Molecular characterization of *Staphylococcus aureus* isolates from various healthcare institutions in Nairobi, Kenya: a cross sectional study

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Abstract

**Background:** *Staphylococcus aureus* (*S. aureus*) has established itself over the years as a major cause of morbidity and mortality both within the community and in healthcare settings. Methicillin resistant *S. aureus* (MRSA) in particular has been a major cause of nosocomial infections resulting in significant increase in healthcare costs. In Africa, the MRSA prevalence has been shown to vary across different countries. In order to better understand the epidemiology of MRSA in a setting, it is important to define its population structure using molecular tools as different clones have been found to predominate in certain geographical locations.

**Methods:** We carried out PFGE, MLST, SCCmec and spa typing of selected *S. aureus* isolates from a private and public referral hospital in Nairobi, Kenya.

**Results:** A total of 93 *S. aureus* isolates were grouped into 19 PFGE clonal complexes (A–S) and 12 singletons. From these, 55 (32 MRSA and 23 MSSA) representative isolates from each PFGE clonal complex and all singletons were spa typed. There were 18 different MRSA spa types and 22 MSSA spa types. The predominant MRSA spa type was t037 comprising 40.6% (13/32) of all MRSA. In contrast, the MSSA were quite heterogeneous, only 2 out of 23 MSSA shared the same spa type. Two new MRSA spa types (t13149 and t13150) and 3 new MSSA spa types (t13182, t13193 and t13194) were identified. The predominant clonal complex was CC 5 which included multi-locus sequence types 1, 8 and 241.

**Conclusion:** In contrast to previous studies published from Kenya, there’s marked genetic diversity amongst clinical MRSA isolates in Nairobi including the presence of well-known epidemic MRSA clones. Given that these clones are resident within our referral hospitals, adherence to strict infection control measures needs to be ensured to reduce morbidity and mortality associated with hospital acquired MRSA infections.

**Keywords:** *Staphylococcus aureus*, MRSA, MSSA, Kenya

Background

*Staphylococcus aureus* (*S. aureus*) has established itself over the years as a major cause of morbidity and mortality globally both within the community and in healthcare settings [1–3]. Its ability to cause disease is aided not only by its impressive repertoire of virulence factors but also its ability to develop resistance to antibiotics used in its treatment epitomized by the emergence of methicillin resistant *S. aureus* (MRSA). Methicillin resistance is conferred by the *mecA* gene that is carried on a staphylococcal cassette chromosome mec (SCCmec) and codes for a modified penicillin binding protein (PBP2a). This binding protein has reduced affinity to all beta-lactam and...
beta-lactam/beta-lactamase inhibitor combination antibiotics [4, 5]. In Africa, the MRSA prevalence has been shown to vary across different countries with a prevalence as low as 7 % reported in Madagascar and as high as 82 % in Egypt [6]. This marked variation could be due to different environmental determinants or simply due to a difference in the genetic diversity of S. aureus. In Kenya, there is a marked difference in reported MRSA prevalence in clinical isolates within Nairobi with one recent study reporting a prevalence of 3.7 % while another reported 87.2 % [7, 8]. In order to better understand the epidemiology of MRSA, it is important to define its population structure. Molecular characterization helps in identifying clonal populations which can help in surveillance and investigation of outbreaks.

