Diversity of SCCmec Elements in Methicillin-Resistant Staphylococcus Aureus (MRSA) Recovered from a Healthy Student Population in Kenya

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Abstract

Background: Methicillin-resistant *S. aureus* continues to be a concern for public health systems, particularly due to infections emerging in non-hospital settings. Resistance to methicillin is presently classified as a serious phenomenon because the majority of methicillin-resistant strains are also multi-drug resistant. The genetic determinant of resistance to methicillin and other β-lactam antibiotics is the *mec-A* gene, which lies in the SCCmec resistance island. In Kenya, studies done previously have shown the existence of SCCmec types in clinical isolates, but similar information on isolates recovered from healthy populations is scanty.

Methods: A cross-sectional study was conducted on healthy university students residing within the university residence halls to determine the carriage of MRSA. MRSA was detected using Cefoxitin (30µg), and *mec-A* gene and Sccmec elements were detected using conventional PCR methods. A total of 237 students were recruited, and 657 swabs were collected using standards methods for recovering *S. aureus*.

Results: A total of 231 *S. aureus* isolates were recovered, out of which 26 (11.3%) were MRSA. Out of the 26 MRSA strains, 17 carried the *mecA* gene in their gene cassettes. SCCmecV was the most prevalent (61.5%), followed by SCCmedI (53.9%) among the MRSA strains. SCCmedVa, SCCmedVb, SCCmedVc and SCCmedVd were absent in all the isolates. SCCmecV was found to be highly prevalent (64.7%) followed by SCCmedI, 8 (47.1%) among the *mecA*-positive MRSA strains. On the other hand, small proportions of *mecA*-negative isolates harbored SCCmed (0.9%), SCCmedII (3.3%), SCCmec III (0.5%) and SCCmecV (2.3%).

Conclusion: This study revealed that the strains recovered from the student population were highly diverse in terms of the SCCmec elements they carried in their gene cassettes.

Background

Methicillin-resistant *S. aureus* infections have become a global public health concern, especially due to infections acquired in non-hospital settings (Qiao et al., 2014). In Kenya, the emergence of MRSA strains accounts for the major skin and soft-tissue infections (Aiken et al., 2014). The development of resistance to methicillin occurred in the 1960s, and this marked the beginning of the second wave of antibiotic resistance after the first wave, which was marked with the development of penicillin resistance. Resistance to methicillin by *S. aureus* is now classified as a serious phenomenon because the majority of such strains are also resistant to other classes of β-lactam antibiotics such as oxacillin, penicillin, amoxicillin, cephalosporins and carbapenems (Chambers & Deleo, 2009). Methicillin resistance is attributed to an extra penicillin-binding protein (PBP2a), which is coded by the *mecA* gene and has been shown to have a significantly reduced affinity for many β-lactam antibiotics (Arède et al., 2012).

The genetic determinant of resistance to β-lactam antibiotics is the *mecA* gene (Fuda et al., 2004). The gene lies in the SCCmec resistance island and is present in about 95% of *S. aureus* isolates, displaying the phenotype of methicillin resistance (Wielders et al., 2002). Resistance to β-lactam has been shown
not to be native to *S. aureus*, but rather, it has been acquired via the *mecA* gene for more than 40 years. The *mecA* gene encodes a protein known as penicillin-binding protein (PBP), which is designated as PBP2a. *S. aureus* produces four PBPs, namely, PBP1, PBP2 PBP3 and PBP4 that are anchored on the cytoplasmic membrane (Navratna et al., 2010). Penicillin-binding proteins function in the assembly and regulation of stages of the synthesis of a bacterial cell wall. Whereas the four PBPs are susceptible to alteration by β-lactam antibiotics resulting in the death of bacterial cells, PBP2a is refractory to the action of all presently used β-lactam antibiotics. The PBP2a can take over the functions of the four staphylococcal PBPs during exposure to β-lactam antibiotics (Kondo et al., 2007).

The *mecA* gene is known to be carried on a peculiar type of mobile genetic element inserted into the staphylococcal chromosome, known as the staphylococcal cassette chromosome *mec* (SCC*mec*) element (Katayama et al., 2000). The SCC*mec* elements share four characteristics. First, they carry the *mec* gene complex, which consists of a methicillin-resistance determinant gene (*mecA*), its regulatory genes and insertion sequences; second, they carry the *ccr* gene complex responsible for the mobility of the element and its associated sequences; third, they have distinct directly repeated nucleotide sequences and inverted complementary sequences at each end; and fourth, they integrate into the 3′ end of an open reading frame (ORF), *orfX*. Despite the similarities identified above, structures of SCC*mec* elements are also divergent (Kondo et al., 2007).

