Diversity of SCCmec elements and spa types in South African Staphylococcus aureus mecA-positive blood culture isolates

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Abstract

Background: The prevalence of Staphylococcus aureus varies depending on the healthcare facility, region and country. To understand its genetic diversity, transmission, dissemination, epidemiology and evolution in a particular geographical location, it is important to understand the similarities and variations in the population being studied. This can be achieved by using various molecular characterisation techniques. This study aimed to provide detailed molecular characterisation of South African mecA-positive S. aureus blood culture isolates by describing the SCCmec types, spa types and to lesser extent, the sequence types obtained from two consecutive national surveillance studies.

Methods: S. aureus blood culture isolates from a national laboratory-based and enhanced surveillance programme were identified and antimicrobial susceptibility testing was performed using automated systems. A real-time PCR assay confirmed the presence of the methicillin-resistance determinant, mecA. Conventional PCR assays were used to identify the SCCmec type and spa type, which was subsequently analysed using the Ridom StaphType™ software. Multilocus sequence typing was performed on selected isolates using conventional methods. MRSA clones were defined by their sequence type (ST), SCCmec type and spa type.

Results: A detailed description of findings is reported in this manuscript. SCCmec type III predominated overall followed by type IV. A total of 71 different spa types and 24 novel spa types were observed. Spa type t037 was the most common and predominated throughout followed by t1257. Isolates were multidrug resistant; isolates belonging to all SCCmec types were resistant to most of the antibiotics with the exception of type I; isolates with spa type t045 showed resistance to all antibiotics except vancomycin. The most diverse SCCmec-spa type complex was composed of the SCCmec type IV element and 53 different spa types.

Conclusion: Although ST data was limited, thereby limiting the number of clones that could be identified, the circulating clones were relatively diverse.

Keywords: Methicillin-resistant Staphylococcus aureus, SCCmec typing, Spa typing

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Introduction

*Staphylococcus aureus* bacteraemia is an important cause of morbidity and mortality in both healthcare-associated (HA) and community-associated (CA) infections worldwide [1, 2]. *S. aureus* is responsible for an extensive range of human diseases, including bloodstream infections, pneumonia, endocarditis, food poisoning, toxic shock syndrome, skin and soft tissue infections, and bone and joint infections [3, 4]. The prevalence of *S. aureus* varies depending on the healthcare facility, region and country. Furthermore, the prevalence of methicillin-susceptible *S. aureus* (MSSA) and methicillin-resistant *S. aureus* (MRSA) may also differ. In order to understand the genetic diversity, transmission, dissemination, epidemiology and evolution of MSSA and MRSA clones in a particular geographical location, it is important to acquire knowledge on the similarities and variations in the population being studied. This is not only important for epidemiological surveys but also for infection prevention and control policies [5]. This can be achieved by employing the use of various molecular characterisation techniques [2]. Reliable molecular techniques that have been used for typing *S. aureus* include Pulsed-field Gel Electrophoresis (PFGE), Multilocus Sequence Typing (MLST), Staphylococcal protein A (*spa*) typing and Staphylococcal Cassette Chromosome *mec* (SCC*mec*) typing [2, 6].

