Molecular Diversity of *Staphylococcus aureus* from the Nares of Hospital Personnel, HIV-Positive and Diabetes Mellitus Patients in Yaoundé Cameroon

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**Abstract**

Nasal carriage of *Staphylococcus aureus* has been identified as a risk factor for the development of staphylococcal infections caused by endogenous colonizing strains. Information on the genotypic diversity of *Staphylococcus aureus* is relevant for managing epidemiological and clinical challenges resulting from the evolutionary differences of this bacterium. The objective of this study was to determine and compare the molecular diversity of *Staphylococcus aureus* isolates from three high-risk populations in Yaoundé, Cameroon. Molecular analysis confirmed that 95% of 100 tested isolates were *S. aureus*. The *meca* and Panton Valentine-Leukocidin (PVL) genes (*lukS/F-PV*) were detected in 37% (35/95) and 43% (41/95) of isolates respectively and 18% (17/95) of the isolates harboured both the *meca* and *lukS/F-PV* genes. A mixed distribution of both methicillin sensitive *S. aureus* (MSSA)/PVL and methicillin resistant *S. aureus* (MRSA)/PVL strains were detected within the study population. Community associated MRSA accounted for 94% (33/35) of the isolates, further classified into allotypes SCCmec type IV 54% (19/35) and SCCmec type V 40% (14/35), while two isolates were hospital associated SCCmec type II strains. A majority of the isolates harboured a single aggressive gene regulator allele *agr* type I. Pulsed Field Gel Electrophoresis (PFGE) generated 18 pulsotypes that grouped isolates irrespective of the study popu-
lation. Multilocus Sequence Typing (MLST) of 12 selected isolates was assigned to six pandemic clonal complexes (CC): CC5 (ST5), CC8 [ST8, (n = 3)], CC15 (ST 15), CC25 (ST 25), CC72 [ST72 (n = 2)] and CC121 [ST 121 (n = 2)] and three atypical sequence types ST 508, ST 699 (CC45) and ST 1289 (CC 88). The study population represents an important reservoir for MRSA, MRSA-PVL and MSSA-PVL which could serve as focal point for further dissemination bringing about significant clinical and epidemiological implications. The predominance of SCCmec IV and agr types in this setting warrants further investigation. Isolates were genetically diverse with MLST indicating that pandemic ST8 was predominant. Detection of atypical STs has provided an insight into the necessity for constant monitoring.

Keywords

Nasal Carriage, Methicillin Resistant *S. aureus*, Methicillin Sensitive *S. aureus*, Panton-Valentine Leukocidin, Multilocus Sequence Typing

1. Introduction

*Staphylococcus aureus* is a Gram positive bacterium that is part of the microbiota of human nares, skin, intestine, upper respiratory tract and vagina [1]. These bacteria can cause serious disease when there is a breach of the mucosal barrier allowing sterile body sites to be infected by tissue invasion or toxin production [2]. The host and bacterial factors that facilitate colonization have not been fully characterized. However, polymorphism of genes that encode the glucocorticoid receptor, interleukin-4, complement inhibitor proteins and the reduced expression of antimicrobial peptides in nasal secretions have been associated with persistent nasal carriage [3] [4].

Bacteria adhesion is facilitated by loricrin, a major component of the squamous epithelium, which is the primary ligand for the clumping factor B (ClfB) expressed on the surface of *S. aureus*. Other bacterial factors like cell wall teichoic acid, capsular polysaccharide and iron-regulator surface determinants are also involved in *Staphylococcus aureus* colonization [5] [6] [7] [8]. Invasiveness of the bacteria is enhanced by the secretion of enzymes (nucleases, proteases, lipases, hyaluronidase and collagenases) and cytokines that inhibit complement activation, cause neutrophil chemotaxis and neutralize antimicrobial defensin peptides [9] [10]. Some strains produce toxic proteins such as enterotoxins, exfoliative toxins and leucocidin, which are responsible for diseases that vary in severity from superficial skin lesions to more serious invasive and life-threatening infections such as pneumonia, endocarditis, septicaemia, acute staphylococcal toxemia syndromes and staphylococcal food poisoning [11].

