# Characterization of Colonizing *Staphylococcus aureus* Isolated from Surgical Wards' Patients in a Nigerian University Hospital

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## Abstract

In contrast to developed countries, only limited data on the prevalence, resistance and clonal structure of *Staphylococcus aureus* are available for African countries. Since *S. aureus* carriage is a risk factor for postoperative wound infection, patients who had been hospitalized in surgical wards in a Nigerian University Teaching Hospital were screened for *S. aureus* carriage. All *S. aureus* isolates were genotyped (*spa, agr*) and assigned to multilocus sequence types (MLST). Species affiliation, methicillin-resistance, and the possession of pyrogenic toxin superantigens (PTSAg), exfoliative toxins (ETs) and Panton-Valentine Leukocidin (PVL) were analyzed. Of 192 patients screened, the *S. aureus* carrier rate was 31.8 % (n = 61). Of these isolates, 7 (11.5%) were methicillin-resistant (MRSA). The isolates comprised 24 *spa* types. The most frequent *spa* types were t064, t084, t311, and t1931, while the most prevalent MLST clonal complexes were CC5 and CC15. The most frequent PTSAg genes detected were *seg/sei* (41.0%) followed by *seb* (29.5%), *sea* (19.7%), *seh* (14.7%) and *sec* (11.5). The difference between the possession of classical and newly described PTSAg genes was not significant (63.9% versus 59.0% respectively; *P* = 0.602). PVL encoding genes were found in 39.3% isolates. All MRSA isolates were PVL negative, SCC*mec* types I and VI in MLST CC 5 and CC 30, respectively. Typing of the accessory gene regulator (*agr*) showed the following distribution: *agr* group 1 (n = 20), group II (n = 17), group III (n = 14) and group IV (n = 10). Compared to European data, enterotoxin gene *seb* and PVL-encoding genes were more prevalent in Nigerian methicillin-susceptible *S. aureus* isolates, which may therefore act as potential reservoir for PVL and PTSAg genes.

## Introduction

*Staphylococcus aureus* is a widely recognized human pathogen that continues to represent a significant public health challenge globally. Owing to its broad spectrum of inherent virulence factors, it has been shown to cause a variety of infectious diseases such as superficial skin infections, endocarditis, septicemia, and toxin-mediated diseases, which are often difficult to treat [1,2]. In addition to its known virulence properties, the ability of methicillin-resistant *S. aureus* strains (MRSA) to consistently evolve resistance mechanisms has led to the emergence of hospital-adapted multi-resistant clones, which became a major cause of nosocomial infections world-wide [3,4,5]. The occurrence of MRSA strains in the community (community-associated MRSA) and in livestock (livestock-associated MRSA), as well as the potential risk of import of such strains into the hospital, are also matters of concern [6,7]. While some data are available on the occurrence of *S. aureus* implicated in various infections [1,4,5,6,8,9,10,13,14,15] there are only few reports describing *S. aureus* colonization pattern in patients from sub-Saharan Africa

[11,12,13]. In particular, for surgical patients who are predisposed to develop a variety of post-operative events, including post-surgical toxic shock syndrome, data concerning the toxigenic equipment, MRSA prevalence, and clonal structure of *S. aureus* are missing in Africa. It is well known that antibiotic-resistance, toxigenic equipment carriage and clonal analyses in *S. aureus* can provide useful insights into the virulence potential and nature of *S. aureus* populations [1,2,16,17,18,19]. Among the several exotoxins secreted by *S. aureus*, Panton-Valentine Leukocidin (PVL), a bi-component cytotoxin encoded by *luk*S-PV and *luk*F-PV genes of the PVL-encoding operon, has been observed in less than 5% of *S. aureus* isolates in Europe and can be associated with necrotic skin lesions including severe necrotizing pneumonia [20,21]. More-over, the expression of most *S. aureus* virulence factors is known to be controlled by a complex regulatory network including the accessory gene regulator (*agr*) system which consists of an approximate 3kb genetic locus containing divergent transcription units [20,21,22,23]. The *agr* locus is characterized by a polymor-phism of its auto-inducing peptide (AIP), and has been shown to

reliably divide *S. aureus* into four major *agr* groups. These groups evolved early in staphylococcal evolution [20,21,22]. Additionally, molecular typing technologies such as staphylococcal protein A (*spa*) typing and multilocus sequence typing (MLST) provide informative typing results which enable the grouping of individual isolates in clonal lineages [20,21,24]. Therefore, the aims of the present study were (i) to prospectively collect *S. aureus* isolates from a hospitalized surgical patient cohort in a Nigerian University Teaching Hospital (ii) to investigate their toxigenic and resistance properties and (iii) to analyze their clonal composition.