PFGE is based on the DNA banding pattern obtained after digesting the bacterial genome with a restriction enzyme [7]. MLST and its clustering algorithm, Based Upon Related Sequence Type (BURST) classifies isolates according to nucleotide variations in seven housekeeping/reference genes (loci) [5]. These genes are sequenced and a unique allele number is assigned using an online programme specific to the MLST scheme. A combination of the allele numbers (i.e. allelic profile) produces a particular sequence type (ST) for a bacterial strain. Those with similar STs are grouped together in a single clonal complex (CC) [6, 8]. *Spa* typing sequences the *S. aureus*-specific staphylococcal protein A (*spa*) gene which is one of the virulence factors on the surface of the organism preventing phagocytosis by the immune system [9]. *Spa* typing and its clustering algorithm, Based Upon Repeat Pattern (BURP) is based on the sequencing of a polymorphic 24 bp region of the *spa* gene. This is a variable-number tandem repeat (VNTR) sequence within the 3′ coding region [4]. The repeat regions are assigned a numerical code and the *spa* type is determined by the order of specific repeats [3]. Studies have shown that *spa* typing produced results that are notably comparable with that of MLST [6, 10]. Due to lower implementation costs and that only a single locus needs to be sequenced, *spa* typing has shown to be more efficient and results are consistent across different settings, specimen type and patient age [6]. Therefore *spa* typing has been shown to be appropriate for use in evolutionary and macro-epidemiology studies [4, 6, 11, 12]. However, as recombination events in a single locus can distort clonal relationships, there is the question of how a method that sequences only a single locus can be used for macro-epidemiology studies [13]. SCC*mec* typing classifies SCC*mec* elements according to their structural differences [5]. It involves the typing of the staphylococcal cassette chromosome *mec*, which is a mobile genetic element and harbours the methicillin-resistance determinant gene. This element is genetically diverse with many types, subtypes and variants being reported [14]. The molecular organisation of the cassette is complex, but it can be broken down into three structural components, which include: i) the cassette chromosome recombinase (*ccr*) gene complex, ii) the *mec* gene complex and iii) the joining (J) regions [15, 16]. The *ccr* gene complex encodes site-specific recombinases for the excision and insertion of the element into the chromosome [14, 16, 17]. This complex therefore affords the SCC*mec* element mobility and thus facilitates its transfer to other staphylococcal species [16]. The *mec* complex confers methicillin resistance as it consists of the *mec* gene, its regulatory genes, the *mecI* and the *mecR* genes and various insertion sequences [14, 18]. A combination of both the *ccr* gene complex and the *mec* gene class is used to assign the specific SCC*mec* type. Thirteen SCC*mec* types (I–XIII) have been defined in MRSA based on complete sequence data [17, 19–21]; International Working Group on the Staphylococcal Cassette Chromosome elements (IWG-SCC) (2015) Available online: http://www.sccmec.org).

Although we have previously described the MRSA population in South Africa [22–25], a detailed description of the SCC*mec* types and *spa* types is lacking. This study therefore reports on the various clones present in our MRSA study population by SCC*mec* and *spa* type combinations (SCC*mec*-spa type complexes). Moreover, although MLST data was lacking for the majority of our sample population, the predominating circulating clones (ST-SCC*mec*-spa type) based on the most common *spa* types were described.

Materials and methods

Bacterial strains and phenotypic methods

A case of *S. aureus* bacteraemia was defined as the isolation of *S. aureus* from a blood culture. Blood culture isolates, which formed part of the GERMS-SA laboratory-based and enhanced antimicrobial resistance surveillance studies from sentinel centres in South Africa were submitted and participation was voluntary. The first was a two-year laboratory-based surveillance study (June 2010 to July 2012); sites represented 13 sentinel
centres from the Gauteng, KwaZulu-Natal, Free State and Western Cape provinces. The second was an enhanced surveillance study (August 2012 to December 2017); sites represented five sentinel centres from six large academic hospitals from the Gauteng and the Western Cape provinces. A 21-day exclusion period was applied to avoid duplicate isolates of the organism from the same patient.

In total, 5820 viable isolates [MSSA (n = 3801) and MRSA (n = 2019)] were submitted on Dorset transport media (Diagnostic Media Products (DMP), National Health Laboratory Service (NHLS), Johannesburg, South Africa). Each isolate was plated onto a 5% blood agar plate (DMP, NHLS, Johannesburg, South Africa) followed by organism identification and antimicrobial susceptibility testing using automated systems. Organism identification was done using VITEK® II (bioMérieux, France) or MALDI-TOF MS (Microflex, Bruker Daltonics, MA, USA) and antimicrobial susceptibility testing (AST) was done using the MicroScan Walkaway system (Gram-positive panel PM33) (Siemens, Sacramento, CA, USA). Interpretation of susceptibility was performed according to the Clinical and Laboratory Standards Institute (CLSI) guidelines [26]. Bacterial cells were lysed at 95 °C for 25 min and the DNA was extracted and used in the genotypic assays.

Polymerase chain reaction (PCR) screening for meca in MRSA isolates
The LightCycler 480 II (Roche Applied Science) instrument was used for the real-time PCR of meca and nuc, which were amplified in a multiplex assay using the LightCycler 480 Probes Master kit (Roche Diagnostics, IN, USA) with previously published primers and probes [27].

SCCmec typing
All 2019 meca-positive MRSA isolates were typed by a multiplex PCR assay using the Qiagen Multiplex PCR kit (Qiagen, Germany) and previously published primers [28].

Spa-typing
Spa-typing was performed on 1467 MRSA isolates. The spa gene was amplified using previously published primers [12] and the AmpliTag Gold DNA Polymerase kit (Applied Biosystems, CA, USA). Purified PCR products (Qiagen Purification kit; Qiagen, Germany) were sequenced (Inqaba Biotech, South Africa). Sequences were assembled using CLC Bio main workbench (Qiagen, Germany) and analysed using the Ridom StaphType™ software, (Ridom GmbH, Würzburg, Germany).