There is a growing interest in the characterization of MRSA isolates and this stems primarily from its role as a major cause of hospital and community acquired infections [1, 9, 10]. There are various molecular methods used, the more common ones include multi-locus sequence typing (MLST), pulse field gel electrophoresis (PFGE), staphylococcal protein A (spa) typing and SCC-mec typing [11]. Despite S. aureus having a very diverse clonal population, MLST studies have shown that a small set of clonal complexes (CC) are associated with most of the MRSA epidemics. These include CC5, CC22, CC30, CC45 and CC80 [6, 12, 13]. A clonal complex can have several sequence types, however the multi-locus sequence types that are regarded as the founders in these clonal complexes are ST5, ST22, ST30, ST45 and ST80 respectively [14]. As regards spa types, it has been shown that particular ones are more predominant in certain regions. For example t030 is quite predominant in hospitals in Turkey [15], t042 and t044 are more common in North Africa while t008 is common in the US [16]. Unfortunately, the molecular epidemiology of MRSA in Africa is not very well described. Most of the studies carried out in Africa characterizing MRSA have emanated from a few countries namely Tunisia, Nigeria, South Africa, Algeria and Egypt [6]. There are very few studies from East Africa that have reported on the molecular characterization of S. aureus presumably due to lack of readily available technical expertise and laboratory facilities. A study done in Kenya looking at carriage of S. aureus by inpatients in a government hospital found that only 6 out of 86 (7 %) S. aureus isolates were MRSA and they all belonged to the same clone (MLST ST239; spa type t037) [17]. This clone is a globally distributed hybrid of ST8 and ST30 and is known to be responsible for several outbreaks in different continents [18–21]. The only other study from Kenya did not report on spa or multi locus sequence types [8].

We set out to characterize selected S. aureus isolates from different hospitals in Nairobi, Kenya in order to identify which clonal lineages are present and further shed light on the molecular epidemiology of both MSSA and MRSA in Kenya.

**Methods**

We obtained archived methicillin susceptible (MSSA) and MRSA isolates from 2 hospitals in Nairobi, Kenya collected between January 2010 and July 2013. The hospitals included a government hospital whose samples we obtained through the Kenya Medical Research Institute (KEMRI) and the Aga Khan University Hospital Nairobi (AKUHN) which is a private referral hospital with a network of satellite clinics and laboratories spread in and around Nairobi as well as different parts of the country. The isolates from the government hospital were part of a previous study done to determine prevalence of MRSA carriage in a paediatric ward and the rest of the isolates were from clinical specimens submitted to the AKUHN laboratory for routine culture and sensitivity. These were convenience isolates that were not collected through a well-structured, formal and documented process. All isolates were stored at −80 °C and grown overnight on sheep blood agar plates at 37 °C.

**S. aureus identification**

All isolates were confirmed to be S. aureus using routine bench identification methods which included growth characteristics on sheep blood agar, gram stain, catalase, coagulase, deoxyribonuclease (DNase) and mannitol fermentation tests. A cefoxitin screen using a 30 µg disc (Oxoid, United Kingdom) was performed to distinguish MSSA from MRSA. Isolates with a diameter ≤21 mm were classified as MRSA.

**Antibiotic susceptibility**

Antibiotic susceptibility was only available for the MRSA isolates obtained from AKUHN. These were performed on Vitek 2 (version 4.01, bioMerieux, Marcy-l’Etoile, France) an automated bacterial identification system that performs antibiotic susceptibility using broth dilution and interpretation based on Clinical Laboratory Standards Institute (CLSI) antimicrobial susceptibility guidelines [22]. Multidrug resistance (MDR) was defined as resistance to three or more drug classes.

**DNA derivation**

Isolates were grown on blood agar plates (National Health Laboratory Services Media Lab, Cape Town, South Africa) at 37 °C overnight. After incubation, 4–5 large colonies were re-suspended in 200 µL nuclease free water. The samples were incubated at 95 °C for 30 min, followed by −80 °C for 30 min and centrifuged for 10 min at 14,000×g when thawed. The supernatant containing
DNA was carefully aspirated without disturbing the pellet of cell debris and stored as DNA aliquots at −20 °C until further use.

**PFGE**

PFGE based on SmaI macrorestriction analysis was performed using the CDC laboratory protocol for *S. aureus* [23]. The PFGE was run on a CHEF DR III system (Bio-Rad, California, United States of America) with optimum settings as follows: initial 5 s, switch 30 s, run time 29 h, voltage 6 V/cm and a SeaKem Gold agarose (Lonza, Rockland, USA) gel concentration of 1.4 %. *S. aureus* NCTC 8325 was used as a control in each gel run. Gels were visualized with ethidium bromide. Analysis of PFGE clusters was performed using the BioNumerics software package (Applied Maths, Sint-Martens-Latem, Belgium), using the Dice coefficient, and visualized as a dendrogram by the unweighted-pair group method, using average linkages with 1 % tolerance and 1 % optimization settings. In order to define a cluster, a cutoff of 80 % similarity was used.