## Materials and Methods

### Population

The Obafemi Awolowo University Teaching Hospitals Complex in Ile-Ife has approximately 500 available beds across seven major hospital wards (internal medicine, surgery, orthopedics, psychiatry, gynecology, obstetrics and pediatrics). The hospital serves the city of Ile-Ife in Osun State and is a major referral hospital in Southwestern Nigeria. Exclusion criteria were (i) referral from other hospitals, (ii) superficial skin and soft tissue infections, and (iii) hospitalization for more than 48 hours at the time of sampling. The most common causes of hospitalization on the basis of clinical records were internal fractures (38.8%), cancers (34.6%), peritonitis (8.3%), osteomyelitis (7.2%), and urinary tract infections (5.5%). Demographic and clinical information was collected by interviews, questionnaires and review of clinical records. Institutional Review Board approval was obtained from Obafemi Awolowo University Teaching Hospitals Complex and all patients gave written informed consent to participate in the study.

### Bacterial strains

Between November 2008 and July 2010 all eligible patients hospitalized in the surgical wards (general surgery, pediatric surgery, orthopedics and gynecology) were prospectively screened for *S. aureus* within 48 hours of admittance. Swabs were collected from both anterior nares and from the skin (back of the wrist) using sterile cotton-tipped swab sticks (MicroPoint Diagnostics). A total of 48 strains were isolated from the nares, and 13 strains were recovered from cutaneous specimens. Identification of *S. aureus* and antimicrobial susceptibility testing was performed using VITEK-2 automated systems (BioMérieux, Marcy l'Etoile, France).

### DNA extraction and polymerase chain reaction approaches

DNA extraction was achieved with a QIAamp tissue kit (Qiagen, Hilden, Germany), by following the manufacturer's recommendations. Confirmation of *S. aureus* and methicillin-resistance was achieved by PCR targeting *nuc* and *mec* genes (*mecA* and recently described *mec*$_{LGA251}$/*mecC*) respectively [25,26,27]. Multiplex PCRs for detection of exotoxin genes including exfoliative toxin genes (*eta*, *etb*, *etd*), classical staphylococcal PTSAgs (*sea*, *seb*, *sec*, *sed*, *see* and *tst*), the subsequently described staphylococcal PTSAgs (*seg*, *seh*, *sei* and *sej*), and *luk*F-PV and *luk*S-PV genes were conducted as previously described [20,28,29].

### *spa* and *agr* typing

To determine the *spa* type of *S. aureus* strains, the polymorphic X-region of the *S. aureus* protein A gene (*spa*) was sequence-typed [18]. Cluster formation of *spa* types was performed by the "based upon repeat patterns" (BURP) algorithm of the StaphType software (Ridom, Münster, Germany) using the default parameters described by Mellmann et al. [19]. Additionally, subtypes of the

accessory gene regulator were detected by multiplex PCR as previously described [20,30]. Multilocus sequence typing (MLST) was performed for *spa* types detected for the first time in this study [24].

### SCC*mec* typing

SCC*mec* typing of MRSA isolates (n = 7) was performed as previously described [31].

### Statistical analysis

Categorical data were compared using the chi-square test. In addition, we report proportions of categorical variables (i.e. virulence factors and antimicrobial resistance). All computations were performed using Epi Info™ 3.5.3 (Centers for Disease Control and Prevention, Atlanta, USA). P values >0.05 were considered not statistically significant.

## Results

### Patient characteristics and *S. aureus* carriage

Among 192 persons tested, the overall prevalence of *S. aureus* carriage was 31.8% (n = 61) with 27.1% (n = 28) among males and 37.1 % (n = 33) among females (*P* = 0.105) (Table 1). The mean age was 39.7 years among carriers of methicillin-susceptible *S. aureus* (MSSA), 31.4 years among MRSA carriers and 44.2 years among *S. aureus* non-carriers (*P* = 0.30). All *S. aureus* were tested *nuc* positive. Seven of the 192 patients (3.7%) were colonized by MRSA and 11.5% of all *S. aureus* were MRSA. The seven MRSA were recovered within the period of sampling from seven patients variously hospitalized in the orthopedic (n = 2), general surgery (n = 4) and pediatric surgery (n = 1) wards. All MRSA isolates were tested positive for *mecA* and negative for *mecC*. Of all 61 *S. aureus* isolates, 73.7% were resistant to penicillin, 42.6% to tetracycline, 3.3% to clindamycin, 16.4% to gentamicin, 9.8% to levofloxacin