In vulnerable persons, colonization increases the risk of subsequent infection usually caused by colonizing strains [12]. Several studies have illustrated a causal association between *S. aureus* nasal carriage and an estimated 4- to 10-fold increase in the odds of carriers developing staphylococcal disease [13] [14].
Human Immunodeficiency Virus/acquired immune deficiency syndrome (HIV/AIDS) associated infections are generally linked to the progressive depletion of cluster of differentiation 4 T (CD4 T) cells. A count lower than 200 cells/mm³ of blood, recent antibiotic use, duration of hospital stay are considered to be risk factors for nasal colonization by methicillin, resistant S. aureus in HIV-infected patients [15] [16]. Diabetes mellitus, a debilitating chronic disease with ensuing complications such as renal failure, dermatological disorders and peripheral neuropathy with risk of foot ulcers, is considered risk factors for MRSA colonization [17]. Colonization of hospital personnel has been identified as a reservoir of healthy carriers of S. aureus in the hospital and community. Previous studies in Ethiopia, recorded a prevalence of 28.8% (34/118) S. aureus colonization of hospital personnel, among which 44.1% (15/34) were resistant to methicillin [18]. A similar study conducted in Yaounde Cameroon, recorded a prevalence of 23.7% [19]. Transmission of resistant strains from personnel to patients is likely to occur during routine patient care in the absence of effective hand hygiene. Acquisition of MRSA on hands by healthcare workers, was common after examination of sites like the chest, abdomen, forearm and hands [20].

Management of staphylococcal infections has become a burden to the global health community leading to increased cost of healthcare, prolonged hospital stay and higher morbidity and mortality among infected individuals [21] [22]. The epidemiology of S. aureus especially MRSA has evolved considerably over the years. Managing the upsurge in prevalence of community associated methicillin resistant Staphylococcus aureus (CA-MRSA) is challenging for several healthcare institutions which are already battling with high levels of hospital-associated methicillin resistant Staphylococcus aureus (HA-MRSA) [23]. Disease severity is further compounded by the presence of mobile genetic elements that confer antibiotic resistance such as Staphylococcus cassette chromosome (SCC) harboring mec elements for methicillin resistance or the A operon for vancomycin resistance. The SCCmec type II strains initially associated to the hospital settings have now been identified among community and animal isolates [24] [25]. Methicillin sensitive S. aureus (MSSA) and MRSA strains harboring Panton-Valentine Leukocidin (PVL), a bi-component cytotoxin encoded by luk S-PV with diverse genetic characteristics, are on the rise in Africa [26]. Given that luk S-PV is phage mediated and can easily be transmitted between isolates, this may explain high prevalence rates of gene among strains in Africa where monitoring is limited. PVL-encoding operon has been detected in 55.9% of S. aureus strains from certain African populations such as the Babongo Pygmies. Data from across Europe, however, depicts lower rates: 1.8% in Ireland, 4% in Turkey, 30% in Germany and 20% in United Kingdom [27] [28]. The expression of PVL and other S. aureus virulence determinants is thought to be controlled by multiple regulatory pathways including the accessory gene regulator (agr) characterized by a polymorphism of its auto-inducing peptide (AIP). Based on this polymorphism, S. aureus has been classified into four agr groups. Furthermore, molecular typing techniques such as pulsed field gel electrophore-
sis (PFGE) and multilocus sequence typing (MLST) are currently being used to
generate genotype information to categorize isolates into sequence types (STs)
and clonal lineages or complexes (CCs) for effective epidemiological surveillance
and for monitoring evolutionary trends [29].

The clinical relevance of characterizing both MRSA and MSSA has been
demonstrated though such data is limited in Cameroon. The objective of this
study was therefore to investigate the genetic diversity of S. aureus isolates ob-
tained from hospital personnel, HIV-positive and diabetes mellitus patients in
Yaounde, Cameroon for disease surveillance and better patient management.

