Constellation of Methicillin-Resistant Genomic Islands (SCCmec) among Nasal Meticillin-Resistant Staphylococcus aureus Isolates

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SUMMARY

The apprehensiveness for the knowledge vacuum on existential threat of nasal carriage of pvl+ healthcare-acquired meticillin-resistant Staphylococcus aureus (HA-MRSA) strains amongst subjects in hospitals have led us to pursue a grasp on the constellation of staphylococcal cassette chromosome mec (SCCmec) types and pvl gene among mecA positive MRSA nasal strains. This was accomplished by phenotypic (catalase, coagulase, Microgen staph ID, ORSAB) and genotypic (polymerase chain reaction) biotyping techniques. All the mecA+ strains harboured the SCCmec gene; SCCmec type I prevailed in 43.75% and pvl was found in 42.1% of the isolates. Dual carriage of mecA and pvl genes occurred in six (37.5%, n = 6/16) strains. Overall, majority of the mecA+ MRSA strains documented in this study carried SCCmec elements of the HA genotype with a hint of community-acquired (CA)- genotype suggesting a possible coexistence of both HA-MRSA and community-acquired- healthcare-acquired meticillin-resistant Staphylococcus aureus (CA-MRSA) strains. Consequently, the implementation of methodical surveillance is needed for the evaluation of potential shifts in directionality of (HA-MRSA/CA-MRSA) pvl+ MRSA clones in our hospitals for effective and prudent antimicrobial stewardship.

Key words: mecA, SCCmec, pvl, healthcare-acquired meticillin-resistant Staphylococcus aureus (HA-MRSA), community-acquired meticillin-resistant Staphylococcus aureus (CA-MRSA), Staphylococcus aureus
INTRODUCTION

Acquisition of mecA gene mediates the acquisition of methicillin resistance by encoding the production of altered penicillin binding protein (PBP2a) housed on a genomic island called staphylococcal cassette chromosome mec (SCCmec), a mobilizable genetic element that integrates into the specific position in the chromosome (1). These elements differ in size from 20 kb to about 60 kb and are classified into types based on the orientation of mecA regulators (mecR1/mecI) and recombinases (ccrAB and ccrC) that ease SCCmec excision, circularization, and insertion in chromosome (2). SCCmec island houses the ccr complex, the mec complex and the ‘Junkyard area’. The first of these cassettes (SCCmec I) was initially identified at the end of the 20th century in Staphylococcus aureus. Subsequently, two others (SCCmec II and III) were found from different MRSA strains (3). Contemporarily, novel types of SCCmec elements, like IV to XI and several new variants of already identified SCCmec types have been documented (4). Anecdotally, the lines of distinction between SCCmec types among community and hospitals is diminishing worldwide. The majority of hospital-acquired MRSA (HA-MRSA) and community-acquired MRSA (CA-MRSA) isolates still carry the SCCmec type’s I–III and SCCmec types IV–V, respectively (4). Nonetheless, the carriage of SCCmec types IV–V is not an all-encompassing characteristic of CA-MRSA because unlike the larger mobile elements of type I to III, genomic islands IV–V are small and easily transferable which may spill-over to HA-MRSA (5).

In the 20th century, Noel Panton and Francis Valentine associated a leukocyte lysing, cytolytic gamma toxin latter called Panton-Valentine leukocidin (Luks) genes found in the genomes of S. aureus associated bacteriophage (8, 15, 16). The PVL toxin targets the cell membrane of leukocytes and increases its permeability (by forming pores) that leads to cellular degradation and necrosis (17, 18). In severe infections, this will result in a decrease in leukocytes count, increases in S. aureus virulence, responsible for intense necrotic skin infections (19, 20). The pvl gene is carried by methicillin susceptible (MSSA) and resistant (MRSA) S. aureus (7, 21). The contribution of pvl in musculoskeletal disorders, necrotizing pneumonia, and brain abscess has been documented. PPSA associated highly transmissible therapy-refractory skin infections, and life-threatening hemoptysis has been on the rise globally (8, 22).

The prevalence of HA-MRSA (based on mecA+ strains) in hospitals in Nigeria may differ from 1.5% to 20% (23). The purpose of this study was to determine the constellation of SCCmec types among mecA positive (mecA+) MRSA nasal isolates in our study center.

MATERIALS AND METHODS

Confidentiality and ethical considerations

Ethical review board of Sokoto State Ministry of Health in Nigeria approved this study (SMH/1580/V. IV). Informed consent was obtained from the study participants and information gathered were documented anonymously.