**Table 1.** Characteristics of the surgical patient cohort studied.

| Characteristic[1] | Males | Females | Total |
|---|---|---|---|
| **Population size, *S. aureus* carriage and age** | | | |
| Number of participants | 103 (53.6) | 89 (46.3) | 192 (100) |
| *S. aureus* carriage | 28 (27.1) | 33 (37.1) | 61 (31.8) |
| Age, years, median (range) | 36 (2–86) | 41 (1–76) | 38 (2–86) |
| **Duration of hospitalization** | | | |
| (≤3 days) | 13 (12.6) | 22 (24.7) | 33 (17.1) |
| (7 days) | 33 (32.0) | 30 (33.7) | 63 (32.8) |
| (≥14 days) | 57 (55.3) | 37 (41.5) | 94 (49.0) |
| **Risk factor for *S. aureus* colonization** | | | |
| Recent hospitalization (<6 months) | 7 (6.8) | 12 (13.4) | 17 (8.8) |
| Recent surgery | 3 (2.9) | 5 (5.6) | 8 (4.1) |
| Diabetes | 4 (3.8) | 3 (3.3) | 7 (3.6) |
| Urinary tract catheter | 20 (19.4) | 12 (13.4) | 32 (16.6) |
| Chest drain | 0 | 2 (2.24) | 2 (1.04) |
| Intravenous fluid (IVF) administration | 27 (26.2) | 30 (33.7) | 57 (29.6) |
| Pre-surgical patient | 48 (46.6) | 75 (84.2) | 123 (64.0) |
| Post-surgical patient | 55 (53.3) | 14 (15.7) | 69 (35.9) |

[1]Data are no.(%) of participants unless otherwise indicated.
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and 44.2% to trimethoprim/sulfamethoxazole, respectively. Vancomycin resistance was not found.

## Exotoxin gene detection

Overall, 70.5% (n = 43) of all isolates tested were PTSAg gene-positive. We did not observe significant differences between the prevalence of classical PTSAg genes and the subsequently described enterotoxin and enterotoxin-like genes tested (63.9% vs 59.0% repspectively; *P* = 0.602). Regarding the classical enterotoxin genes, *seb* gene was the most prevalent followed by *sea* gene and *sec* gene. The *sed* gene was detected in two isolates, while *see* gene was not observed. Altogether, three isolates harbored the *tst* gene. The *seg-sei* genes occurred most frequently and strictly in combination with one another while the *seh* gene was observed in nine isolates (Table 2). Several enterotoxin gene combinations were observed including isolates with a combination of two (n = 14, 22.9%), three (n = 20, 32.7%), four (n = 2, 3.6%) and five (n = 1, 1.6%) different genes. The most frequent combination of genes detected was *seg – sei – seb* genes occurring in 19.6% (n = 12) of all isolates. In contrast, seven isolates (11.4%) harbored a single PTSAg gene. Overall, three (4.9%) of the isolates were positive for the exfoliative toxin genes; *etb* gene was not detected. While 99% of isolates tested PCR positive to γ–hemolysin encoding genes, the *luk*F-PV and *luk*S-PV genes of the PVL operon were detected in 24 isolates (39%), all of which were MSSA strains. Moreover, 75.9% (n = 41) of the MSSA population were PTSAg gene positive (Table 3): 13% were positive for the classical PTSAg genes (n = 7); 13% exclusively encoded the newly described PTSAg genes (n = 7) while 50% of the MSSA population encoded both classical and newly described PTSAg genes. The *seb* gene was the most frequently observed classical PTSAg gene in the MSSA group (n = 17, 31.4%) while *seg-sei* genes occupied this position (n = 24, 44.4%) among the subsequently described PTSAg genes. The percentage of exfoliative toxin gene-positive MSSA strains was 5.5% (n = 3).