2. Materials and Methods

2.1. Sample Collection

Nasal samples were collected from hospital personnel, HIV-positive patients
and diabetes mellitus out-patients in three health institutions over a period of
12 months, from January 2016 to January 2017. Samples were collected from
participants by introducing a sterile cotton swab into both nostrils one after
the other. Collected nasal samples were transported in an enrichment medium
(m-Staphylococcus broth) containing 10% Sodium Chloride (NaCl) to the ba-
cteriology unit of the Centre for the Study and Control of Communicable Diseas-
es, Faculty of Medicine and Biomedical Sciences of the University of Yaounde 1,
for culture and identification. One hundred randomly selected multidrug resi-
sistant and biochemically identified Staphylococcus aureus isolates were further
analyzed. Isolates were selected according to study population and study site as
follows: hospital personnel: [Yaounde General Hospital (n = 6), Yaounde Cen-
tral Hospital (n = 10) and Biyem-Assi District Hospital (n = 4)]. HIV-positive
patients: [Yaounde Central Hospital (n = 60)] and diabetes mellitus patients:
[Yaounde Central Hospital (n = 20)].

2.2. Culture and Identification of Staphylococcus aureus Isolates

Identification was based on mannitol fermentation after incubation of plates at
37°C for 24 to 48 hours. Isolates that fermented mannitol were further analysed
using API staph (BioMérieux, Marcy l’Etoile, France). Antimicrobial susceptibil-
ity testing was carried out according to the disk diffusion method by Kirby Bau-
er. Identified isolates were stored at −20°C pending molecular analyses per-
formed at the Department of Medical Microbiology, University of Pretoria,
South Africa.

2.3. Total Genomic DNA Extraction of Staphylococcus aureus
Isolates

Total bacterial DNA was extracted from a 2 ml tryptone soya broth (Oxoid Ltd.,
Basingstoke, UK) bacteria suspension. DNA extraction was performed using the
Zymo Fungal/Bacterial DNA Mini Prep™ (Zymo Research Corp CA-USA.)
DNA extraction kit according to instructions referenced by the manufacturer.
Extracted DNA was stored at ~20°C.

2.4. Molecular Identification of *Staphylococcus aureus* Isolates

The isolates were subjected to a multiplex Polymerase Chain Reaction (M-PCR) assay using specific primers to confirm biochemical identification. *Staphylococcus* species and genus specific upstream sense and downstream antisense primers shown in Table 1 were used to amplify the 16S rRNA and the *nuc* fragments of isolates respectively [30]. The PCR reaction mixture, volume and cycling conditions were adapted as previously described [30] [31]. Generated amplicons were run on 1.5% agarose gel electrophoresis after staining with 5 µl ethidium bromide (10 mg/ml; Sigma Aldrich) and visualized under UV light (Transilluminator Ultra-violet products incorporated, USA). A 100 bp DNA ladder (Promega, Madison, USA) was used as DNA molecular weight standard.

2.5. Detection of Antibiotic Resistance and Virulence Genes by M-PCR Assays

Primers specific for *mecA* were used to amplify the genes responsible for methicillin resistance. The ability to produce Panton-Valentine leukocidin (PVL) was determined by the presence of *lukS-PV-lukF-PV* and virulence regulation by the different *agr* (I to IV) groups, according to previously described protocols [28] [32]. Details of primer sequences, expected sizes of the amplified products, reaction volume and specific annealing temperatures are listed in Table 1.

