin, RIF = Rifampicin, TET = Tetracycline, TGC = Tigecycline, TM = Tobramycin, and SXT = Trimethoprim/Sulfamethoxazole, CL = Clinical, US = Urethral swab, + = Positive, − = Negative.

Table 3
Genotypes and resistant phenotypes of nasal methicillin susceptible *Staphylococcus aureus*.

Isolate ID	Sample Source	Genotype				Resistant Profile
		PVL	*hlg*A	*hlg*B	*luk*E	
VT6	Nasal Swab	+	–	–	+	Pen
VT9	Nasal Swab	+	–	–	+	Pen
VT13	Nasal Swab	+	–	–	+	Pen
VT24	Nasal Swab	+	–	–	+	Pen, SXT
VT25	Nasal Swab	+	–	–	+	Pen, SXT
VT32	Nasal Swab	–	–	–	+	Pen, SXT
VT37	Nasal Swab	+	–	–	+	Pen, SXT
VT48	Nasal Swab	+	–	–	–	Pen, SXT
VT22	Nasal Swab	+	–	–	–	Pen, SXT
VT50	Nasal Swab	+	–	–	–	Pen, SXT
VT51	Nasal Swab	+	–	–	+	Pen, Tet
VT58	Nasal Swab	+	–	–	+	Pen, Tet
VT71	Nasal Swab	+	–	–	+	Pen, Tet
VT64	Nasal Swab	+	–	–	+	Pen, Tet
VT75	Nasal Swab	–	–	–	+	Pen, Tet, SXT
VT77	Nasal Swab	+	–	–	+	Pen, Tet, SXT
VT83	Nasal Swab	+	–	–	+	Pen, Tet, SXT
VT86	Nasal Swab	+	–	–	+	Pen, Tet, SXT
VT95	Nasal Swab	+	–		–	Pen, Tet, SXT
VT80	Nasal Swab	+	–	–	+	Pen, Tet, SXT, MXF, LEV
VT91	Nasal Swab	+	–	–	+	Pen, Tet, SXT, MXF, LEV
VT69	Nasal Swab	+	–	–	+	Pen, Tet, SXT, MXF, LEV

Key: PEN = Penicillin, CEF = Cefoxitin, OXA = Oxacillin, CM = Clindamycin, E = Erythromycin, FOS = Fosfomycin, FA = Fusidic acid, GEN = Gentamycin, MXF = Moxifloxacin, TEC = Teicoplanin, VAN = Vancomycin, LEV = Levofloxacin, LNZ = Linezolid, MUP = Mupirocin, NIT = Nitrofurantoin, RIF = Rifampicin, TET = Tetracycline, TGC = Tigecycline, TM = Tobramycin, and SXT = Trimethoprim/Sulfamethoxazole, CL = Clinical, US = Urethral swab, + = Positive, − = Negative. VT = Apparently healthy.

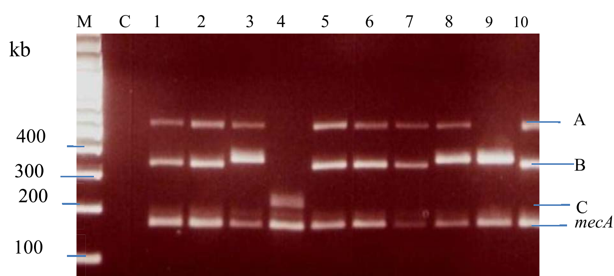
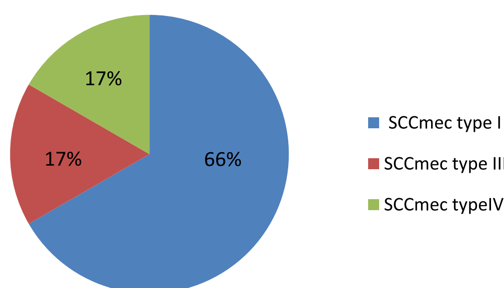


Fig. 2. a: Gel electrophoresis of SCCmec types identified by multiplex PCR. **b**: Percentage of SCCmec types identified by multiplex PCR.