Regarding the MRSA subset, two isolates were PTSAg gene positive (28.5%). One of the MRSA isolates was associated with five PTSAg genes, namely: *seb*, *sed-sej* and *seg-sei* genes. Meanwhile the second MRSA isolate harbored the *sea* gene alone. Exfoliative toxin and PVL encoding genes were not observed in any of the MRSA isolates tested.

## spa typing

All 61 *S. aureus* isolates were *spa*-typeable (Table 3), and were associated with distinct *spa* types. Four hitherto undescribed *spa*-types were found, designated t7834, t7835, t8034 and t8037. Among 54 MSSA isolates, we detected 20 *spa* types (Table 3): the most frequently observed *spa* types were t064, t084, t311, t1931 and t2304, accounting for 54% of all isolates tested. Seven MRSA were associated with 6 *spa* types (t002, t007, t037, t194, t729 and t1842).

The assignment of isolates to clonal lineages by applying *spa* and MLST mappings (http://spa.ridom.de/mlst.shtml), in combination with a literature search and our own MLST analysis revealed that among MSSA isolates, CC15 (ST1, ST15, ST188, ST2355, ST25 and ST97), CC121 (ST121), and CC 5 (ST5, ST8 and ST72) were the most prevalent (Table 3). The MRSA isolates were associated with MLST CC5 (ST5, ST250 and ST2419) CC30 (ST39) and CC88 (ST88). Additionally, BURP clustering of *spa* types resulted in 5 *spa* CCs, 30 Singletons and two excluded *spa* types (t458 and t2304) (Table 3).

## SCCmec typing

Two distinct *SCCmec* types (I and VI) were observed among the seven MRSA isolates. However, five of the seven MRSA isolates were not typeable (NT, Table 3).

## agr typing

Typing of the accessory gene regulator (*agr*), revealed the following distribution: *agr* group I (n = 20, 32.7%), *agr* group II

**Table 2.** Results of testing 61 *S. aureus* isolates for staphylococcal PTSAg and ET genes by multiplex PCR.

| Positive result of multiplex PCR testing[1] | Nasal (n = 48) | | Skin (n = 13) | | Total (n = 61) | |
|---|---|---|---|---|---|---|
| | n | % | n | % | n | % |
| sea | 7 | 14.5 | 5 | 38.4 | 12 | 19.7 |
| seb | 12 | 25.0 | 6 | 46.1 | 18 | 29.5 |
| sec | 7 | 14.5 | 0 | 0.0 | 7 | 11.5 |
| sed | 2 | 4.1 | 0 | 0.0 | 2 | 3.2 |
| see | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 |
| tst | 2 | 4.1 | 1 | 7.7 | 3 | 4.9 |
| seg-sei | 20 | 41.6 | 6 | 12.5 | 26 | 42.6 |
| seh | 5 | 10.4 | 4 | 30.7 | 9 | 14.6 |
| sej | 1 | 2.1 | 0 | 0.0 | 1 | 1.6 |
| eta | 2 | 4.1 | 0 | 0.0 | 2 | 3.2 |
| etb | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 |
| etd | 1 | 2.1 | 0 | 0.0 | 1 | 1.6 |
| lukS-PV and lukF-PV | 20 | 41.6 | 5 | 38.4 | 25 | 41.0 |
| hlg | 48 | 100.0 | 12 | 92.3 | 60 | 99.0 |

[1]*sea*, staphylococcal enterotoxin A gene; *seb*, staphylococcal enterotoxin B gene; *sec*, staphylococcal enterotoxin C gene; *sed*, staphylococcal enterotoxin D gene; *see*, staphylococcal enterotoxin E gene; *tst*, toxin shock toxin gene; *seg-sei*, staphylococcal enterotoxin G and staphylococcal enterotoxin I genes; *seh*, staphylococcal enterotoxin H gene; *sej*, staphylococcal enterotoxin J gene; *eta*, exfoliative toxin A gene; *etb*, exfoliative toxin B gene; *etd*, exfoliative toxin D gene; *luk*S-PV and *luk*F-PV, Panton-Valentine leukocidin genes; *hlg*, gamma-hemolysin gene.
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**Table 3.** Characteristics of nasal and cutaneous *S. aureus* isolates.