Multilocus sequence typing (MLST)
Multilocus Sequence Typing was performed on 48 isolates, which were selected randomly based on the most common spa-types. Primers [29] amplifying seven reference genes were used. Amplification was done using the AmpliTag Gold DNA Polymerase kit (Applied Biosystems, CA, USA). Purified PCR products were sequenced (Inqaba Biotech, South Africa). Sequences were assembled using the CLC Bio main workbench (Qiagen, Germany) and analysed using the online database (https://pubmlst.org/.saureus/).

Results
SCCmec typing
The distribution of SCCmec types per year in 2019 meca-positive isolates is seen in Fig. 1. SCCmec type III predominated every year followed by type IV with the exception of 2011 where the opposite was seen. Type II was seen in multiple isolates throughout the study period and sporadic cases of types V and VI were noted from 2011 onwards. Only two cases of type I were seen in 2014 and 2015. A number of unknown types was noted from 2010 to 2017. We subsequently investigated a proportion (n = 52) of the unknown types from 2013 to 2016 and found that the majority of the isolates were interpreted as type I-like, type II-like and type III-like [30].

The distribution of SCCmec types per province per year is seen in Fig. 2. Type IV predominated in KwaZulu-Natal whereas type III predominated in the remaining three provinces. All six SCCmec types including unknown types were observed in Gauteng and the Western Cape provinces.

Antibiotic non-susceptible phenotypes were examined and the distribution of SCCmec types per non-susceptible phenotype is seen in Table 1. Isolates belonging to all SCCmec types were resistant to most of the antibiotics with the exception of type I. All isolates were susceptible to vancomycin. Type III predominated in azithromycin-, erythromycin-, oxacillin-, cefoxitin-, penicillin-, trimethoprim/sulfamethoxazole-, daptomycin-, tetracycline-, ciprofloxacin-, levofloxacin-, moxifloxacin- and gentamicin-non-susceptible isolates. Type II predominated in clindamycin-non-susceptible isolates and type IV predominated in rifampicin-non-susceptible isolates.

Majority of the isolates cultured were from adult patients (959/2019, 47.5%). Isolates from paediatric patients were represented by 44.8% (904/2019); the data for the remaining isolates (156/2019, 7.7%) was unknown. The predominating SCCmec type in isolates from adults was type IV (478/2019, 23.7%) followed by type III (265/2019, 13.1%), II (123/2019, 6.1%), unknown type (81/2019, 4.0%), V (4/2019, 0.2%) and VI (8/2019, 0.4%). Type I was not seen in isolates...
cultured from adult patients. The predominating SCCmec type in isolates from paediatric patients was type III (569/2019, 28.2%) followed by unknown types (188/2019, 9.3%), type IV (129/2019, 6.4%), II (13/2019, 0.6%), V (3/2019, 0.1%) and I (2/2019, 0.1%). Type VI was not seen in isolates cultured from paediatric patients. The predominating SCCmec types in isolates obtained from male and female patients were very similar. The predominating SCCmec type could not be correctly established from isolates obtained from patients that died versus those that recovered or were discharged due to the majority of
cases having unknown data. The same is applicable for diagnosis.

**Spa typing**

*Spa* typing was performed on 1467 isolates; the remaining 552 isolates from the period 2010 to 2012 do not have *spa* types assigned. A total of 71 different *spa* types and 24 novel *spa* types were observed. Five isolates were untypable even upon repeat processing. Table 2 shows the distribution of predominating *spa* types over the seven and a half-year period. *Spa* type t037 was the most common and predominated throughout followed by t1257. *Spa* types t012, t045 and t064 were also constantly present over this time period. *Spa* type t4864 was seen only in 2014, t1467 was seen only in 2015, t718 was seen only in 2016 and t5691 emerged in 2017. The remaining *spa* types were seen in small numbers and not consistently throughout the seven and a half-year period.

Table 3 shows the variation of *spa* types over the seven and a half-year period. The most number of *spa* types were seen in 2011 and the most number of novel *spa* types occurred in 2014, which also showed a high variation in the number of different *spa* types observed. No novel *spa* types were found in 2013.