Study center, sample collection and processing

This study was conducted in three Sokoto state-owned hospitals in 2018 (Maryam Abacha Women and Children Hospital, Specialist Hospital and Orthopedic hospital Wamakko). Nasal swabs were randomly collected from 378 participants (healthcare workers, inpatients, outpatients, security men and cleaners) using commercially available swab sticks and processed as per standard microbiological procedures for the recovery of S. aureus (24). Biotyping for S. aureus was based on growth
Mannitol salt agar, Gram staining characteristics, spot tests (catalase and coagulase) and the Microgen™ Staph ID kit.

**Oxacillin resistance screening agar base (ORSAB) test**

A standardized suspension (0.5 McFarland) of *S. aureus* isolates were prepared and inoculated onto ORSAB medium pre-supplemented with 6 μg/ml oxacillin and 4% sodium chloride and incubated at 35°C overnight. The emergent bluish colonies from the overnight cultures were considered methicillin resistant (25).

**Polymerase chain reaction**

The presumptive isolates were additionally queried by two different multiplex PCRs. The first multiplex PCR was done for the detection of methicillin resistance (*mecA* gene) and leukocidin toxin gene (*pvl*) (Table 1). The second multiplex PCR was done for typing SCCmec elements of mecA positive MRSA strains (Table 1). The primers used were calculated using Primer3Plus® based on partial coding sequences of genes of interest obtained from GenBank/NCBI (26). PCR simulation was executed using Snap Gene™ software (version 1.1.3) to determine the efficiency and the validity of the expected amplicon sizes of the proposed primers before its production (Assumption-free). Total bacterial genomic DNA was extracted from 24 hours culture on nutrient agar using the Qiagen™ DNA extraction kit (Qiagen, Hilden, Germany) in accordance with the manufacturer’s protocols. The first PCR for the detection of meca and *pvl* gene was done using the protocol given by (27) and the second PCR for the typing and subtyping of the SCCmec elements was carried out as follows. The reaction was performed in the final volume of 25 μL, involving 4 μL of DNA template, 12.5 μL of Qiagen master mix, 2.5 μL of Q-reagent, 0.5 μL of each primer pair (3 μL totally) and 3 μl molecular grade water. DNA was amplified with a thermocycler (Applied bio systems 9700), and multiplex PCR conditions were as follows: initial denaturation for 3 minutes at 94°C, 35 cycles of denaturation at 94°C for 40 seconds, annealing at 56°C for 45 seconds, and extension at 72°C for 1 minute. The final extension was carried out at 72°C for 4 minutes. The PCR amplicons were visualized on 1.5% agarose gel pre-stained with ethidium bromide using a 100 bp+ ladder (Bio-labs, New England, UK). They were documented with BIO-RAD gel-doc (Milan, Italy) under a UV transilluminator and analyzed with image lab™ 6.01 software (BIO-RAD, Milan, Italy) and Applied Maths Bionumerics version 7.0 (Sint-Martens-Latem, Belgium).

**Table 1. The primer sequence of the studied genes**

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence (5' -- &gt; 3')</th>
<th>Product</th>
<th>Tm</th>
<th>Accession</th>
</tr>
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<tbody>
<tr>
<td>SCCmec I</td>
<td>F TCGGGTGAAAAGTGATGACAC</td>
<td>495 bp</td>
<td>59.5°C</td>
<td>CCJ25736</td>
</tr>
<tr>
<td></td>
<td>R GCGGTAATTGATATCCAGCAA</td>
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<td></td>
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</tr>
<tr>
<td>SCCmec IIa</td>
<td>F TGGCGATGACGATATTGAAG</td>
<td>284 bp</td>
<td>59.65°C</td>
<td>AB774377</td>
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<tr>
<td></td>
<td>R ACCGCAGAAGATGACGAAACT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SCCmec IIb</td>
<td>F AGGTTTAGGAGCGGTTTTTCA</td>
<td>381 bp</td>
<td>59.72°C</td>
<td>AB127982</td>
</tr>
<tr>
<td></td>
<td>R CTTCTAACGCTTCGATTCC</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>SCCmec III</td>
<td>F TCCCCATATCGGAAAGAACCG</td>
<td>313 bp</td>
<td>59.86°C</td>
<td>AB047089</td>
</tr>
<tr>
<td></td>
<td>R ACTTGCTGCATCCACTGTTG</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>SCCmec IVa</td>
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<td>450 bp</td>
<td>59.95°C</td>
<td>AB266531</td>
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<tr>
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<td>R TGCAATGCAACGATGATAACGA</td>
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<tr>
<td>SCCmec IVb</td>
<td>F TGCATGCACAGTGATAACGA</td>
<td>1kb</td>
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<td></td>
<td>R TTTGAGGTTTTCCGGGTGTTT</td>
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<td><em>mecA</em></td>
<td>F TGGTAAGGTTGGCCAAAAAGA</td>
<td>533 bp</td>
<td>59.6°C</td>
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<tr>
<td></td>
<td>R TTGTCCGTAAACCAGGAATCA</td>
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<tr>
<td><em>pvl</em></td>
<td>F TAAGGGGCAAACACTTGTGGA</td>
<td>433 bp</td>
<td>59.8°C</td>
<td>HQ020533</td>
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<tr>
<td></td>
<td>R CCATTGATCAGACGAGCA</td>
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Statistical method