In Kenya, the few studies which sort to examine the diversities of SCC*mec* elements have revealed the existence of different types of SCC*mec* elements. In a recent study that analyzed isolates obtained from a government hospital and a private referral hospital in Nairobi County, SCC*mec*III was found to be more prevalent, followed by SCC*mec*IV. The predominance of these two SCC*mec* elements was attributed to their small sizes, which facilitated their transmission in both community and hospital settings (Omuse et al., 2016). Another study that focused on isolates recovered from a government hospital setting also revealed the existence of SCC*mec*II among all the six MRSA isolates obtained from inpatient screens (Aiken et al., 2014). In a study done by Maina et al., (2013), out of 69 MRSA strains obtained from patients with skin and soft tissue infections, 52 (75.4%) harbored SCC*mec*II, 5 (7.2%) harbored SCC*mec*I, 2 (2.9%) harbored SCC*mec* and II, while 1 (1.4%) harbored SCC*mec*II in their gene cassettes. Based on this information, it is clear that previously published studies done in Kenya on the diversity of SCC*mec* elements focused on isolates recovered from patients only. Thus MRSA circulating in healthy populations outside hospital settings remain under-investigated. The previous studies have revealed the existence of SCC*mec* types II, III and IV, making this information available specifically for clinical MRSA isolates. As such, there is a lack of information on the SCC*mec* types harbored by *S. aureus* isolates circulating in otherwise healthy persons outside hospital settings in Kenya, and this is what this study sort to determine.

**Methods**

**Recruitment of participants**
Participants were recruited from the hostels. Students who resided at the university and agreed to provide written consent for participation by providing samples and filling the questionnaire were included in the study. Students who were on antibiotics and those who consented, but did not provide a nasal swab were excluded from the study.

This study was conducted in accordance with the regulations of the scientific and ethics review unit (SERU) of the Kenya Medical Research Institute (KEMRI) (Protocol No. KEMRI/SERU/CMR/0024/3123).

**Study design**

This cross-sectional study was conducted in 2016 on healthy students residing in a university's residence halls. A systematic random sampling procedure was used to select the students' hostel rooms where all occupants were recruited. A total of 237 healthy students were included in the study.

**Collection of swabs**

Swabs were collected from the nostrils, mobile phones, and pens used by participants using sterile swab sticks pre-moistened with sterile normal saline 0.85% (NaCl). All swabs were put in Amies transport media for transportation to the laboratory within a maximum of 4 hours. For enrichment, the swabs were transferred to trypticase soy broth (TSB) and incubated for 18 to 24 hours (Coia et al., 2006).

**Isolation and identification of S. aureus isolates**

A loopful of inoculum from the TSB cultures was inoculated in on Mannitol Salt Agar (Oxoid) plates and incubated aerobically at 37°C for 24 hours. Standard methods of identifying S. aureus, as described by Baron (1996), were employed. The plates were assessed for colony morphology and yellow pigment formation associated with S. aureus. Suspect colonies were subjected to Gram staining, coagulase, and catalase tests. Spa typing was also done for confirmation of all S. aureus isolates.

**Antimicrobial susceptibility testing**

The antimicrobial susceptibility tests were done using a modified Kirby-Bauer disk diffusion method describe by Rongpharpi et al. (2013). Commercially available antibiotic discs for Gram positive bacteria were used (Oxoid, CB, UK). The discs included ampicillin (10µg), amoxicillin-clavulanic acid (30), ciprofloxacin (10µg), erythromycin (15µg), gentamicin (10µg), cefoxitin (30µg), linezolid (30µg), norfloxacin (10µg), nitrofurantoin (300µg), chloramphenicol (30µg) and trimethoprim-sulfamethoxazole (25µg) (CLSI, 2015). S. aureus (MRSA) ATCC® 33591 (Oxoid) was used as control. Zones of inhibition were measured after overnight incubation at 37°C (Rongpharpi et al., 2013). Methicillin-resistant S. aureus was detected phenotypically using cefoxitin (30µg). S. aureus isolates that displayed zone sizes
of less or equal to 21mm were characterized as MRSA, while *S. aureus* isolates that displayed zone sizes of more than 21mm were characterized as MSSA (CLSI, 2015). *S. aureus* isolates resistant to β-lactams, and antibiotics from at least three non-β-lactam classes were classified as MDRs.