### Table 1.

| Primer name  | *Primer sequence*                               | Target gene | Amplicon size (bp) | Annealing temp (˚C) |
|--------------|-------------------------------------------------|-------------|--------------------|---------------------|
| Staph756     | F/-5AACTCTGTATTTAGGGAAGAACA-3 R/-5 CCACCTCTCCTCGGTTTG TCACC-3 | 16S rRNA    | 756                |                     |
| Staph750     | F/-5GACCTATTATTTGATGCCCACCT-3 R/-5GCCTTGAAGCAATCGGATTCG-3 | *nuc*       | 218                |                     |
| Tn1 (+)      | F/-5GACTATTATTGGGTGATCCACCTG-3 R/-5GCCTTGAAGCAATCGGATTCG-3 | *mecA*      | 310                | 57                  |
| Tn2 (−)      | F/-5GACCTATTATTGGGTGATCCACCTG-3 R/-5GCCTTGAAGCAATCGGATTCG-3 | *mecA*      | 310                | 57                  |
| mecA1        | F/-5 GATATCGATGTTAAGGGAAGTGGG-3 R/-5 ATGCTCTCACTACCGGATTTG-3 | *mecA*      | 310                | 57                  |
| MecA2        | F/-5 GATATCGATGTTAAGGGAAGTGGG-3 R/-5 ATGCTCTCACTACCGGATTTG-3 | *mecA*      | 310                | 57                  |
| LukS-PV      | F/-5 ATCATTAGTTAATGTCCTGACATGATCCA-3 R/-5 GCATCAAGTGTATTTGATAGC-3 | *LukS/F*    | 151                | 57                  |
| LukF-PV      | F/-5 ATGCACATGTTGACATGGG-3 R/-5 GCATCAAGTGTATTTGATAGC-3 | *agrI*      | 440                |                     |
|              | F/-5 ATGCACATGTTGACATGGG-3 R/-5 GCATCAAGTGTATTTGATAGC-3 | *agrI*      | 440                |                     |
| agrI         | F/-5 ATGCACATGTTGACATGGG-3 R/-5 GCATCAAGTGTATTTGATAGC-3 | *agrII*     | 572                | 55                  |
| agrII        | F/-5 ATGCACATGTTGACATGGG-3 R/-5 GCATCAAGTGTATTTGATAGC-3 | *agrII*     | 572                | 55                  |
| agrIII       | F/-5 ATGCACATGTTGACATGGG-3 R/-5 GCATCAAGTGTATTTGATAGC-3 | *agrIII*    | 4064               |                     |
| agrIV        | F/-5 ATGCACATGTTGACATGGG-3 R/-5 GCATCAAGTGTATTTGATAGC-3 | *agrIV*     | 588                |                     |

*Legend: F/Forward primer; R/Reverse primer.*
2.6. Identification of the SCCmec Types of MRSA Isolates

The diversity of MRSA strains was established by multiplex PCR assays of the chromosomal cassette recombinase (ccr) types I to V and the Staphylococcal cassette chromosome mec allotypes (classes A to C). SCCmec types were assigned based on the combination of ccr type and mecA classes detected [33].

2.7. Pulsed Field Gel Electrophoresis

Pulsed Field Gel Electrophoresis analysis of 95 molecularly confirmed isolates (MSSA n = 60 and MRSA n = 35) was performed in accordance with CDC (Centers for Disease Control and Prevention) Pulse Net protocol (https://www.cdc.gov/pulsenet/pathogens/protocol-images.html). The reference strain ATCC 12,600 was used for quality control. Briefly, to 400 μl, each bacterial suspension (optical density 1.2) of an overnight culture was added 20 μl of lysozyme (20 mg/ml) and incubated at 56˚C for 20 minutes. To this was added 20 μl of thawed proteinase K and 5 μl of lysostaphin (100 μg/ml, Sigma). The bacterial suspension for each isolate was thereafter mixed with 1.2% (400 μl) low melting point agarose gel (Seakem) and casted into plugs. Plugs were prepared by cutting genomic DNA embedded within them with Serratia marcescens (SmaI) (Fermentas Life Sciences, St. Leon-Rot, Germany) restriction enzyme. Electrophoresis of DNA fragments was performed on 1% Seakem Gold agarose gel in 1X tris-Boronic Acid-EDTA buffer, using the Rotaphor® (Biometra, Gottingen, Germany) at 13˚C for 25 hours, with a reorientation angle of 120˚ and a linear increase of switch time from 5 to 40 seconds. The gels were stained with ethidium bromide (2 mg/mL, Sigma) for 30 minutes, destained in ultra-pure water for 15 minutes, viewed under UV light (Transilluminator, Ultra-violet products incorporated, USA), and resulting images photographed and analyzed.

2.8. Multilocus Sequence Typing (MLST) of Staphylococcus aureus

Twelve representative isolates based on pulsotypes generated by PFGE were selected for sequencing by MLST. PCR primers designed by Inqaba Biotech South Africa were used to amplify the following highly conserved seven housekeeping gene (carbamate kinase (arcC), shikimate dehydrogenase (aroE), glycerol kinase (glp), guanylate kinase (gmk), phosphate acetyltransferase (pta), triosephosphate isomerase (tpi), and acetyl coenzyme A acetyltransferase (yqiL) as previously described (34)). Each pair of primers amplified an internal fragment of a housekeeping gene of approximately 500 bp. Sequence analysis was performed by Inqaba Biotech South Africa. The identified loci and corresponding alleles were then compared with those from the S. aureus database.