Descriptive statistics were used to present the study outcomes. Each study variable was compared between SCCmec types. Variables were summarized as proportions (%). Statistical analysis was done using SAS version 9.4 software (SAS, Cary, NC, USA).

RESULTS

We studied eighty-one phenotypic *S. aureus* nasal isolates from state-owned hospitals and arrived at thirty-eight phenotypically confirmed MRSA strains out of which 16 were PCR-positive for mecA and 17 for *pvl* gene (Figure 1). Dual carriage of mecA and *pvl* occurred in six (37.5%, n = 6/16) strains. The isolates were resolved into four clades: A, B, C and D with decreasing PCR profile similarity. Cluster A housed mecA+–*pvl*+ strains. Clade B comprised mecA+ stains, C housed *pvl*+ and D was PCR-negative. Further, we amplified SCCmec from all strains (100%) that were PCR-positive for the mecA gene (Figure 2). SCCmec type I prevailed in 43.75%, type IIA in 18.75%, type IIB in 12.50%, type

![Figure 1. A clustering tree of DNA gel fingerprint resolved from electropherograph showing MRSA strains segregated based on their SCCmec type in groups of various sizes. In the figure, clustering identified four clades based on banding profiles; a scale bar indicates a degree of similarity. Note: Red = PCR-positive for dual carriage of mecA and *pvl*; Yellow = PCR-positive for mecA only; Green = PCR positive for *pvl* gene only, and Blue = PCR negative strains.]
Figure 2. Three-dimensional electrophoretogram of ethidium bromide-stained gel showing amplification of SCCmec I (495 bp), SCCmec Ila (284 bp), SCCmec IIb (381 bp), SCCmec III (313 bp), SCCmec IVa (450bp), and SCCmec IVb (1kb) from 16 mecA positive MRSA strains. In the figure, Lane 1 and 20 = 100 bp+ DNA ladder, Lane 2 = S. aureus ATCC 25923 (Positive control), Lane 3 - 18 = Contain all 16 MRSA strains and Lane 19 = Negative control (Nuclease-free water)

Figure 3. Percentage distribution of SCCmec and pvl genes of mecA positive MRSA strains
III in 18.75% and type IVA in 6.25% of the MRSA isolates. None of the isolates were typable for SCCmec type IVA (Figure 3). The pvl gene was documented in 6.25% of strains typed SCCmec I, IIb and IVb, and 12.5% of SCCmec IIa and III typed strains. Overall, the prevalence of SCCmec and pvl gene was 42.1% and 44.7%, respectively. We encountered SCCmec gene among healthcare workers (43.75%), inpatients (18.75%), outpatients (25%), cleaners (6.25%) and security men (6.25%). Most of the mecA positive isolates that harbored both SCCmec gene and pvl genes originated from healthcare workers and inpatients. Almost all the mecA+ strains were HA-MRSA (Figure 4).

**DISCUSSION**

In this work, we provided genotypic data on 38 phenotypic MRSA strains discovered during a six-month period in Sokoto state-owned hospital in order to elucidate the genetic pool and potential emergence of unexpected MRSA strains. The mecA carrying MRSA strains prevailed in 42.1% of phenotypic methicillin resistant strains amongst the hospital with a 93.75% presence of HA-MRSA. This prevalence of HA-MRSA strains carrying a HA genotype in this study was higher than previous rates reported by Okwu et al. (28), Gebremedhin et al. (29), Pathare et al. (30), Mohajeri et al. (31), and Parvez et al. (32) that documented the occurrence rates of 59%, 52.85%, 15.1%, 36.8% and 40%, respectively.