### Extraction of DNA

The extraction of DNA was done using a 10% Chelex solution prepared using 1X TE buffer following a modification of the procedure used by (HwangBo et al., 2010). A 300µl volume of 10% Chelex solution was added to fresh sterile Eppendorf tubes. To each Eppendorf tube filled with 300µl volume of 10% Chelex solution, a loopful of 24 hours old bacterial colonies were emulsified. The Chelex/sample tubes were then vortexed for 5-10 seconds. The tubes were then transferred to a heating block set at 95 ºC for 20 minutes. After boiling for 20 minutes, the tubes were left to cool for 10 minutes before centrifugation at 14700 rpm for 10 minutes. After that, 50µl of the supernatant was transferred to fresh Eppendorf tubes prefilled with 450µl of RNase DNase free PCR water. All the tubes were labeled carefully using the respective isolate codes.

### Spa typing

Amplification and detection of *mecA* gene were done on all *S. aureus* isolates using conventional PCR methods. The primers used are listed in Table 1. In every PCR tube, 4µl of the ready to mix 5x FIREPol® Master Mix (Solis Biodyne, UK), 0.4µl of both forward and reverse primers at a concentration of 10pmol of each primer, an aliquot of 1µl of BSA, 12.2 µl of RNase DNase free PCR water and 2µl of sample DNA was added. A single control strain of *S. aureus* was incorporated in the DNA amplification process. PCR reactions were set for 5 minutes at 80°C; 35 cycles of 45 seconds at 94°C, 45 seconds at 60°C, and 90 seconds at 72°C; a single final extension at 72°C for 10 minutes and a holding temperature at 4°C. The excepted band sizes were to be variable (Strommenger et al., 2008).

### Amplification and detection of *mecA* gene

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**SCCmec typing**

SCCmec typing was done for all isolates using PCR. Primers specific for SCCmec, SCCmecI, SCCmecII, SCCmecIVA, SCCmecVb, SCCmecVc, SCCmecVd and SCCmecV elements were used as published previously (Zhang et al., 2005). The primer sequences are provided in Table 1. PCR procedures were similar to those for the mecA gene, but the annealing temperature was set at 65°C for 45 seconds. A control strain of *S. aureus* known to be positive for the gene was incorporated in all the PCR sets.

| Type of primer/gene | Primer sequences | Annealing temperature | Amplicon size | Reference |
|---------------------|------------------|-----------------------|---------------|-----------|
|                      | Forward primer   | Reverse primer        |               |           |
| *spa*               | 5’ TAA AGA CGA  | 5’ CAG CAG TAG         | 60°C          | Variable  |
|                     | TCC TTC GGT GAG | TGC CGT TTG CTT3’      |               | (Strommenger et al., 2008) |
| *mecA*              | 5’AAA ATC GAT   | 5’AGT TCT GCA GTA     | 56°C          | 533bp     |
|                     | GGT AAA GGT TGG | CCG GAT TTG C3’       |               | (Kondo et al., 2007) |
| SCCmecI             | 5’GCT TTA AAG   | 5’GTT CTC TCA TAG TAT| 65°C*, 55°C   | 613bp     |
|                     | AGT GTC GTT ACA | TAT GAC GTC C3’       |               | (Zhang et al., 2005) |
| SCCmecII            | 5’ CGT TGA AGA  | 5’-CGA AAT TGG TTA   | 65°C*, 55°C   | 398bp     |
|                     | TGA TGA AGC G 3’| ATG GAC C3’          |               | (Zhang et al., 2005) |
| SCCmecIII           | 5’CCA TAT TGT  | 5’ CCT TAG TTG TCG   | 65°C*, 55°C   | 280bp     |
|                     | GTA CGA TGC G 3’| TAA CAG ATC G 3’     |               | (Zhang et al., 2005) |
| SCCmecIVA           | 5’GCC TTA TTC  | 5’-CTA CTC TTT TGA  | 65°C*, 55°C   | 776bp     |
|                     | GAA GAA ACC G 3’| AAA GCG TCG 3’       |               | (Zhang et al., 2005) |
| SCCmecIVb           | 5’TCT GGA ATT  | 5’AAA CAA TAT TGC    | 65°C*, 55°C   | 493bp     |
|                     | ACT TCA GCT GC’ | TCT CCC TC 3’        |               | (Zhang et al., 2005). |
| SCCmecIVc           | 5’ACA ATA TTT  | 5’TGG GTA TGA GGT  | 65°C*, 55°C   | 200bp     |
|                     | GTA TTA TCG GAG | ATT GCT GG 3’         |               | (Zhang et al., 2005). |
| SCCmecIVd           | 5’CTC AAA ATA  | 5’TGC TCC AGT TGA   | 65°C*, 55°C   | 881bp     |
|                     | CGG ACC CCA ATA | TGC TAA AG 3’       |               | (Zhang et al., 2005). |
| SCCmecV             | 5’GAA CAT TGT  | 5’ TGA AAG TTA TAC | 65°C*, 55°C   | 325bp     |
|                     | TAC TTA AAT GAG | CCT TGA CAC C3’     |               | (Zhang et al., 2005). |