**SCCmec typing**

SCCmec typing was performed using multiplex PCR as described by Milheirico et al. [24]. All assays were performed in a GeneAmp 9600 thermocycler (Applied Biosystems). The optimal cycling conditions were the following: 95 °C for 5 min; 35 cycles of 95 °C for 45 s, 57 °C for 45 s, and 72 °C for 1 min; and a final extension at 72 °C for 10 min. Each PCR mixture contained 0.5 µL of the primers listed in Table 1, KAPA2G Robust HotStart ReadyMix PCR (KAPA biosystems) which contains KAPA2G Robust HotStart DNA Polymerase (1 U per 25 µL reaction) in a proprietary reaction buffer containing dNTPs (0.2 mM of each dNTP at 1X), MgCl2 (2 mM at 1X), 0.3 µL (3 mM) additional MgCl2, 10.7 µL of PCR grade water and genomic DNA in a final volume of 25 µL. The following *S. aureus* isolates were used as controls: BAA-38, BAA-1681, BAA-39, BAA-1680, BAA-1688 and BAA-42 for SCCmec types 1–VI respectively. The PCR products were resolved in a 1 % SeaKem Gold Agarose (Lonza, Rockland, USA) gel in 0.5 % Tris–borate-ethylene-diamine-tetra-acetic acid (EDTA) buffer (Bio-Rad, Hercules, CA) at 4 V/cm for 2.5 h and were visualized with ethidium bromide.

**spa typing**

This was done using the following primers: 1095 F: 5′-AGACGATCCTTCGTTGAGC-3′ and 1517R: 5′-GCTTTTGGCAATGTCATTACTG-3′. PCR reactions consisted of 12.5 µL of KAPA2G Robust HotStart ReadyMix PCR (KAPA biosystems) which contains KAPA2G Robust HotStart DNA Polymerase (1 U per 25 µL reaction) in a proprietary reaction buffer containing dNTPs (0.2 mM of each dNTP at 1X), MgCl2 (2 mM at 1X), 0.5 µM of primers and genomic DNA in a final volume of 25 µL. PCR conditions were 95 °C for 6 min; 30 cycles each of 95 °C for 45 s, 64 °C for 45 s, and 72 °C for 60 s; and a final extension at 72 °C for 6 min. Sequencing was outsourced to inqaba biotec, a biotechnology company based in Pretoria, South Africa. Using the Ridom spa server (http://www.spa.server.ridom.de), spa sequences were automatically assigned to spa types. Sequence types and clonal complexes (spa-CC) were assigned where possible using Based Upon Repeat Patterns (BURP) grouping analysis from the Ridom StaphType software (version 1.4; Ridom GmbH, Würzburg, Germany). For BURP analysis, default parameters were used which allows spa types with maximum 4 genetic differences to be grouped into one cluster resulting in a calculated cost between members of a group being less than or equal to 4.

**MLST**

MLST was done on representative isolates from each PFGE clonal complex and selected singletons according to the protocol published by Enright et al. [25]. The PCRs were carried out as uniplex reactions consisting of 1 µM of the forward and reverse primers, 12.5 µL of 2× KAPA Taq ReadyMix (KAPA Biosystems), 2.5 mM MgCl2, 1 µL of template DNA and nuclease free water up to 25 µL. The PCR conditions were 95 °C for 5 min, followed by 30 cycles of 95 °C for 45 s, 56 °C for 45 s and 72 °C for 1 min. A final elongation step was carried out at 72 °C for 10 min. 5 µL of the PCR product was visualised with gel electrophoresis at 120 V for 1 h. Sequencing was performed on the remainder of the PCR product by Inqaba Biotechnical Industries (Pty) Ltd (Pretoria, South Africa). Sequences were inspected and trimmed in BioEdit Sequence Alignment Editor using reference sequences for each of the seven loci. A consensus sequence was generated from the forward and reverse sequences and used to generate sequence types (STs) on the *S. aureus* MLST database (http://www.saureus.beta.mlst.net/). Isolates that were not typed by MLST were assigned STs using BURP analysis. Isolates with the same PFGE clonal complex and spa type were assigned the same STs. MLST clonal complexes (MLST-CC) were determined using a Java applet found at http://www.eburst.mlst.net that uses the eBURST algorithm. The default setting was used in which STs that share identical alleles at 6 or 7 of MLST loci are put in the same group. Where there was a discrepancy between the CC determined using eBURST and BURP, we considered the MLST-CC as the correct one.
**Table 1** Primers used in the updated version of SCCmec multiplex PCR