Lane M contained 100 kb molecular weight marker. Lane C was a negative S. aureus control. Lane 1-10 had isolates within the loci specific to SCCmec types: (A) SCCmec types I; (B) SCCmec type III; and (C) SCCmec type IV.

a



b

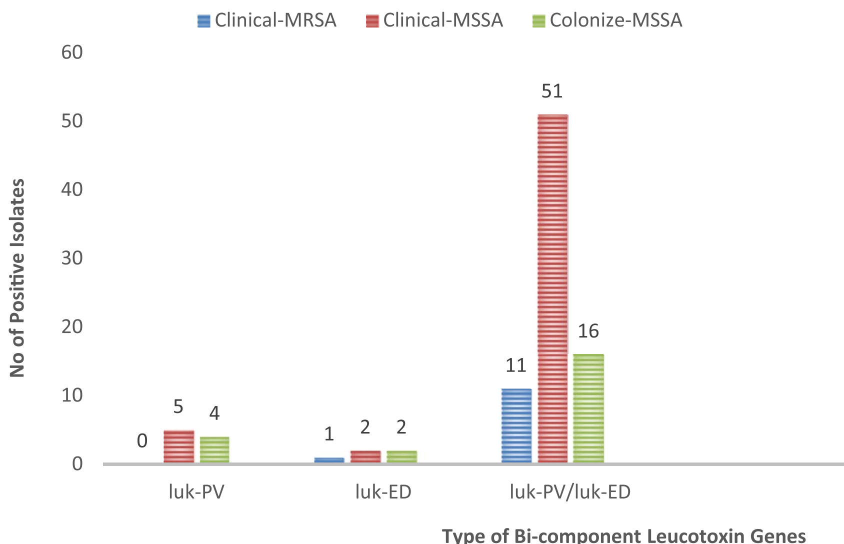


Fig. 3. Prevalence of Panton–Valentine Leucocidin and Leucotoxin E/D genes.
Note: Fig. 3 is deduced from Tables 1–3 above. Four (4) of the MSSA colonizing/community isolates had luk-pv alone, as well as five (5) of the clinical MSSA; one of the clinical MRSA isolate presents no (0) luk-pv gene. Again, 1and 2 each of clinical MRSA, clinical MSSA and colonizing MSSA had luk-ED gene alone respectively. Many of the isolates (11, 51 and 16) of clinical MRSA, MSSA and colonizing MSSA had combined presence of lukPV/luk-ED respectively.

existence of acquired resistance genes. We considered that the proportion of methicillin susceptible *S. aureus* detected in this study corroborates the findings of Kolawole et al. [39] where 11.5% of 61 colonizing *S. aureus* from surgical patients were MRSA. This could indicate that some MSSA genetic backgrounds may not provide a stable environment for SCC*mec* integration [28,39].

Notably, the 12 CL-MRSA identified within this study period were SCC*mec*-typeable. On the contrary, Ayepola group [27] showed that five of their seven MRSA were non-typeable; suggesting that certain genes are yet to be identified or not identifiable with the existing assays during their study period. Equally, only 2.9% (2/7) of MRSA isolates analyzed by Kolawole and colleagues [39] could be assigned SCC*mec* types. The difference between these studies and the observation presented here might have resulted from the different experimental approaches used. Nevertheless, we discovered that our SCC*mec* types correlate with the classic HA-MRSA (SCC*mec* types I, III) and community-associated MRSA (CA-MRSA) (SCC*mec* types IV). SCC*mec* type I was the most predominant and associated with isolates from wound-related samples. The predominance of other SCC*mec* types has been documented in some studies in Nigeria [28,39]. In Zambia, SCC*mec*

type III was detected in similar frequency as obtained in this study [42].

PVL toxin is known to have a cytolytic effect on polymonuclear neutrophils, and been identified as important indicators of staphylococcal virulence [8,24]. In this present work, over ninety percent (88/92; 95.7%) of the isolates harboured the *luk*PV gene. Although, it is widely assumed that PVL is a common virulence factor of CA-MRSA but HA-MRSA isolates in our study showed a considerable rate of *luk*PV positivity. Some studies have also shown an association between PVL genes and HA- MRSA. For instance, Shrestha et al. [43] found an elevated PVL-positivity among Panton-Valentine Leucocidin (PVL) genes in MRSA from nosocomial infections in Nepal. They provided evidence to support the circulation of a limited number of clones of PVL-positive MRSA and argued for the adaptability of these isolates to a hospital setting. We therefore opined that *luk*PV gene may not be a reliable indicator of CA-MRSA virulence. Regarding *lukPV*-positive MSSA, studies by other authors have shown contrasting frequencies of 42.7% [17], 40% [26] and 24% [29]. Moderately high PVL-positivity (≥50%) has also been identified in some African countries [44,45].