2.9. Data Analysis

Data collected were entered into a spreadsheet and analyzed using Stata (version 13.0 STATA corps, Texas, USA). Proportions were compared using Chi-Square tests. The level of statistical significance was set at a p-value ≤ 0.05. PFGE images
were analysed with GelCompare II software (Applied maths, Kortrijk, Belgium). The percentage of relatedness was determined by Dice coefficient with 1.5% band tolerance and 1% optimization and the unweighted pair group method with arithmetic averages (UPGMA). The coefficient of similarity was set at ≥80% to show clonal relationships. Sequence analysis was done using CLC workbench software and serotypes assigned using the online database S. aureus (http://saureus.mlst.net/).

3. Results

In total, 95 (95%) of the 100 isolates were confirmed as S. aureus based on 16SrRNA and nucA genes (Figure 1). The other five isolates were not subjected to any further analysis. These 95 confirmed S. aureus were distributed according to study population as follows; hospital 20, HIV-positive patients 57 and diabetes mellitus patients 18.

The mecA gene for MRSA, was detected in 36.8% (35/95) of the analyzed isolates. The other isolates, (60) were identified as MSSA. Distribution of MRSA according to study population was as follows; hospital personnel 45% (9/20), HIV-positive patients 33% (19/57) and diabetes mellitus patients 39% (7/18). For the lukS/F-PV gene, 40% was detected amongst isolates from hospital personnel, 40% from HIV-positive patients and 56% from diabetes mellitus patients resulting in an overall prevalence of 43% (41/95). The combined prevalence for MRSA/PVL was 49% (17/35) distributed as follows: hospital personnel 41% (7/17), HIV-positive patients 35% (6/17) and diabetes mellitus patients 24% (4/17), while that for MSSA/PVL was 40% (24/60) with the highest proportion 81% (17/24) detected among isolates from the HIV-positive population, followed by diabetes mellitus 25% (6/24) and hospital personnel 4% (1/24). Based on study site, the Yaounde Central Hospital registered the highest prevalence of 86% and 90% for both mecA and lukS/F-PV genes respectively.

Figure 1. The PCR amplification products of the S. aureus encoding 16S rRNA (756 bp), nuc (218 bp), mecA (310 bp) and the lukS/F-PV (151 bp) genes. Lanes 1 and 18:100 bp molecular weight marker. Lanes 2 - 17 S. aureus isolates.
Overall, 71% (25/35) of the MRSA were typed as community acquired CA-MRSA SCCmec type IV, SCCmec type V 23% (8/35) and two isolates (6%) typed as SCCmec type II (hospital acquired-MRSA). Analyses revealed that 55% (5/9), 79% (15/19) and 71% (5/7) of MRSA from hospital personnel, HIV-positive and diabetes mellitus patients respectively, were typed as SCCmec type IV. All the MRSA/PVL positive isolates (n = 6) from HIV-positive subjects belonged to SCCmec type IV. The two hospital acquired-MRSA SCCmec type II was detected among isolates from the Yaounde Central Hospital. According to study population, the predominant agr type was agr type I distributed as follows; hospital personnel 65% (13/20), HIV-positive patients 52% (30/57) and diabetes mellitus patients 33% (6/18). These results and more are shown in Figure 2.

3.1. Pulsed Field Gel Electrophoresis

Of the 95 (35 MRSA and 60 MSSA) isolates that underwent restriction digestion with SmaI and electrophoresis on agarose gel, 93 had PFGE detected patterns. Thus 2 MSSA isolates were untypable with SmaI. These two isolates were not analysed any further. Relatedness of strains was analyzed by constructing a dendrogram based on distance value and 80% homology. The dendrogram showed 18 distinct pulsotype designated here as CI to CVIII (Figure 3) and several singletons. Twelve representative isolates based on generated pulsotypes were sequenced using MLST.