| spa[1] | | MLST[2] | | Source for MLST classification | PVL[3] | PTSAg/ET gene profile[4] | agr[5] | Methicillin resistance[6] | SCCmec[7] | Source | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Type (n) | *spa*-CC | CC | ST | | | | | | | Nasal | Skin |
| t159 (3) | singleton | CC121 | ST121 | 39, 41 | Pos. | *seb, seg, sei* | IV | MSSA | ND | 2 | 1 |
| t2304 (6) | excluded | CC121 | ST121 | 41 | Pos. | *seb, seg, sei* | IV | MSSA | ND | 3 | 3 |
| t2304 (1) | excluded | CC 121 | ST 121 | 41 | Pos. | *sed, seg, sei* | IV | MSSA | ND | 1 | – |
| t084 (2) | spaCC084/2216 | CC15 | ST15 | 39, 41 | Neg. | – | II | MSSA | ND | 2 | – |
| t084( 2) | spaCC084/2216 | CC15 | ST15 | 39, 41 | Neg. | *sec* | II | MSSA | ND | 2 | – |
| t084 (1) | spaCC084/2216 | CC15 | ST15 | 39, 41 | Neg. | *eta* | II | MSSA | ND | 1 | – |
| t2216 (2) | spaCC084/2216 | CC 15 | ST15 | 39, 41 | Neg. | – | I, II | MSSA | ND | 2 | – |
| t2216 (1) | spaCC084/2217 | CC15 | ST15 | 39, 41 | Neg. | *eta* | II | MSSA | ND | 1 | – |
| t127(1) | spaCC127/321 | CC 15 | ST1 | 39, 41 | Pos. | *sea, seh* | III | MSSA | ND | 1 | – |
| t1931(6) | spaCC1931/7835 | CC15 | ST1 | 39 | Neg. | *sea, seh* | III | MSSA | ND | 3 | 3 |
| t7835 (1) | spaCC1931/7835 | CC15 | ST1 | This study | Pos. | *tst, seh* | III | MSSA | ND | 1 | – |
| t321(1) | spaCC127/321 | CC15 | ST1 | 41 | Neg. | *tst, sea, seh* | III | MSSA | ND | 0 | 1 |
| t3772 (1) | singleton | CC15 | ST25 | 39, 41 | Neg. | *sec, seg, sei/etd* | I | MSSA | ND | 1 | – |
| t458 (1) | excluded | CC15 | ST97 | 41 | Neg. | – | I | MSSA | ND | 1 | – |
| t3086 (1) | singleton | CC15 | ST188 | 37 | Pos. | – | I | MSSA | ND | 1 | – |
| t7834 (1) | singleton | CC15 | ST2355 | This study | Neg. | *sec, seg, sei* | II | MSSA | ND | 1 | – |
| t355 (4) | singleton | CC152 | ST152 | 39, 41 | Pos. | – | I | MSSA | ND | 3 | 1 |
| t8037 (1) | singleton | CC152 | ST152 | This study | Pos. | – | I | MSSA | ND | 1 | – |
| t318 (2) | singleton | CC30 | ST30 | 41 | Neg. | *seg, sei* | III | MSSA | ND | 1 | 1 |
| t007 (1) | singleton | CC30 | ST39 | 45 | Neg. | – | III | MRSA | VI | 1 | – |
| t095 (1) | singleton | CC45 | ST45 | 34 | Neg. | *tst, sec, seg, sei* | I | MSSA | ND | 1 | – |
| t002 (1) | spaCC002/311 | CC5 | ST5 | 39, 41 | Neg. | *seb, sed, seg, sei, sej* | II | MRSA | NT | 1 | – |
| t1842(1) | singleton | CC5 | ST5 | 39, 41 | Neg. | *sea* | I | MRSA | NT | 1 | – |
| t311(1) | spaCC002/311 | CC5 | ST5 | 39, 41 | Neg. | *seb, seg, sei* | II | MSSA | ND | 1 | – |
| t311(2) | spaCC002/311 | CC5 | ST5 | 39, 41 | Pos. | *sea, seg, sei* | II | MSSA | ND | 2 | – |
| t311 (2) | spaCC002/311 | CC5 | ST5 | 39, 41 | Neg. | *seg, sei* | II | MSSA | ND | 2 | – |
| t688(2) | singleton | CC5 | ST5 | 46 | Pos. | *seb, seg, sei* | II | MSSA | ND | 1 | 1 |
| t688(1) | singleton | CC5 | ST5 | 46 | Pos. | *seg, sei* | II | MSSA | ND | 1 | – |
| t064 (1) | singleton | CC5 | ST8 | 36 | Neg. | *seb, seg, sei* | I | MSSA | ND | 1 | – |
| t064(3) | singleton | CC5 | ST8 | 36 | Neg. | *sea, seb* | I | MSSA | ND | 2 | 1 |
| t064(2) | singleton | CC5 | ST8 | 36 | Neg. | *seb* | I | MSSA | ND | 2 | – |
| t8034 (1) | singleton | CC5 | ST72 | This study | Neg. | *sec, seg, sei* | I | MSSA | ND | 1 | – |
| t037(1) | singleton | CC5 | ST241 | 41 | Neg. | – | I | MRSA | I | 1 | – |
| t194(1) | singleton | CC5 | ST250 | 47 | Neg. | – | I | MRSA | NT | 1 | – |
| t729(2) | singleton | CC88 | ST88 | 39 | Neg. | – | III | MRSA | NT | 2 | – |