Table 1

Primers used for amplification of selected genes
Results

Frequency of recovery of S. aureus

A total of 237 students, 120 (50.6%) males and 117 (49.4%) females were recruited, and 657 swabs were collected. The recovered S. aureus isolates were 231 in number, out of which 26 (11.3%) were MRSA. Overall, a large proportion of the participants 87 (36.7%) harbored S. aureus at least on their mobile phones compared to the proportions that carried the bacteria at least on their nostrils, 78 (32.9%), and pens, 66 (27.9%). It was also noted that a large proportion of MRSA strains were harbored in the nostrils (20, 76.9%), followed by mobile phones (7, 26.9%) and pens (5, 19.2%). In several cases, MRSA was also found to be harbored in the nostrils, mobile phones and pens used by the same student at the same time, as shown in Table 2.

| Type of sample/s analyzed | Number of S. aureus per sample type | Number of MRSA per sample type |
|--------------------------|----------------------------------|-------------------------------|
|                          | S. aureus (N=231)                |                               |
|                          | Frequency (%)                    | MRSA (N=26)                  | Frequency (%) |
| Nostril                  | 78                               | 20                            | 76.9          |
| Phone                    | 87                               | 7                             | 26.9          |
| Pen                      | 66                               | 5                             | 19.2          |
| Nostril + Phone          | 40                               | 4                             | 15.4          |
| Nostril + Pen            | 29                               | 1                             | 3.8           |
| Phone + Pen              | 31                               | 1                             | 3.8           |
| Nostril + Phone + Pen    | 17                               | 9                             | 34.6          |

Table 2
Number of S. aureus and MRSA recovered from nostrils and fomites

Antimicrobial resistance phenotypes

All 231 isolates of S. aureus were tested against a panel of 11 antimicrobials. Resistance to ampicillin was very high (84%). Lower resistances of less than 20% were recorded on other isolates. Precisely, resistances to amoxicillin, chloramphenicol, gentamicin, ciprofloxacin, nitrofurantoin and norfloxacin were below 10%. None of the isolates was resistant to linezolid, and only a single isolate showed intermediate resistance to nitrofurantoin, as shown in Figure 1.

Following the screening of the 231 isolates for antimicrobial resistance, 26 (11.3%) were found to be MRSA, while 16 (9.9%) were MDRs. Out of the 26 MRSA strains, 13 (50%) were also MDRs. All the MRSA and MDR strains showed total resistance to ampicillin. Resistances above 40% were observed for
erythromycin, norfloxacin and trimethoprim-sulfamethoxazole among the MRSAs. Similar observations were made for the MDRs, which also showed over 40% resistance levels to ciprofloxacin (50%) and norfloxacin (75%). For both MRSA and MDRs, less than 20% of resistance levels were recorded for amoxicillin-clavulanic acid, chloramphenicol and gentamicin, as shown in Figure 2. Statistical analysis revealed that MRSA strains were more likely to display multi-drug resistance phenotypes compared to the MSSA strains (P-value = 0.001).

**mecA and Diversity of SCCmec types**

All 231 S. aureus isolates were screened for the presence of the mecA gene and SCCmec elements. Only 17 (7.4%) of all the S. aureus isolates harbored the mecA gene. mecA gene was found in MRSA 17 (65.4%) of the MRSA strains. SCCmed was more prevalent among the MRSA strains, 4 (15.4%), compared to MDR strains, 1 (6.3%), and MSSA strains, 2 (1%). Small proportions (less than 5%) of isolates recovered from the nostrils and fomites were also found to harbor SCCmed. The prevalence of SCCmedI was found to be higher among the MDRs, 11 (68.8%) than in MRSA strains, 14 (53.9%) and the MSSA, which did not harbor SCCmedI. A large proportion of the isolates from the nostrils, 10 (12.8%), also harbored SCCmed compared to the proportion of isolates from fomites, 5 (3.3%). SCCmedII was only present in 3 (11%) of the MRSA isolates, and only 2 (2.6%) of the isolates recovered from the nostril and fomites respectively. Similar to SCCmedII, the prevalence of SCCmedV was found to be slightly higher among the MDRs, 10 (62.5%), compared to MRSAs, 16 (61.5%), as shown in Table 3. SCCmedIVA, SCCmedVb, SCCmedVc and SCCmedVd were absent in the pool of isolates.