| Primer name | Primer sequence (5'-3') | Primer specificity (SCCmec type, region) | Amplicon size (bp) | Conc. (µM) |
|-------------|------------------------|----------------------------------------|--------------------|-----------|
| CIF2 F2     | TTCGAGTGTGCTGATGAGAAGG  | I, J1 region                           | 495                | 0.4       |
| CIF2 R2     | ATTTACCACAAAGGACTACCCGC |                                        |                    | 0.4       |
| ccrC F2     | GTACTGTTGAAACTCAAGGG    | V, ccr complex                         | 449                | 0.8       |
| ccrC R2     | ATATGGCTTGGCTGTCCTAC    |                                        |                    | 0.8       |
| RIFF F10    | TTCTAGTACAGAGAATACTCC   | III, J3 region                         | 414                | 0.4       |
| RIFF R13    | ATGAGATGAATTCAAGGGG     |                                        |                    | 0.4       |
| SCCmec V J1 F | TTTCATCTTGTTCATCC     | V, J1 region                           | 377                | 0.4       |
| SCCmec V J1 R | AGAGACTCTGACTTAAGGG   |                                        |                    | 0.4       |
| dcs F2      | CATCTTATGATGGTACCTG    | I, II, IV, and VI, J3 region           | 342                | 0.8       |
| dcs R1      | CTCAATCATAGCTAGTGGCC   |                                        |                    | 0.8       |
| ccrB2 F2    | AGTTCTCAGATACGAGGGG    | II and IV, ccr complex                 | 311                | 0.8       |
| ccrB2 R2    | CCGATATGAAWGGTTAGGC    |                                        |                    | 0.8       |
| kdp F1      | AATATCTGTCATGGGATTTGC  | II, J1 region                          | 284                | 0.2       |
| kdp R1      | CAATAGACTGAAAGAGAGTTTC |                                        |                    | 0.2       |
| SCCmec III J1 F | CATTGATGAACACTGACG   | III, J1 region                         | 243                | 0.4       |
| SCCmec III J1 R | GTATTTAGACCTCTAAAGCC |                                        |                    | 0.4       |
| mecP2       | ATCAAGACTTGACATGCAGGC  | II and III, mec complex                | 209                | 0.8       |
| mecP3       | GCAGTTCTATATCCAGTGC    |                                        |                    | 0.8       |
| mecA P4     | TCACATTTACATCCTAACAGG  | Internal positive control               | 162                | 0.8       |
| mecA P7     | CCACATTTATCCTTGAAAGG   |                                        |                    | 0.8       |

**Results**

A total of 93 *S. aureus* isolates underwent PFGE. These were subsequently grouped into 19 PFGE clonal complexes (A–S) and 12 singletons. From these, 55 (32 MRSA and 23 MSSA) representative isolates from each PFGE clonal complex and all singletons were *spa* typed. This comprised 41 isolates from AKUHN and 14 from KEMRI. In total, there were 18 different MRSA *spa* types and 22 different *spa* types amongst the MSSA. The predominant MRSA *spa* type was t037 comprising 40.6% (13/32) of all MRSA. In contrast, the MSSA were quite heterogeneous, only 2 out of 23 MSSA shared the same *spa* type. Two new MRSA *spa* types (t13149 and t13150) and 3 new MSSA *spa* types (t13182, t13193, t13194) were identified as shown in Table 2. Three *spa* types (t005, t318 and t476) were found in both MSSA and MRSA. BURP analysis for both MSSA and MRSA revealed 7 *spa*-clonal complexes and 14 singletons as shown in Fig. 1. The predominant *spa*-CC was *spa*-CC005 which included the new MRSA *spa* type 13149. SCCmec type-III [3A] was the predominant type followed by SCCmec-IV [2B]. Only one MRSA isolate was non-typeable using the SCCmec protocol published by Milheiro et al. [24].