[1]*spa*, staphylococcal protein A gene; spaCC, *spa* clonal complex inferred by BURP analysis; n, indicates number of isolates with similar identity for all characteristics tested;
[2]MLST, multilocus sequence typing; CC, clonal complex; ST, sequence type;
[3]PVL, Panton-Valentine leukocidin; pos., positive; neg., negative;
[4]PTSAg/ET gene profile, pyrogenic toxin superantigen gene/exfoliative toxin gene profile; -, no PTSAg/ET gene detected;
[5]*agr*, accessory gene regulator type;
[6]MSSA, methicillin-susceptible *S. aureus*; MRSA, methicillin-resistant *S. aureus*;
[7]ND, not done; NT, not type able.
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(n = 17, 27.8%), *agr* group III (n = 14, 22.9%) and *agr* group IV (n = 10, 16.4%). We observed 78% of *seb* gene-positive isolates in two *agr* groups, *agr* group I (n = 6, 33.3%) and *agr* group IV (n = 8, 44.4%). However, *seg-sei* gene-positive isolates dominated in *agr* group IV (n = 10, 40%) and *agr* group II (n = 10; 40%). All *seh* gene-positive isolates belonged to *agr* group III (n = 9) and (MLST) ST1. Similarly, *S. aureus* isolates that tested positive for *tst* gene were observed in *agr* group III. Exfoliative toxin gene-positive isolates observed in this study belonged to *agr* group I and *agr* group II. Moreover, PVL genes were mostly associated with *agr* group IV (44%) and (MLST) ST121 while being least associated with *agr* group III (8%). Furthermore, *agr* grouping of MRSA

strains revealed three MRSA strains in *agr* group I (42.8%) and *agr* group III (42.8%) while one MRSA strain (14.2%) belonged to *agr* group II. In contrast, MSSA strains more frequently belonged to *agr* group I (n = 17, 31.4%) and *agr* group II (n = 16, 29.6%) than *agr* group III (n = 11, 20.3%) and *agr* group IV (n = 10,18.5%).

## Discussion

We analyzed the clonal structure, toxin gene equipment and resistance pattern of 61 *S. aureus* carriage isolates obtained from a surgical patient cohort (n = 192) in Nigeria. While 3.9% of the patients in the cohort studied were colonized by MRSA, Heysell et al. [11] reported on a high prevalence of multidrug-resistant MRSA nasal carriage (29%) in a district hospital in South Africa. Interestingly, in epidemiological studies involving persons on the Cape Verde islands as well as Central African Babongo Pygmies, no MRSA isolate was found [12,32]. However, in studies characterizing infection-related *S. aureus* isolates, higher and increasing MRSA rates (11.1–47.4%) have been reported in the North and sub-Saharan African region [9,13,33]. Reports on the prevalence of PTSAg genes and the detection of respective toxin genes differ greatly depending on the geographic affiliation, the population structure tested, and the range of staphylococcal PTSAg genes included. In particular, the recent detection of enterotoxins and enterotoxin gene-like toxin genes beyond the classical spectrum (such as enterotoxins SEG – SEJ, SEK – SER and SEU) has increased the percentage of those *S. aureus* isolates bearing at least one of the PTSAg encoding genes [28,34]. Moreover, like SEC (staphylococcal enterotoxin C), several of the newly described enterotoxins are polymorphic, being character-ized by the occurrence of nucleotide variants, additionally challenging their precise detection [29]. In this study, 11.4% of the *S. aureus* isolates were shown to harbor at least one of the PTSAg gene family members. This is in accordance with other studies of different geographic origin [23,28,35]. However, these studies differ from our investigation because they included *S. aureus* isolates obtained from the clinical care setting. It is well documented that the smallest amounts of staphylococcal entero-toxins may induce T-cell stimulation resulting in systemic illness such as staphylococcal enterotoxin-induced shock and autoimmu-nity [28,36]. Given the detection of a significant amount of toxin genes including *tst* gene in the present study, post-operative patients hospitalized in the surgical wards may be at risk of post-surgical toxic shock syndrome [16,17]. For SEH, emetic activity has been shown and this PTSAg is considered a potential causative agent for food poisoning [37]. Importantly, a high percentage of *seh* gene-positive isolates (14.7%) was found in our study relative to a low prevalence reported for this enterotoxin gene in other studies investigating non-food borne-related *S. aureus* isolates: 5.4% [28], 4% [35], and 4.3% [38]. Peck et al. [39] reported significant differences in the prevalence of selected enterotoxin genes, including *seh* gene in *S. aureus* isolates obtained from blood compared to nasal isolates (7.2% vs. 30.5%). However for food borne isolates, higher percentages of *seh* gene-positive isolates have been observed. Aydin et al. [40] reported on 16.3% *seh* gene-positive *S. aureus* isolates obtained from food samples, particularly from meat.