3.2. Molecular Diversity of S. aureus Based on MLST

Six pandemic STs (ST5, ST8, ST15, ST25, ST72 and ST121) and three atypical STs (ST508, ST669 and ST1289) were identified among the 12 isolates analyzed by MLST (Table 2). Results illustrates that the six pandemic STs belonged to six major clonal complexes [CC5 (ST5), CC8 (ST8), CC15 (ST15), CC25 (ST25), CC72 (ST72 and CC121 (ST121)]. The predominant STs were ST8 (n = 3) and ST72 (n = 2). The sequenced isolates and the characteristics of the identified STs are shown below in Table 3 and Table 4 respectively.
Table 2. Comparison of methicillin-resistant *S. aureus* and PLV harboring carrier state among study groups.

|                           | Hospital personnel (n = 20) | HIV-positive Subjects (n = 57) | Diabetes Mellitus patients (n = 18) | p-value |
|---------------------------|-----------------------------|-------------------------------|------------------------------------|---------|
| Methicillin-resistant *S. aureus* n (%) | 9 (45%) | 19 (33%) | 7 (39%) | 0.635 |
| Panton Valentine leucocidin producing strains n (%) | 8 (40%) | 23 (40%) | 10 (56%) | 0.498 |

Table 3. Allele numbers and assigned clonal complexes of MLST sequenced *S. aureus* isolates.

| Isolate ID | Host               | ST based on MLST-7 | Clonal complex | Loci and corresponding alleles |
|------------|--------------------|--------------------|----------------|---------------------------------|
|            |                    | arc,a,roE,glp,gnk,pta,pti,yqil |
| BA 07      | Hospital personnel | 72                 | 72             | 1 4 1 8 4 4 3                   |
| BA 19      | Hospital personnel | 669                | -              | 3 1 94 1 29 5 3                 |
| HDJ 15     | HIV-positive patient | 15                | 15             | 13 13 1 1 12 11 13             |
| HDJ 16     | HIV-positive patient | 25                | 25             | 4 1 4 1 5 5 4                 |
| HDJ 24     | HIV-positive patient | 121               | 121            | 6 5 6 2 7 14 5               |
| HDJ 88     | HIV-positive patients | 8                 | 8              | 3 3 1 1 4 4 3                   |
| HDJ 127    | HIV-positive patient | 508               | 45             | 10 40 8 6 10 3 2              |
| HDJ 142    | HIV-positive patient | 8                 | 8              | 3 3 1 1 4 4 3                   |
| HDJ 219    | HIV-positive patient | 8                 | 8              | 3 3 1 1 4 4 3                   |
| HDJ 213    | HIV-positive patient | 72                | 72             | 1 4 1 8 4 4 3                   |
| HJD 229    | HIV-positive patient | 5                 | 5              | 1 4 1 4 1 4 2 10 10             |
| HDJ 245    | HIV-positive patient | 1289              | 88             | 22 1 14 10 12 4 31             |

Legend: Sequence Type (ST) Multilocus Sequence Type (MLST).

Table 4. Genetic characteristics of the twelve sequenced isolates.

| Isolate ID | (STs) | Cc | MLST allelic profile | mecA | SCC mec | *LukS*/*lukF*-PV | agr | Study site | Origin of isolates     |
|------------|-------|----|-----------------------|------|---------|-----------------|-----|------------|------------------------|
| HJD 229    | 5     | 5  | 1-4-1-4-12-1-10       | -    | IV      | -               | I   | YCH        | HIV-positive patients   |
| HDJ 219    | 8     | 8  | 3-3-1-1-4-4-3         | -    | V       | -               | I   | YCH        | HIV-positive patients   |
| HDJ 142    | 8     | 8  | 3-3-1-1-4-4-3         | -    | -       | -               | I   | YCH        | HIV-positive patients   |
| HJD88      | 8     | 8  | 3-3-1-1-4-4-3         | +    | V       | +               | II  | YCH        | HIV-positive patients   |
| HDJ15      | 15    | 15 | 13-13-1-1-12-11-13    | -    | -       | +               | III | YCH        | HIV-positive patients   |
| HDJ 16     | 25    | 25 | 4-1-1-5-5-5-4         | -    | -       | -               | YCH | Diabetes Mellitus |
| HDJ213     | 72    | 72 | 1-4-1-8-4-4-3         | -    | -       | +               | IV  | YCH        | HIV-positive patients   |
| BA07       | 72    | 72 | 1-4-1-8-4-4-3         | -    | -       | -               | Biyem Assi | Hospital personnel |
| HDJ 24     | 121   | 121| 6-5-6-2-7-14-5        | +    | IV      | +               | I   | YCH        | Diabetes Mellitus       |
| HDJ127     | 508   | 45 | 10-40-8-6-10-3-2      | -    | -       | -               | I   | YCH        | HIV-positive patients   |
| BA19       | 669   |    | 3-1-94-1-29-5-3       | -    | -       | IV              | Biyem Assi | Hospital personnel |
| HDJ245     | 1289  | 88 | 22-1-14-109-12-4-31   | +    | V       | +               | I   | YCH        | HIV-positive patients   |