MLST STs were determined and extrapolated for 31 isolates. A total of seven different MRSA and MSSA MLST-CC were identified with the predominant one being MLST-CC 5. This clonal complex comprised STs 1, 5, 8 and 241 as shown in Table 2. An isolate belonging to ST241 (t2029) that was detected in a pus sample from AKUHN hospital was found to harbor SCCmec type IV [2B].

Out of the 16 MRSA from AKUHN, 13 were MDR including the two new *spa* types. Resistance was commonly seen to clindamycin, erythromycin and trimethoprim/sulfamethoxazole (TMP/SMX). A number of isolates had intermediate resistance to levofloxacin. However, two isolates were only resistant to beta lactams but susceptible to all other antibiotics including TMP/SMX as shown in Table 3. None of the MRSA was resistant to vancomycin, linezolid, mupirocin, teicoplanin or tigecycline.

**Discussion**

This study reveals a markedly heterogeneous population of *S. aureus* isolates as well as the presence of well described MRSA clonal complexes 5, 22 and 30 that are responsible for several outbreaks worldwide [13, 26]. CC5 has been identified as the major clonal complex causing HA-MRSA in Africa with MRSA ST239/ST241-III [3A] having been identified in several African countries [6]. The main clonal complex in our study was CC5 that included ST 241, a single locus variant of ST 239 also known as the “Brazilian/Hungarian clone”. ST 239 and ST 239 like isolates are well-known epidemic clones responsible for several healthcare associated MRSA outbreaks globally. They have been found to be a cause of hospital
Table 2 Molecular characterization of methicillin susceptible and resistant *Staphylococcus aureus*