The Gauteng province showed the most variation with 44 different *spa* types and 14 novel *spa* types followed by the Western Cape (n = 40 and n = 14), respectively. In KwaZulu-Natal 12 different *spa* types were seen and in the Free State eight different *spa* types were observed. One novel *spa* type was found in both KwaZulu-Natal and the Free State provinces but these *spa* types differed from each other. Only t012, t045, t064 and t1257 were observed in all four provinces; t037 was seen in all provinces except in KwaZulu-Natal and t1971 was seen in all provinces except in the Free State; t9061 was seen only in the Free State and t3165, t1555, t4268 and t951 were seen only in KwaZulu-Natal. Two *spa* types (t209 and t2293) were found in the Gauteng and Free State provinces, which also had one novel *spa* type. Three *spa* types (t148, t451 and t891) were found in the Gauteng and KwaZulu-Natal, which also had one novel *spa* type. Nine *spa* types (t008, t018, t021, t022, t032, t1443, t1476, t304, t718) and two novel *spa* types were observed in Gauteng and the Western Cape provinces. Twenty-four different *spa* types (t10304, t105, t1096, t1107, t118, t127, t174, t186, t1943, t272, t274, t355, t421, t4410, t463, t4833, t4864, t5961, t701, t729, t7962, t840, t913 and t932) and 10 novel *spa* types were seen in Gauteng alone. Twenty-two different *spa* types (t015, t0121, t0379, t059, t11775, t1467, t1774, t1813, t223, t230, t238, t2409, t2526, t294, t324, t432, t498, t5483, t578, t6330, t6931 and t8636) and 10 novel *spa* types were seen in the Western Cape alone.

**Antibiotic non-susceptibility phenotypes**

Antibiotic non-susceptible phenotypes were examined and the distribution of *spa* types representing majority of the isolates is seen in Table 4. One isolate belonging to *spa* type t10304 was non-susceptible to penicillin only (data not shown in table). All three isolates typed as t0379 displayed the same phenotypic profile and were non-susceptible to the fluoroquinolones and beta-lactam antibiotics only (data not shown in table). All four

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**Table 1** Distribution of SCCmec types according to antibiotic non-susceptibility phenotypes for MRSA isolates (n = 2019)

| Antibiotic non-susceptibility phenotype | SCCmec type | I | II | III | IV | V | VI | Untypeable | Negative |
|----------------------------------------|-------------|---|----|-----|----|---|----|-----------|----------|
| Erythromycin                           |             | 133 (6.58%) | 920 (45.56%) | 391 (19.36%) | 4 (0.19%) | 2 (0.09%) | 249 (12.33%) | 2 (0.09%) |
| Clindamycin                            |             | 129 (6.38%) | 106 (5.25%) | 31 (1.53%) | 0 | 4 (0.19%) | 30 (1.48%) | 0 |
| Oxacillin                              |             | 135 (6.68%) | 916 (45.36%) | 648 (32.09%) | 7 (0.34%) | 9 (0.44%) | 271 (13.42%) | 2 (0.09%) |
| Penicillin                             |             | 138 (6.83%) | 926 (45.86%) | 651 (32.24%) | 7 (0.34%) | 9 (0.44%) | 274 (13.57%) | 2 (0.09%) |
| Trimethoprim/Sulfamethoxazole          |             | 12 (0.59%) | 883 (43.73%) | 542 (26.84%) | 2 (0.09%) | 3 (0.14%) | 42 (2.08%) | 2 (0.09%) |
| Daptomycin                             |             | 0 | 7 (0.34%) | 2 (0.09%) | 0 | 0 | 2 (0.09%) | 0 |
| Linezolid                              |             | 3 (0.14%) | 3 (0.14%) | 3 (0.14%) | 0 | 0 | 3 (0.14%) | 0 |
| Tetracycline                           |             | 14 (0.69%) | 913 (45.22%) | 573 (28.38%) | 3 (0.14%) | 2 (0.09%) | 79 (3.91%) | 2 (0.09%) |
| Rifampin                               |             | 14 (0.69%) | 62 (3.07%) | 571 (28.28%) | 1 (0.04%) | 2 (0.09%) | 19 (0.94%) | 2 (0.09%) |
| Ciprofloxacin                          |             | 134 (6.63%) | 917 (45.41%) | 589 (29.17%) | 4 (0.19%) | 3 (0.14%) | 76 (3.76%) | 2 (0.09%) |
| Levofloxacin                           |             | 134 (6.63%) | 916 (45.36%) | 512 (25.35%) | 3 (0.14%) | 3 (0.14%) | 75 (3.71%) | 2 (0.09%) |
| Moxifloxacin                           |             | 133 (6.58%) | 918 (45.46%) | 510 (25.26%) | 3 (0.14%) | 2 (0.09%) | 75 (3.71%) | 2 (0.09%) |
| Gentamicin                             |             | 133 (6.58%) | 912 (45.17%) | 556 (27.53%) | 6 (0.29%) | 3 (0.14%) | 242 (11.98%) | 2 (0.09%) |