| Type/source of isolate | Number of isolates tested (N) | Frequency of mecA presence n (%) | Frequency of SCCmec elements n (%) |
|------------------------|-------------------------------|----------------------------------|-----------------------------------|
|                        |                               |                                  | I (n, %)                          |
|                        |                               |                                  | II (n, %)                         |
|                        |                               |                                  | III (n, %)                        |
|                        |                               |                                  | V (n, %)                          |
| MRSA                   | 26                            | 17 (65.4%)                       | 4 (15.4%, 4, 53.9%, 3 (11.5%), 16 (61.5%) |
| MSSA                   | 205                           | 0                                | 2 (1.0%, 0, 0, 0)                 |
| MDR                    | 16                            | 9 (56.3%)                        | 1 (6.3%, 11 (68.8%), 0, 10 (62.5%) |
| Nostrils               | 78                            | 8 (10.3%)                        | 3 (3.9%, 10 (12.8%), 2 (2.6%), 10 (12.8%) |
| Fomites                | 153                           | 9 (5.9%)                         | 4 (2.6%, 5 (3.3%), 1 (0.7%), 6 (3.9%) |

**SCCmec elements in mecA positive isolates**
All the *meca*-positive strains were screened for the presence of selected SCC\textit{mec} elements in their gene cassettes. Accordingly, SCC\textit{mec}V was found to be highly prevalent, 11 (64.7%), compared to SCC\textit{mec}I, 8 (47.1%), SCC\textit{mec}I, 5 (29.4%), and SCC\textit{mec} III, 2 (11.8%). Contrary to the *meca*-positive MRSA strains, the SCC\textit{mec}I were harbored in the majority, 7 (77.8%), of the *meca*-positive MDR strains. A majority of *meca*-positive isolates recovered from the nostrils, 6 (75%), and fomites, 5 (55.6%), were also found to harbor SCC\textit{mec}V. The unidentified SCC\textit{mec} elements were found in small proportions of the *meca*-positive MRSA strains, 3 (17.6%). See Table 4.

Table 4
Proportion of *meca* positive isolates carrying SCC\textit{mec} elements

| Type/source of isolate | Number of *meca*-positive isolates (N) | SCC\textit{mec} type, n(%) | Unidentified SCC\textit{mec} elements, n (%) |
|------------------------|----------------------------------------|-----------------------------|---------------------------------------------|
| MRSA                   | 17                                     | 5 (29.4%)                   | 3 (17.6%)                                   |
|                        |                                        | 8 (47.1%)                   |                                             |
|                        |                                        | 2 (11.8%)                   |                                             |
|                        |                                        | 11 (64.7%)                  |                                             |
| MDR                    | 9                                      | 1 (11.1%)                   | 0                                           |
|                        |                                        | 7 (77.8%)                   | 3 (33.3%)                                   |
| Nostril                | 8                                      | 3 (37.5%)                   | 0                                           |
|                        |                                        | 5 (62.5%)                   |                                             |
|                        |                                        | 1 (12.5%)                   |                                             |
| Fomites                | 9                                      | 2 (22.2%)                   | 3 (33.3%)                                   |
|                        |                                        | 3 (33.3%)                   | 1 (11.1%)                                   |
|                        |                                        | 5 (55.6%)                   | 3 (33.3%)                                   |

**SCC\textit{mec} elements in *meca* negative isolates**

Generally, the majority of the *meca*-negative MRSA isolates harbored SCC\textit{mec}I, 6 (66.7%), and SCC\textit{mec}V, 5 (55.6%), compared to SCC\textit{mec}II, 1 (11.1%). The SCC\textit{med} was the only gene detected in 2 (1.0%) of the *meca*-negative MSSA strains. The SCC\textit{med}I and SCC\textit{mec}V were present in 7 (57.1%) and 2 (28.6%) of the *meca*-negative MDR strains, respectively. Small proportions of *meca*-negative isolates recovered from nostrils (less than 10%) were found to harbor SCC\textit{med}, II, III and V while those recovered from fomites harbored SCC\textit{med}, II and V as presented in Table 5.
Table 5
Proportion of mecA negative isolates carrying SCCmec elements