A pertinent proportion of mecA positive MRSA strains with HA genotype was associated with healthcare workers carrying SCCmec I, II, and III confirming that most of carried strains emerged from health care settings. Possible reasons for the assortment of SCCmec type might be due to differences in geographical regions, the study population, and detection methods. SCCmec types IVb was rare and carried by 6.25% of the strains. This cassette type is traditionally attributed to CA-MRSA (10, 33). Our result is dissident with those documented by mathematical models that posited the replacement of traditional HA-MRSA strains by CA-MRSA strains, due to their higher growth rate and greater fitness (3), but conforms with the models that hospital-community interactions provoke coexistence among methicillin resistant strains of *S. aureus*. (34 – 36). HA and CA strains of MRSA can coexist if the wider resistance range of the HA strains is balanced by transitional fitness-disadvantages in the presence of resistant strains. The HA strains are better selected in the hospital, where antibiotics are regularly used, but community-associated strains thrive (higher fit-
ness) in the community where the use of antibiotics is relatively low (opposite directions of selection) (34). Despite conflicting directions of selection, these strains exist in both settings because of the high rates of hospitalization and discharge, which rotates individuals between the hospital and the community. Besides, our results also specify that opposite directions of selection are not adequate for maintaining coexistence (34).

The carriage of *pvl* gene (*pvl+*) in 44.7% of MRSA isolates in this report is consistent with 58.8% reported by Govindan et al. (37) and in dissidence with reports from Nigeria (16%) (38), Egypt (2.2%) (39), Uganda (73%) (40). In our study, among the tested mecA+ MRSA isolates, *pvl+* was chiefly associated with HA genotype (37.5%). Previous studies have recognized that the *pvl* genes are carried mostly by CA-MRSA (41, 42). Paradoxically, *pvl-*carrying HA-MRSA strains have also been previously described (23).

Lastly, this study had a few limitations. First, the study was conducted in a few healthcare centers and may have biased the representativeness of the isolates herein studied. Second, clinical features were not considered in characterizing both CA-MRSA and HA-MRSA. We used only genotypic characteristics to classify MRSA.

**CONCLUSION**

Overall, majority of MRSA recovered in our study centres carried diverse SCC*mec* elements of the HA genotype with a hint of CA- genotype. We documented the dominance HA-MRSA-*pvl+*. Our data also revealed the presence of CA-MRSA strains suggesting a possible coexistence of both HA-MRSA and CA-MRSA strains. Consequently, implementing methodical surveillance is needed for the evaluation of shift in directionality of (HA-MRSA/CA-MRSA) *pvl+* clones in our hospitals for effective and prudent antimicrobial stewardship.

**Conflict of interest**

All authors declare to have no conflict of interest.
References


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Konstelacija genomskih ostrva (SCC mec) rezistentnih na meticilin kod nazalnih izolata *Staphylococcus aureus* rezistentnog na meticilin

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**SAŽETAK**

Zabrinutost zbog vakuuma u znanju o egzistencijalnoj opasnosti nazalnog nosilaštva *pv* HA-MRSA sojeva kod hospitalizovanih navela nas je da bolje istražimo konstelaciju SCC mec tipova i *pv* gena kod mecA pozitivnih sojeva u slučaju nazalnog klicenoštva meticilin-rezistentnog *Staphylococcus aureus*. Iz ovog razloga urađene su fenotipske (catalaza, coagulaza, Microgen staph ID, ORSAB) kao i genotipske (lančana reakcija polimeraze) tehnike biotipizacije. Svi mecA+ sojevi sadržali su SCC mec gen; SCC mec tip I preovladavao je kod 43,75% izolata, a *pv* kod 42,1% izolata. Dualno nosilaštvo mecA i *pv* gena zabeleženo je kod 6 (37.5%, n = 6/16) sojeva. Većina mecA+ MRSA sojeva registrovanih u ovoj studiji nosili su SCC mec elemente HA genotipa sa naznakom CA - genotipa, što je ukazivalo na moguću koegzistenciju HA-MRSA i CA-MRSA sojeva. Kao rezultat ovoga, uvodenje metodičnog nadzora potrebno je zbog procene mogućih promena usmerenosti (HA-MRSA/CA-MRSA) *pv* MRSA klonova u našim bolnicama kao i efikasnog i pažljivog praćenja antimikrobnih lekova.

*Ključne reči: mecA, SCC mec, pv, HA-MRSA, CA-MRSA, Staphylococcus aureus*