Susceptibility was classified according to CLSI guidelines [26]

Suggested antibiotics approved by the US Food and Drug Administration (FDA) for clinical use were included in the table. Antibiotics excluded were azithromycin as erythromycin is a surrogate for macrolides, cefoxitin as oxacillin is included for MRSA, those that are recommended for urine only as well as those that were not tested for using the MicroScan Gram-positive PM-33 panel. In addition, vancomycin was excluded as all isolates were susceptible.
isolates typed as t2029 showed resistance to all antibiotics listed except for daptomycin, linezolid and rifampin (data not shown in table). All four isolates belonging to type t238 and t294 showed the same phenotypic profile and two isolates belonging to t304 and t421 displayed the same phenotypic profile (data not shown in table).

Of the known adult vs paediatric information, the predominating spa type in isolates from adults was t1257 (195/1467, 13.3%) followed by t037 (189/1467, 12.7%), t012 (136/1467, 4.8%), t064 (32/1467, 2.2%), t1971 (20/1467, 1.4%), t032 (19/1467, 1.3%) and t045 (15/1467, 1%). The remaining spa types within this group individually represented less than 1%. This group consisted of 55 different spa types and 18 novel spa types. Two isolates were untypeable. The predominating spa type in isolates from paediatric patients was t037 (446/1467, 30.4%) followed by t045 (115/1467, 7.8%) and t1257 (53/1467, 3.6%). The remaining spa types within this group individually represented less than 1%. This group consisted of 32 different spa types and 10 novel spa types.

The following spa types were seen in isolates from adult patients only: t008, t0121, t018, t021, t0379, t059, t064, t1175, t118, t1467, t174, t1774, t1813, t2029, t223, t2293, t230, t2409, t2526, t294, t304, t324, t379, t432, t4410, t463, t4864, t578, t6931, t701, t729, t7962, t840, t8636, t9061 and t913. There were 14 novel spa types in this group. The following spa types were seen in isolates from paediatric patients only: t10304, t1096, t127, t13165, t1555, t186, t1943, t272, t355, t4286, t498, t5483, t6330 and t932; six novel spa types were observed in this group. The predominating spa types in isolates obtained from male and female patients were very similar. Furthermore, the predominating spa type could not be correctly established from isolates obtained from patients that died versus those that recovered or were discharged due to the majority of cases having unknown data. The same is applicable for diagnosis.

### SCCmec and spa types complexes

The SCCmec-spa type combinations are referred to as complexes. A total of 1467 SCCmec-spa type complexes were obtained. The five isolates that were not typeable for spa type were excluded from the analysis; SCCmec types for each of these varied (SCCmec II, III, IV, V and unknown type). The most diverse complex was composed of the SCCmec type IV element and 53 different spa types. Next were the isolates with unknown SCCmec type; these were associated with 28 different spa types. SCCmec type III was associated with 25 different spa types and SCCmec type II was associated with 20 different spa types. There were smaller numbers of SCCmec type I, V and VI isolates and predominance was therefore inconsequential; the isolates varied with regard to spa type. The SCCmec-spa type combinations constituting the complexes are shown in Table 5.

### Predominating circulating clones

MRSA clones were defined by their sequence type (ST), SCCmec type and spa type. Multilocus Sequence Typing was performed on 48 isolates only. Isolates were selected randomly based on the most common spa-types (t037, t1257, t037, t012, t064, t032, t045, t1971, t2029, t223, t2293, t230, t2409, t2526, t294, t304, t324, t379, t432, t4410, t463, t4864, t578, t6931, t701, t729, t7962, t840, t8636, t9061 and t913). There were 14 novel spa types in this group. The following spa types were seen in isolates from paediatric patients only: t10304, t1096, t127, t13165, t1555, t186, t1943, t272, t355, t4286, t498, t5483, t6330 and t932; six novel spa types were observed in this group. The predominating spa type could not be correctly established from isolates obtained from patients that died versus those that recovered or were discharged due to the majority of cases having unknown data. The same is applicable for diagnosis.