| Type/source of isolate | Number of mecA-negative isolates (N) | SCCmec type, n (%) |
|------------------------|-------------------------------------|--------------------|
|                        |                                     | I      | II          | III         | V         |
| MRSA                   | 9                                   | 0      | 6 (66.7%)   | 1 (11.1%)   | 5 (55.6%) |
| MSSA                   | 202                                 | 2 (1.0%)| 0           | 0           | 0         |
| MDR                    | 7                                   | 0      | 4 (57.1%)   | 0           | 2 (28.6%) |
| Nostril                | 70                                  | 5 (7.1%)| 1 (1.4%)    | 1 (1.4%)    | 4 (5.7%)  |
| Fomites                | 144                                 | 2 (1.4%)| 2 (1.4%)    | 0           | 1 (0.7%)  |

### Multiple SCCmec elements

This study revealed four different combinations, each consisting of two distinct SCCmec elements. These included; SCCmecI and SCCmecIII, SCCmecI and SCCmecV, SCCmecI and SCCmecII and SCCmecV. Almost half of the MRSA strains, 11 (42.3%), carried SCCmecII and SCCmecV compared to those that harbored SCCmecI and SCCmecV, 3 (11.5%), SCCmecI and SCCmecII, 2 (7.7%), and SCCmecIII and SCCmecV, 1 (3.9%). Only two combinations of the SCCmec elements were seen among the MDR strains, where 8 (50%) of these strains carried SCCmecII and SCCmecV, and only a single MDR varied SCCmecI and SCCmecV. See Table 6 below.

Table 6
Proportion of S. aureus isolates carrying multiple SCCmec elements

| Type/source of isolate | Number of S. aureus isolates tested (N) | SCCmec elements combination, n (%) |
|------------------------|-----------------------------------------|-----------------------------------|
|                        |                                         | SCCmecI  | SCCmecII  | SCCmecV  | SCCmecI  | SCCmecII  | SCCmecV  |
| MRSA                   | 26                                      | 2 (7.7%) | 3 (11.5%) | 11 (42.3%)| 1 (3.9%)  |
| MDR                    | 16                                      | 0        | 1 (6.3%)  | 8 (50%)   | 0         |
| Nostril                | 78                                      | 1 (1.3%) | 2 (2.6%)  | 7 (9.0%)  | 1 (1.3%)  |
| Fomites                | 153                                     | 1 (0.7%) | 1 (0.7%)  | 4 (2.6%)  | 0         |

### Discussion
Carriage of S. aureus in a population that is otherwise considered as healthy has shown global variation. Previous studies have revealed colonization prevalence ranging between 20%-40%, which is reported as the prevalence limit in literature, in different study populations (Chambers & Deleo, 2009; Sivaraman et al., 2009). In this study, the prevalence of nasal, phone and pen colonization fell within the range of 20-40%. Similar results have also been reported by other studies which focused on college students in Tanzania (Okamo et al., 2016), China (Du et al., 2011), Nepal (Ansari et al., 2016) as well as the general population in Nigeria (Onanuga & Temedie, 2011), Gabon (Schaumburg et al., 2011), Brazil (Pires et al., 2014), Northern Manhattan (Miller et al., 2009) and Mexico (Hamdan-Partida et al., 2010). On the contrary, other published reports have indicated prevalences outside the 20-40% limit. A study conducted on healthy volunteers in Tunisia reported a nasal colonization prevalence of 13% (Ben Slama et al., 2011) while another study focused on a remote population in Guiana reported a colonization prevalence of 57.8% (Ruimy et al., 2008). As reported by Onanuga & Temedie (2011), these variations in the prevalence of nasal colonization could be linked to the characteristics of study populations. Therefore, the high prevalence of colonization observed on nasal cavities and phones could be attributed to specific aspects of this study’s participants.

In the present study, a prevalence of MRSA (11.3%) was found among a healthy population of university students aged between 18-30 years in Central Kenya. The MRSA strains were detected using cefoxitin (30 μg) discs, which, according to Rasheed & Ahmed (2010), is an alternative method to PCR in the detection of MRSA strains. So far in Kenya, this is the first study to present results on the prevalence of MRSA among healthy university students residing within institution-based residence halls. Other recent studies like (Zakai, 2015) have reported a higher MRSA prevalence (18.7%) among student populations in Jeddah, Saudi Arabia while others, Okamo et al. (2016) in Tanzania and Kitt et al. (2011) in Thailand, have reported very low prevalences of 0.3% and 1% respectively. These reports indicate the varied prevalence of MRSA strains in student populations. Even though other reports reveal MRSA prevalence that is as low as 1%, Kitt et al. (2011), the prevalence remains higher in different populations in other areas, thus posing a serious public health issue due to frequent contact with colonized individuals (Zakai, 2015). Based on the results reported by Kitt et al. (2011) (an MRSA prevalence of 1% in a student population), it could be possible that non-exposure to MRSA may result in reduced risks of MRSA colonization. It can be speculated that continued exposure to MRSA colonized environments may result in an increased risk of MRSA colonization. In addition, considering the case of this study where significantly high chances of MRSA colonization were reported in particular residence halls, it could be possible that other students visiting these residence halls would be increasing their risk for MRSA colonization.