ST: Sequence Type; YCH: Yaoundé Central hospital; Cc: Clonal Complexes.
Figure 3. Hierarchical clustering analysis of the pulsed field gel electrophoretic (PFGE) pattern showing the genetic relatedness of the *S. aureus* strains isolated from hospital personnel, HIV-positive and diabetes mellitus patients. The dendrogram was generated by BioNumerics Software with the Band-matching coefficient and the unweighted pair-group method with arithmetic mean (UPGMA).

4. Discussion

The present study has demonstrated the importance of molecular identification by revealing that community acquired-MRSA-PVL and community acquired-MSSA-PLV are common amongst *S. aureus* isolates colonizing the nares of hospital personnel, HIV-positive and diabetes mellitus patients in Yaounde. Three atypical sequence types were detected while pandemic strains were found to cluster irrespectively of studied genetic characteristics analysed or study population. Molecular identification and sequence typing of bacterial isolates is essential to confirm speciation, to monitor evolutionary trends and to determine clonal relatedness within the study population.

The prevalence of colonization with MRSA has been reported to vary according to the population studied as well as study site [34]. MRSA colonization rate (45%) amongst isolates from hospital personnel identified in this study was higher than the 11.4% registered for a similar group in 2013 by Rongpharpi and collaborators in Assam, Nigeria [34] [35]. The 33% prevalence recorded for HIV-positive patients was higher than 2.4% reported in Mekelle, Northern Ethiopia [36]. Likewise the prevalence of MRSA amongst isolates from diabetes mellitus patients (39%) was higher than 9.9% registered by Kutlu and collaborators in 2012 [36]. These MRSA rates however fall within the 0% - 59% range docu-
mented in a systematic review 104 studies describing MRSA nasal carriage in several countries in Africa [37]. Discrepancy in results has been attributed to the level of compliance to control measures such as hand washing and techniques used for MRSA detection. The highest prevalence was recorded at the Yaounde Central Hospital which was the most crowded hospital facility among the study sites. Overcrowding is considered a favourable condition for the spread of MRSA through hand contact with contaminated surfaces or otherwise through the air. MRSA are resistant to several classes of antibiotics, rendering treatment of simple infections complicated and costly. Antimicrobial resistance is enhanced by ease in drug acquisition, automedication and poor hygiene.

Molecular characterisation of MRSA isolates revealed community-associated SCCmec type IV as the predominant type among the study population and study sites. A similar result was reported by Abdul et al., 2015 who identified SCCmec type IV as the leading type between SCCmec type IV and type V [38]. SCCmec type IV has been identified as the most virulent type because it carries a mecA gene, it has a functional recombinase and it is most mobile thus can be transmitted easily to MSSA within the community. Contrary to these findings, SCCmec V was reported as being the major type among MRSA isolated from the nares of Egyptian and Saudi Arabian outpatients. This variation could be due to the different multiplex-polymerase chain reaction (PCR) methods used in the assignment of SCCmec types as highlighted by Antiabong et al., 2016 [33]. The detection of hospital associated SCCmec type II among isolates from HIV positive and diabetes mellitus patients has been previously reported. This implies that community acquired-MRSA and hospital acquired-MRSA isolates might no longer be differentiated based only on SCCmec genotypes [29]. The circulation of MRSA strains within communities and hospitals may be driven by routine visits of patients and their family members (patients’ care givers) to the hospital.