We have found a high prevalence (44%) of *luk*S-PV and *luk*F-PV genes of the PVL operon in MSSA carriage isolates. For *S. aureus*

isolates obtained from wound infection, similar results have been reported [15,37]. Similarly, Schaumburg et al. [13] reported on Gabonese *S. aureus* isolates from both, asymptomatic carriers and infections with high percentages of PVL-positive MSSA isolates. Moreover, for a remote Central African population with limited access to health care facilities, a high prevalence of PVL-positive MSSA was also detected [12]. Since PVL-encoding genes are carried on prophages as mobile genetic elements, which enable incorporation into *S. aureus* lineages via horizontal transfer, the Central and West African region may act as a potential major reservoir for the PVL virulence factor with considerable impact on regional as well as global health care systems. Molecular typing of the MRSA carriage isolates characterized in this study revealed that they belonged to *spa* types indicative for the clonal complexes *spa*-CC5 (t002, t037, t194, t1842), *spa*-CC30 (t007) and *spa*-CC88 (t729), all of which have been previously described in African countries [12,15,41,42].

In contrast, among MSSA isolates, *spa* types indicative for MLST CC15 (ST1/ST215; mostly *spa* types t084, t1931) and MLST CC5 (ST1/ST8; mostly *spa* types t064, t311) were predominant. While similar findings have been reported from other studies on the molecular structure of African MSSA [10,43], we did not observe *S. aureus* strains of MLST sequence types ST2250, and ST2277 in the present study. Of note, four hitherto undescribed *spa* types: t7834, t7835, t8034 and t8037, associated with ST2355, ST1, ST72 and ST152 respectively, were detected in this study. Furthermore, a high prevalence of PVL-positive MSSA isolates (16.4%) of *spa* types t159 and t2304 (clonal lineage MLST ST121/CC121), which belonged to *agr* group IV, was observed in our study. These findings are consistent with previous reports from Africa and other regions of the world including Europe and arctic Russia [10,15,44].

In summary, we documented a 3.9% prevalence of MRSA colonization among 192 patients screened. Analysis of the toxin gene content of the Nigerian *S. aureus* isolates revealed a relatively high overall prevalence of PTSAg genes. In particular, the *seh* gene encoding for an enterotoxin which can induce emetic disease was found in association with ST1. While no PVL-positive MRSA isolates were detected, PVL-encoding genes were highly frequent among MSSA isolates of different clonal lineages. Since we characterized carriage isolates of *S. aureus* obtained from surgery wards' patients in Nigeria, this study represents an important reference point for understanding the virulence potential and clonal epidemiology of the strains studied. While the role of the accessory gene regulator in *S. aureus* colonization is yet unclear, our study nonetheless provides further insight in to the clonal nature of the strains studied given the well-demonstrated relationship between accessory gene regulator groups and *S. aureus* clonal evolution [20,22].

## Author Contributions

Conceived and designed the experiments: DOK AA OOL ALA YBA KB. Performed the experiments: AA FS RK. Analyzed the data: AA FS RK KB. Contributed reagents/materials/analysis tools: DOK AA FS RK KB. Wrote the paper: DOK AA FS RK KB.

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