**Antimicrobial resistance phenotypes**

This study established that MRSA strains were more likely to display multi-drug resistance compared to MSSA strains, a finding which has also been reported by Gupta et al. (2013) in India. Among the published studies done in Kenya (all of which focused on clinical isolates), none reported this property
among the MRSA strains. Therefore, this study highlights crucial findings regarding the circulation of multi-drug resistant MRSA and MSSA strains among a healthy student population. Given the fact that S. aureus is transmitted via direct or indirect person-to-person contact, there is the possibility of an increased prevalence of multi-drug resistant MRSA and MSSA strains among the student population, which is a worrisome scenario. It is also worth noting that the multi-drug resistance property renders multi-drug resistant strains as one of the most intractable pathogenic organisms in the history of antibiotic therapy. Therefore, the circulation of these multi-drug resistant S. aureus among students presents a challenge to the management of Staphylococcal infections that may develop, particularly in regards to acquiring effective antibiotics, which may be expensive.

**Carriage of mecA gene**

Molecular analysis in this study showed that the S. aureus isolates were genetically diverse. A majority of the MRSA strains carried the mecA gene, while none of the MSSA strains carried this gene (Table 4-6). Among the studies done in Kenya, carriage of the mecA gene has been reported among the MRSA strains. In their study, Maina et al. (2013) reported that all the MRSA strains harbored the mecA gene, and similar to this study, none of the MSSA strains were found to harbor this gene. In a recent study conducted in Zambia, all the MRSA were found to harbor the mecA gene (Samutela et al., 2017). Similar to this study also, a majority of MRSA (90.2%) strains carried mecA gene, while 9.8% were negative for the gene in a study conducted in Sudan (Elhassan et al., 2015). According to available literature, the presence of the mecA gene forms the basis of resistance to beta-lactam antibiotics (Murakami et al., 1991; Sharma et al., 1998; Wongwanich et al., 2000). Therefore, basing on suggestions by the majority of researchers in this field, the mecA gene was linked to cefoxitin resistance among the mecA-positive isolates recovered in this study. Other genetic elements may also be considered for the explanation of cefoxitin-resistance mechanisms (Elhassan et al., 2015). Hence, the basis of cefoxitin-resistance among the mecA-negative MRSA isolates characterized in this study could be attributed to two possible reasons. Firstly, the hyperproduction of β-lactamases, as described by Opanyika et al., (2009) and secondly, development of specific alterations in the variable amino acids found in the PBPs cascades as highlighted by (Ba et al., 2014).

**Diversity of SCCmec elements**

Screening of all the S. aureus isolates for the presence of SCCmec elements revealed the existence of SCCmecV as the most prevalent mec type, followed closely by SCCmecII, then SCCmecI and lastly, SCCmecIII, which was only present in three isolates. Contrary to the clinical isolates recovered in Kenya, the non-clinical isolates examined in this study did not harbor any of the SCCmecIV types. SCCmecV has been shown to be of small molecular size, and this could be the possible reason for its high prevalence among isolates recovered in this study (Omuse et al., 2016). No other published study done in Kenya with similar findings was found. Previously, SCCmecIV has been reported to circulate highly in community
settings, possibly because of its small size, which renders it more mobile and thus can be inserted into multiple S. aureus lineages (Omuse et al., 2016; Tong et al., 2012). It was unclear why none of the isolates recovered in this study harbored SCCmecIV.