The frequency of MSSA/PVL and MRSA/PVL isolates from the three study populations were higher than reported prevalences of less than 10% in European countries, but falls within the 17% to 70% range reported for Madagascar, Morocco, Niger and Senegal [29]. While the MRSA/PVL prevalence for hospital personnel (41%) was comparable to the rate in Algiers (44%), rates for HIV-positive patients (35%) and diabetes mellitus patients (24%) were higher than has been documented in other parts of Africa [39]. Given that luk S-PV is phage mediated and can easily be transmitted between isolates, this may explain high prevalence rates of gene among strains in Africa where monitoring is limited. The MRSA/PVL strains sequenced were categorized as CC8, CC121 and CC1289. Most MSSA/PVL isolates were obtained from HIV-positive patients and those sequenced were classified in clonal complexes CC8, CC15, CC72 and CC121. The CC121 clone is a known African pandemic clone common in Nigeria, Togo, South Africa and Cameroon [38]. The relatively high rates of both MSSA/PVL and MRSA/PVL strains among asymptomatic carriage isolates may serve as important endogenous reservoirs for subsequent transmission to sterile body sites and an important epidemiological focal point for possible dissemination within
the community. This may worsen clinical outcomes in financially over burdened individuals and healthcare facilities.

The predominant agr type in all three populations was agr type I, same as results by Meysam et al., 2014 who reported 43.3% for agr type I [40]. In another study conducted by Van Leeuwen et al., 2000, 71% of 192 S. aureus carrier strains were classified as agr 1 [41]. The role played by agr group I in staphylococcal infections in high risk populations need to be elucidated in future studies.

The eighteen different pulsotypes generated, grouped isolates irrespective of study population. Three isolates from hospital personnel from the Yaounde General Hospital [n = 2] and the Biyem-Assi District Hospital [n = 1], clustered in the same pulsotype with the same band patterns at 80% homology. This indicates that intra- and inter-hospital transmission of strains, highlighting the need for improvement of hospital hygiene and hand hygiene of hospital personnel.

Twelve representative isolates were classified into six pandemic STs (5, 8, 15, 25 72 and 121) based on pulsotype patterns generated by PFGE. All of these clones have been described as pandemic clones in previous studies from other African countries, with ST5 and ST15 being predominant in West and Central Africa. ST15 was reported in Mali, Gabon and Germany as the most prevalent serotype in asymptomatic carriers. The PVL-positive ST121 (MRSA-IV) clone detected, is prevalent in Africa and was also reported in Asia as a paediatric epidemic clone. [31] In addition to typical STs, three atypical STs (508, 699, and 1289) were identified. ST1289 (SCCmecV/PLV) which is a single-locus variant of ST88, with characteristics of an epidemic clone, was detected in 2008 among clinical isolates [15]. Similarly, Schaumburg et al., 2011, found ST508 to be significantly associated with asymptomatic carriage (p-value = 0.024) amongst isolates from Gabon.

5. Conclusion

Hospital personnel, HIV-positive and diabetes mellitus patients in Yaounde represent an important reservoir for MRSA, MRSA-PVL and MSSA-PVL strains. The virulence factor agr type I was common among study populations. Studying the possible role of agr I in staphylococcal infections may provide insight for controlling risk factors among high risk patients. Isolates were genetically diverse with MLST revealing pandemic ST88 as being predominant and three atypical STs (508, 699, and 1289). The detection of uncommon STs especially ST1289 with characteristic of an epidemic clone has provided an insight into the necessity for routine monitoring to prevent spread and disease outbreaks. The clonal diversity of S. aureus reported in this study and the detection of some epidemic strains serve as the basis for informed decisions on better patient management.

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**Availability of Data and Materials**

The data used and or analysed during the current study are available from the corresponding author on reasonable request.

**Authors' Contributions**

AE designed the study with input from ME and ELM SKS. EM, GI, MM. CH collected samples from participants and isolated *Staphylococcus aureus*. AE, ELM, MCOA, MT and HGK did the molecular analysis. AE and MK did the PFGE. AE, JA and CF performed the MLST. MH and CF did the statistical analysis. AE, ME, ELM and JA wrote the first draft of the manuscript and all authors contributed to and had final approval of the article.

**Consent for Publication**

Not applicable.

**Ethics Approval**

Ethical clearance (No. 2014/07/475/CEA/CNERSH/SP) was obtained from the Cameroon National Ethics Committee for Human Health Research. Authorizations were obtained from recruitment sites and only participants who signed the assent form were enrolled in the study.

**Conflicts of Interest**

The authors declare that they have no competing interests.

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