### Table 2 Distribution of predominating spa types per year

| Spa type | 2010 | 2011 | 2012 | 2013 | 2014 | 2015 | 2016 | 2017 |
|----------|------|------|------|------|------|------|------|------|
| t012     | 11   | 11   | 3    | 18   | 12   | 14   | 14   |      |
| t037     | 136  | 116  | 53   | 28   | 82   | 114  | 85   | 83   |
| t045     | 31   | 10   | 4    | 1    | 17   | 27   | 22   | 19   |
| t064     | 18   | 15   | 2    | 4    | 3    | 3    | 2    | 2    |
| t1257    | 52   | 57   | 12   | 15   | 29   | 43   | 28   | 33   |
| t022     | 0    | 1    | 1    | 2    | 0    | 0    | 0    | 1    |
| t118     | 0    | 1    | 1    | 2    | 0    | 0    | 0    | 0    |
| t018     | 1    | 1    | 1    | 0    | 5    | 0    | 1    | 0    |
| t032     | 5    | 0    | 0    | 0    | 2    | 6    | 7    | 4    |
| t1971    | 0    | 0    | 0    | 1    | 1    | 4    | 7    | 7    |
| t1443    | 5    | 4    | 0    | 0    | 1    | 2    | 0    | 0    |
| t1467    | 0    | 0    | 0    | 0    | 0    | 5    | 0    | 0    |
| t1476    | 0    | 1    | 0    | 0    | 0    | 3    | 6    | 1    |
| t5691    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 4    |
| t021     | 0    | 0    | 1    | 1    | 0    | 1    | 0    | 2    |
| t148     | 3    | 1    | 0    | 0    | 0    | 0    | 0    | 1    |
| t238     | 3    | 0    | 0    | 0    | 1    | 0    | 0    | 0    |
| t294     | 0    | 0    | 0    | 0    | 1    | 2    | 0    | 1    |
| t451     | 1    | 2    | 0    | 0    | 0    | 0    | 0    | 0    |
| t718     | 0    | 0    | 0    | 0    | 0    | 0    | 2    | 0    |
| t891     | 1    | 1    | 0    | 0    | 2    | 0    | 1    | 0    |
| t4833    | 1    | 2    | 1    | 0    | 0    | 0    | 0    | 0    |
| t4864    | 0    | 0    | 0    | 0    | 2    | 0    | 0    | 0    |
| t2029    | 1    | 3    | 0    | 0    | 0    | 0    | 0    | 0    |

| Spa type | 2010 | 2011 | 2012 | 2013 | 2014 | 2015 | 2016 | 2017 |
|----------|------|------|------|------|------|------|------|------|
| t2029    | 1    | 3    | 0    | 0    | 0    | 0    | 0    | 0    |

**Table 3** Variation of spa type per year

| Year | No. of different spa types | No. of novel spa types |
|------|----------------------------|------------------------|
| 2010 | 18                         | 2                      |
| 2011 | 29                         | 7                      |
| 2012 | 15                         | 4                      |
| 2013 | 11                         | 0                      |
| 2014 | 21                         | 10                     |
| 2015 | 23                         | 6                      |
| 2016 | 19                         | 5                      |
| 2017 | 22                         | 2                      |
### Table 4 Distribution of Spa types according to antibiotic non-susceptibility phenotypes for MRSA isolates (n = 1467)