| Isolate No. | Hospital | Sample Type | ID | spa type | spa-CC | SCCmec type | MLST/Spa ST | MLST CC | PFGE CC | PFGE pulsotype |
|-------------|----------|-------------|----|----------|--------|-------------|-------------|---------|---------|----------------|
| 36          | AKUHN    | Pus swab    | MSSA | t645     | sng    | 1841        | 121         | A       |        | A2             |
| 84          | AKUHN    | Pus swab    | MSSA | t314     | sng    | 121         | 121         | B       |        | B2             |
| 78          | AKUHN    | Pus swab    | MSSA | t355     | sng    | 152         | 152         | C       |        | C1             |
| 48          | AKUHN    | Pus swab    | MSSA | t355     | sng    | 152         | 152         | C       |        | C4             |
| 91          | AKUHN    | Nasal swab  | MRSA | t005     | S      | IV          | 22          | D       |        | D2             |
| 28          | AKUHN    | Nasal swab  | MRSA | t005     | S      | IV          | 22          | D       |        | D3             |
| 83          | AKUHN    | Sputum      | MRSA | t005     | S      | IV          | 22          | D       |        | D3             |
| 89          | AKUHN    | Pus swab    | MRSA | t13149    | S      | IV          | ND          | D       |        | D1             |
| 22          | KEMRI    | Nasal swab  | MRSA | t022     | S      | IV          | 22          | E       |        | E2             |
| 75          | AKUHN    | Blood       | MRSA | t9622    | sng    | IV          | ND          | E       |        | E1             |
| 15          | AKUHN    | Pus swab    | MSSA | t005     | S      | 22          | 22          | F       |        | F6             |
| 12          | AKUHN    | Pus swab    | MSSA | t223     | 5      | 22          | 22          | G       |        | G2             |
| 71          | AKUHN    | Pus swab    | MSSA | t122     | sng    | 30          | 30          | H       |        | H1             |
| 88          | AKUHN    | Tracheal aspirate | MSSA | t318 | sng    | 30          | 30          | I       |        | I5             |
| 16          | AKUHN    | Pus swab    | MSSA | t021     | 21     | 30          | 30          | I       |        | I7             |
| 23          | AKUHN    | Pus swab    | MSSA | t1339    | 3202/186 | UT | 88        | 88      | J       | J2             |
| 49          | KEMRI    | Nasal swab  | MRSA | t3202    | 3202/186 | UT | ND        | J       |        | J1             |
| 19          | AKUHN    | Axillary swab | MSSA | t3841    | sng    | 672         | 672        | K       |        | K2             |
| 6           | AKUHN    | Nasal swab  | MRSA | t091     | NF3    | V           | 789        | L       |        | L2             |
| 69          | AKUHN    | Pus swab    | MSSA | t2505    | NF3    | 789         | 7          | L       |        | L1             |
| 14          | AKUHN    | Tracheal aspirate | MSSA | t002    | sng    | 5          | 5          | M       |        | M3             |
| 79          | AKUHN    | Pus swab    | MRSA | t13150    | sng    | II          | 5          | M       |        | M2             |
| 52          | AKUHN    | Blood       | MSSA | t2473    | sng    | 72          | 72        | N       |        | N3             |
| 92          | AKUHN    | Blood       | MSSA | t852     | 5      | IV          | ND        | O       |        | O1             |
| 87          | AKUHN    | Blood       | MSSA | t127     | sng    | 1          | 5          | P       |        | P3             |
| 81          | AKUHN    | Ear swab    | MRSA | t1476    | NF2    | V           | 8          | 5       |        | Q1             |
| 45          | AKUHN    | Pus swab    | MSSA | t1476    | NF2    | 8          | 5          | Q       |        | Q2             |
| 31          | AKUHN    | Pus swab    | MSSA | t064     | sng    | 8          | 5          | R       |        | R4             |
| 33          | KEMRI    | Nasal swab  | MRSA | t104     | NF1    | IV          | 8         | 5       |        | R2             |
| 7           | KEMRI    | Nasal swab  | MRSA | t689     | NF1    | I           | ND        | R       |        | R1             |
| 25          | KEMRI    | Blood       | MRSA | t852     | 5      | IV          | ND        | R       |        | R5             |
| 4           | AKUHN    | Sputum      | MRSA | t037     | NF4    | III         | 241        | S       |        | S1             |
| 2           | KEMRI    | Nasal swab  | MRSA | t037     | NF4    | III         | 241        | S       |        | S10            |
| 3           | KEMRI    | Nasal swab  | MRSA | t037     | NF4    | III         | 241        | S       |        | S10            |
| 1           | AKUHN    | Blood       | MRSA | t037     | NF4    | III         | 241        | S       |        | S11            |
| 47          | KEMRI    | Nasal swab  | MRSA | t037     | NF4    | III         | 241        | S       |        | S2             |
| 38          | KEMRI    | Nasal swab  | MRSA | t037     | NF4    | III         | 241        | S       |        | S3             |
| 34          | KEMRI    | Nasal swab  | MRSA | t037     | NF4    | III         | 241        | S       |        | S4             |
| 20          | KEMRI    | Nasal swab  | MRSA | t037     | NF4    | III         | 241        | S       |        | S5             |
| 18          | KEMRI    | Nasal swab  | MRSA | t037     | NF4    | III         | 241        | S       |        | S6             |
| 37          | KEMRI    | Nasal swab  | MRSA | t037     | NF4    | III         | 241        | S       |        | S7             |
| 27          | KEMRI    | Nasal swab  | MRSA | t037     | NF4    | III         | 241        | S       |        | S9             |
| 29          | AKUHN    | Pus swab    | MRSA | t2029    | NF4    | IV          | 241        | S       |        | S8             |
| 13          | AKUHN    | Pus swab    | MRSA | t037     | NF4    | III         | 239/240/241 | Sng12  |
| 11          | AKUHN    | Pus swab    | MRSA | t037     | NF4    | III         | 239/240/241 | Sng6  |
| 17          | AKUHN    | Pus swab    | MSSA | t13182    | sng    | ND          | Sng3      |
| 44          | AKUHN    | Urine       | MSSA | t13194    | sng    | ND          | Sng7      |
| 73          | AKUHN    | Pus swab    | MSSA | t1839    | 345    | ND          | Sng5      |
| 58          | AKUHN    | Blood       | MSSA | t186     | 3202/186 | 88        |            |        |        |                |
acquired infections in other African countries including Algeria, Ghana, Morocco, South Africa and Nigeria [6]. A study done by Aiken et al. [17] in a public hospital that is approximately 40 km from Nairobi identified t037-ST239 as the predominant clone carried by inpatients in a surgical ward. Most of the nasal swabs in our study were obtained from children in a paediatric ward situated in a public referral hospital. The high proportion of t037-ST241 among our MRSA isolates is not necessarily reflective of the true prevalence of this spa type in Nairobi due to a selection bias in the manner in which the isolates were collected. Nevertheless, it is quite concerning that a clone known to be associated with MRSA epidemics is resident within hospitals in Nairobi indicating an urgent need for proper infection control interventions and regular surveillance.