Nonetheless, the high frequency of PVL supports those of Ayepola [27] and consistent with the results of a study which demonstrated an association between *S. aureus*-producing PVL and furuncles [11]. The strains from clinical setting (68/70, 97.1%) and the carrier strains (20/22, 90.9%) harboured this gene and there was no obvious difference in the proportion of PVL-positive isolates from both groups. Presumably, there is "cross-traffic" of *luk*PV gene between hospital and community which enables incorporation of the PVL-encoding genes through horizontal transfer into *S. aureus* lineages. This concern has also been raised by Kolawole et al. [39]. Other authors have proposed factors which may contribute to higher *luk*PV gene among African *S. aureus* [28,45,46].

In this study, we focused on isolates from series of infections including carrier strains and found that 35.9% (33/92) of the PVL-positive strains were from wound specimens. Also, 18.2% (4/22) PVL-carrying *S. aureus* was identified among the carrier group. In a study in Tehran, *luk*PV positive *S. aureus* isolates were more recovered from patients with blood, pneumonia and cutaneous infections [5]. Another study [24] equally established strong link between PVL genes and skin and soft tissue infections. The authors revealed that 70% of *S. aureus* isolates from skin, soft tissue, and bone related infections produced PVL. Ayepola and colleagues [27], however, did not find any significant association between PVL production and skin and soft tissue infections. Taken together, it could be deduced that there is no predisposition of PVL-positive *S. aureus* isolates to any specific infections or colonization site.

In the present study, we also found that none of the 92 *S. aureus* isolates possessed γ-hemolysin (*hlg* A/B) genes. Previously, it was observed that *hlg*A and B genes were present in most of the *S. aureus* analyzed in Nigeria [28]. This suggests that detection of these virulence factors in *S. aureus* is variable and the characteristic of the toxins could be specific to geographical locations and MRSA status [47]. Apparently, *hlg*A/B-negative isolates are more likely to show positivity for other bicomponent genes since the occurrence of *luk*E/D positive isolates was substantial (CL-MSSA; 91%, CL-MRSA; 100% and NL-MSSA; 82%). Similar results were described by Al-Hassnawi et al. [15] who observed high rate of *lukE/D* genes in (CA-MRSA) from Hilla/Iraq. *Staphylococcus aureus luk*E/D had been detected in isolates from cases of skin dermatitis, furuncles, impetigo and systemic infection [5,6]. Although, workers in Tehran commented that isolates from blood and trachea harboured no *lukE/D* genes [5], there have been speculations that *lukE/D* is produced during the course of human infection.

Although, the co-existence of *luk*PV and *luk*E/D is uncommon in the literature, the characterization of our isolates revealed significant combination of *luk*PV and *luk*E/D genes. It has been shown that *luk*E/D reduced the frequency of IL-17 producing cells during *S. aureus* infections [29]. Thus, this suggests that the co-occurrence of *luk*PV and *luk*E/D toxins may contribute to the incidence and severity of *S. aureus* infection. It may also accelerate the emergence of a hybrid that could result into an epidemic. However, our study has some limitations. First, the isolates were limited to two tertiary healthcare institutions. Second, we were unable to capture data the demographic profiles associated with the isolates. These data could have shed more light on the molecular epidemiological features of the *S. aureus* population. Nonetheless, the information deduced herein, together with previous relevant data established an obvious diversity amongst the MRSA and MSSA population in Nigeria.

In conclusion, the high proportion of SCC*mec* type I, *luk*PV and *luk*E/D MSSA, and the presence of *luk*PV-*luk*E/D cluster with their resistance phenotypes in the two health institutions is a major concern, both as a source of severity of infections and a repository of possible epidemic strains. These further highlight the need for re-enforcement of antibiotic stewardship, continued antibacterial surveillance policy and strong public health disease control strategy.

Conflicts of interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.micpath.2017.12.030.

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