| Spa type | Antibiotic non-susceptibility phenotype |
|----------|----------------------------------------|
|          | Azithromycin | Erythromycin | Clindamycin | Oxacillin | Cefoxitin | Penicillin | Trimethoprim/ Sulfamethoxazole | Daptomycin | Linezolid | Tetracycline | Rifampin | Ciprofloxacin | Levofloxacin | Moxyflaxacin | Gentamicin |
| t012 (n = 84) | 28 (1.90%) | 81 (5.52%) | 71 (4.83%) | 84 (5.72%) | 84 (5.72%) | 84 (5.72%) | 11 (0.74%) | 0 | 4 (0.27%) | 14 (0.95%) | 7 (0.47%) | 84 (5.72%) | 84 (5.72%) | 84 (5.72%) | 83 (5.69%) | 17 (1.19%) |
| t018 (n = 9) | 4 (0.27%) | 8 (0.54%) | 7 (0.47%) | 8 (0.54%) | 9 (0.61%) | 9 (0.61%) | 0 | 0 | 0 | 0 | 1 (0.06%) | 1 (0.06%) | 9 (0.61%) | 9 (0.61%) | 9 (0.61%) | 2 (0.13%) |
| t032 (n = 24) | 4 (0.27%) | 12 (0.81%) | 3 (0.20) | 24 (1.63%) | 24 (1.63%) | 24 (1.63%) | 1 (0.06%) | 0 | 0 | 0 | 2 (0.13%) | 43 (2.93%) | 23 (1.56%) | 23 (1.56%) | 23 (1.56%) | 3 (0.20) |
| t037 (n = 697) | 327 (22.29%) | 690 (47.03%) | 81 (5.52%) | 693 (47.23%) | 695 (47.37%) | 698 (47.58%) | 659 (44.92%) | 5 (0.34%) | 1 (0.06%) | 679 (46.28%) | 0 | 691 (47.10) | 688 (46.89%) | 690 (47.03%) | 681 (46.42%) |
| t045 (n = 131) | 53 (3.6%) | 130 (8.86%) | 13 (0.88%) | 130 (8.86%) | 130 (8.86%) | 130 (8.86%) | 11 (0.74%) | 2 (0.13%) | 4 (0.27%) | 18 (1.22%) | 6 (0.40%) | 25 (1.70%) | 24 (1.63%) | 26 (1.77%) | 123 (8.38%) |
| t064 (n = 49) | 21 (1.43%) | 29 (1.97%) | 0 | 49 (3.34%) | 49 (3.34%) | 49 (3.34%) | 44 (2.99%) | 0 | 0 | 47 (3.20%) | 47 (3.20%) | 30 (2.04%) | 25 (1.70%) | 26 (1.77%) | 47 (3.20%) |
| t1257 (n = 269) | 79 (5.3%) | 164 (11.17) | 14 (0.95%) | 268 (18.2%) | 268 (18.2%) | 268 (18.2%) | 249 (16.97%) | 0 | 0 | 2 (0.13%) | 263 (17.92) | 255 (17.38%) | 264 (17.99%) | 231 (15.74%) | 231 (15.74%) | 252 (17.17%) |
| t1443 (n = 14) | 1 (0.06%) | 0 (0.06%) | 0 | 14 (0.95%) | 13 (0.88%) | 14 (0.95%) | 13 (0.88%) | 0 | 0 | 13 (0.88%) | 14 (0.95%) | 13 (0.88%) | 13 (0.88%) | 13 (0.88%) | 13 (0.88%) |
| t1476 (n = 12) | 0 | 5 | 0 | 12 (0.81%) | 12 (0.81%) | 12 (0.81%) | 2 (0.13%) | 0 | 0 | 9 (0.61%) | 0 | 10 (0.68%) | 7 (0.47%) | 7 (0.47%) | 11 (0.74%) |
| t1197 (n = 23) | 2 (0.13%) | 19 (1.29%) | 1 (0.06%) | 23 (1.56%) | 23 (1.56%) | 23 (1.56%) | 23 (1.56%) | 0 | 0 | 23 (1.56%) | 23 (1.56%) | 23 (1.56%) | 21 (1.42%) | 21 (1.42%) | 23 (1.56%) |
| Novel spa types (n = 38) | 9 (0.61%) | 24 (1.63%) | 7 (0.47%) | 37 (2.52%) | 37 (2.52%) | 38 (2.59%) | 23 (1.56%) | 0 | 0 | 25 (1.70%) | 16 (1.09%) | 31 (2.11%) | 29 (1.97%) | 27 (1.84%) | 27 (1.84%) |
| Untypeable (n = 5) | 3 (0.20%) | 4 (0.27%) | 4 (0.27%) | 5 (0.34%) | 5 (0.34%) | 5 (0.34%) | 3 (0.20%) | 0 | 0 | 3 (0.20%) | 1 (0.06%) | 4 (0.27%) | 4 (0.27%) | 3 (0.20%) | 3 (0.20%) |

The following spa types were excluded from the table as they accounted for a small number of isolates: t008, t0121, t021, t022, t0379, t059, t10304, t110, t1096, t1107, t11175, t1118, t127, t113165, t1147, t1148, t1555, t174, t1774, t1813, t186, t1994, t2029, t223, t2293, t230, t238, t2409, t2526, t272, t2724, t294, t304, t324, t355, t379, t421, t4268, t432, t4410, t451, t463, t4833, t4864, t498, t5483, t5691, t578, t6330, t6931, t718, t796, t840, t891, t9061, t913, t9192, t951

Susceptibility was classified according to CLSI guidelines [26]