Table 2 continued

| Isolate No. | Hospital  | Sample        | ID  | spa type | spa-CC | SCCmec type | MLST/spa ST | MLST CC | PFGE CC | PFGE pulsotype |
|-------------|-----------|---------------|-----|----------|--------|--------------|-------------|---------|---------|----------------|
| 70          | AKUHN     | Blood         | MSSA| t224     | 345    | V            | 97c         | Sng8    |         |                |
| 21          | AKUHN     | Pus swab      | MRSA| t293     | sng    | IV           | NDc         | Sng 2   |         |                |
| 43          | KEMRI     | NASAL SWAB    | MRSA| t318     | sng    | IV           | 30c         | Sng 1   |         |                |
| 35          | AKUHN     | Pus swab      | MRSA| t345     | 345    | V            | NDc         | Sng10   |         |                |
| 40          | AKUHN     | Urine         | MRSA| t648     | NF2    | IV           | NDc         | Sng11   |         |                |
| 85          | AKUHN     | Vulval swab   | MSSA| t131     | 345    | NDc          | Sng9        |         |         |                |

sng singleton, NF no founder, UT untypeable, ND not defined on the Ridom database Accessed on 07/10/2015

* MLST ST
b MLST ST extrapolated based on similar spa type and pulsotype
c spa ST
d New spa type

Fig. 1 Based upon repeat pattern clustering analysis for all identified S. aureus isolates: the clustering analysis resulted in seven spa-clonal complexes and 14 singletons. The blue circle represents the group founder and the circle size is proportional to the frequency of the spa type
The multi-drug resistant patterns for the MRSA in this study are in keeping with what has been described in other countries in Africa [17, 32, 36, 37]. Most of the MRSA were resistant to macrolide–lincosamide,
tetracycline and sulphonamide group of antibiotics which is fairly common amongst MRSA especially those that are healthcare associated. However two of the isolates showed resistance to only beta lactam antibiotics suggesting that they may be community acquired (based on their molecular structure) given that they belonged to SCCmec type IV which has been associated with CA-MRSA.

The major limitation of this study is that the isolates characterized were not collected in a structured and consistent manner and as such the proportions reported do not necessarily represent a true picture of the relative distributions of different clones in Nairobi due to a selection bias. The over representation of nasal swab specimens from a paediatric population from one hospital may have exaggerated the prevalence of t037-ST 241. We also did not carry out MLST and spa typing on all isolates due to financial constraints. However, we did ensure that a representative isolate from each PFGE clonal complex was included in the isolates that were further characterized using MLST and spa typing.

Conclusion
To the best of our knowledge, this is the largest study from Kenya that has carried out PFGE, MLST, spa and SCCmec typing on a diverse collection of MRSA isolates. This study highlights the marked genetic diversity of MSSA and MRSA isolates in Nairobi including the presence of well-known epidemic MRSA clones and new MRSA spa types. Given the evolution of S. aureus over the years, there is need for continuous surveillance in order to keep track of emerging clones. The existence of epidemic MRSA clones further justifies the need to strengthen infection control measures within our hospitals so as to avoid nosocomial S. aureus infections.

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Competing interests
The authors declare that they have no competing interests.

Availability of data and materials
The datasets analysed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate
This being a low risk study, a waiver from full ethics review was granted by the AKUHN research ethics committee (2016/REC-33).

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