The prevalence of SCCmecI was high among the MRSA strains compared to MDR strains. This observation was not unique to this study since SCCmecI is known to encode solely for resistance to β-lactam antimicrobials (Deurenberg et al., 2007). The prevalence of SCCmecII was found to be high among the MDRs compared to MRSA strains. Also, this observation was not unique to the present study because the SCCmecII cassettes are determinants of antimicrobial multi-resistance. The SCCmecII cassette carries additional genes for drug resistance, which are integrated into plasmids (pI258, pUB110 and pT181) and a transposon (Tn554) (Deurenberg et al., 2007). Similar to SCCmecII, the prevalence of SCCmecV was found to be slightly higher among the MDRs than MRSAs by 1%. SCCmecII (as stated before) determines antimicrobial multi-resistance, while SCCmecV encodes for resistance to β-lactam antimicrobials. The high numbers of SCCmecV strains, which were also found to be MDRs, could be attributed possibly to the fact that half the population of MDRs carried both SCCmecII and V in their genomes.

**Multiple SCCmec elements**

In this study, carriage of multiple SCCmec elements was reported on several occasions. Maina et al. made a similar observation in Kenya, where a single isolate was found to carry both SCCmec I and II. Almost half of the MRSA strains analyzed in the present study carried SCCmecII and SCCmecV compared to those that carried SCCmecI and SCCmecV (11.5%), SCCmecI and SCCmecIII (7.7%) and SCCmecII and SCCmecV (3.9%). Half the proportion of MDRs carried SCCmecII and SCCmecV. Characteristically, this study established that the SCCmecII and SCCmecV combination was more prevalent among the MRSA and MDR strains, and therefore, a likely genotypic characteristic of MRSA strains that are also MDRs. More studies could also be done to ascertain the prevalence of the existence of this combination of SCCmec elements among MRSA strains that are also MDRs.

**Variants of SCCmec elements**

In the present study, two MSSA strains were also found to carry SCCmecI. Additionally, among the mecA-negative MRSA strains, six carried SCCmecII, five carried SCCmecV while only a single isolate carried SCCmecIII. None of the previously published studies done in Kenya reported a similar finding. This finding was unclear, though other research reports demonstrated the existence of similar observations (Chlebowicz et al., 2010; Donnio et al., 2005; International Working Group on the Classification of Staphyloococal Cassette Chromosome Elements (IWG-SCC), 2009; Shore et al., 2014; Vandendriessche et al., 2014; Wong et al., 2010). Donnio et al. (2005) characterized MSSA strains, which had the IS431: pUB110: IS431::dcs structure (SCCmec-associated elements), but did not carry mecA and ccrAB. Wong et al. (2010) characterized seven isolates, which resembled USA100 and carried SCCmecII but not mecA. For
the two MSSA isolates that carried SCCmecI, it could be possible that the isolates lost the methicillin-resistant phenotype due to partial excision of the SCCmec Stamper et al. (2011). On the other hand, the mecA-negative MRSA carrying the various SCCmec types retained their methicillin-resistant phenotype possibly due to other genetic factors, which have been described previously in this context.

**Conclusions**

In summary, the present study demonstrated the existence of varied SCCmec types among *S. aureus* strains circulating in the studied student population. The SCCmecV was the most prevalent. Some strains carried multiple SCCmec elements in the gene cassettes, while other strains lacked the mecA gene but still carried the SCCmec elements in their gene cassettes.

**List Of Abbreviations**

SCCmec  Staphylococcal cassette chromosome mec

MRSA  Methicillin-resistant *S. aureus*

MDR  Multi-drug resistance

MSSA  Methicillin-susceptible *S. aureus*

PBP  penicillin-binding protein

ORF  Open reading frame

KEMRI  Kenya Medical Research Institute

SERU  Scientific and Ethics Review Unit

TSB  Tryptone Soya Broth

PCR  Polymerase Chain Reaction

UK  United Kingdom

CLSI  Clinical & Laboratory Standards Institute

AMP  Ampicillin

AMC  Amoxicillin clavulanic acid

FOX  Cefoxitin

C  Chloramphenicol
E  Erythromycin
CN  Gentamicin
CIP  Ciprofloxacin
F  Nitrofurantoin
SXT  Sulphamethoxazole-trimethoprim
NOR  Norfloxacin
LZD  Linezolid

Declarations

Ethics approval and consent to participate

This study was conducted in accordance with the regulations of the scientific and ethics review unit (SERU) of the Kenya Medical Research Institute (KEMRI) (Protocol No. KEMRI/SERU/CMR/0024/3123).

Consent for publication

Authors have approved the review and publication of this manuscript.

Availability of data and materials

Data collected in this study is available for perusal.

Competing interests

Authors have to competing interests to declare.

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Authors' contributions
Osborn Khasabuli performed the laboratory work and wrote the manuscript. Dr. Caroline Ngugi and Dr. John Kiiru provided expert advice in designing the study and manuscript writing process.

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**Authors' information (optional)**

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