Suggested antibiotics approved by the US Food and Drug Administration (FDA) for clinical use were included in the table. Antibiotics excluded were those that are recommended for urine only as well as those that were not tested for using the MicroScan Gram-positive PM-33 panel. In addition, vancomycin was excluded as all isolates were susceptible.
within a hospital environment [31]. Important in identifying a link to specific genotypes, susceptibility profiles were also reported; apart from standing of the circulating strains in a geographical surveillance population. We can therefore not comment the circulating clones that are representative of entire types are seen in Table 6. Although only data for 48 isolates are present, the circulating clones are relatively diverse. As MLST was only done on a few selected isolates we could not confidently establish the circulating clones that are representative of entire surveillance population. We can therefore not comment on the evolution of MRSA clones in our setting.

Discussion

This study is a detailed description of the molecular characterisation of MRSA isolates with specific focus on SCCmec types and spa types and, to a lesser extent, sequence types. It is important to have a genetic understanding of the circulating strains in a geographical region to establish genetic diversity, transmission, dissemination, epidemiology and evolution. Antimicrobial susceptibility profiles were also reported; apart from using antimicrobial susceptibility results for treatment regimens, antimicrobial susceptibility profiles are also important in identifying a link to specific genotypes, which could potentially identify virulence patterns. Antimicrobial selection may potentially also be a key factor in the dissemination of predominating MRSA clones within a hospital environment [31].

SCCmec type III was the most predominant SCCmec type followed by type IV. Type III was also the most frequent SCCmec type in studies in Iran [32, 33], Serbia [34], Brazil [35] and Europe [36]. The most prevalent

| SCCmec type, n | Spa type, n (%) |
|----------------|----------------|
| SCCmec type I isolates (n = 2) | t015, t1186 (n = 1, 50%, each) |
| SCCmec type II (n = 104) | t012 (n = 71, 67.6%); t037 (n = 7, 6.7%); t021 (n = 4, 3.8%); t238 (n = 4, 3.8%); t1257 (n = 3, 2.9%); t018 (n = 2, 1.9%); t0121 (n = 1, 0.9%); t045 (n = 1, 0.9%); t064 (n = 1, 0.9%); t2526 (n = 1, 0.9%); t4864 (n = 1, 0.9%); t6330 (n = 1, 0.9%); t729 (n = 1, 0.9%); t840 (n = 1, 0.9%); t8563 (n = 1, 0.9%); t913 (n = 1, 0.9%); novel spa types: txAF, txAK, txAQ, txAQ (n = 1, 0.9%, each) |
| SCCmec type III (n = 709) | t037 (n = 656, 92.5%); t045 (n = 12, 1.7%); t1257 (n = 8, 1.1%); t012 (n = 8, 1.1%); t0209 (n = 4, 0.6%); t0421 (n = 2, 0.3%); t1476 (n = 2, 0.3%); t032 (n = 1, 0.1%); t127 (n = 1, 0.1%); t2293 (n = 1, 0.1%); t355 (n = 1, 0.1%); t932 (n = 1, 0.1%); t7962 (n = 1, 0.1%); t701 (n = 1, 0.1%); t19453 (n = 1, 0.1%); t4140 (n = 1, 0.1%); t5691 (n = 1, 0.1%); novel spa types: txAI, txF, txAI, txAQ, txAQ, txBD (n = 1, 0.1%, each) |
| SCCmec type IV (n = 451) | t41257 (n = 255, 56.5%); t064 (n = 47, 10.4%); t1973 (n = 23, 5.1%); t032 (n = 21, 4.7%); t1443 (n = 14, 3.1%); t037 (n = 12, 2.7%); t022 (n = 5, 1.1%); t1467 (n = 5, 1.1%); t118 (n = 4, 0.9%); t294 (n = 4, 0.9%); t4833 (n = 4, 0.9%); t451 (n = 3, 0.7%); t891 (n = 3, 0.7%); t012 (n = 2, 0.4%); t105 (n = 2, 0.4%); t2293 (n = 2, 0.4%); t304 (n = 2, 0.4%); t718 (n = 2, 0.4%); t908 (n = 1, 0.2%); t105 (n = 1, 0.2%); t018 (n = 1, 0.2%); t0379 (n = 1, 0.2%); t045 (n = 1, 0.2%); t059 (n = 1, 0.2%); t1555 (n = 1, 0.2%); t1774 (n = 1, 0.2%); t320 (n = 1, 0.2%); t272 (n = 1, 0.2%); t2724 (n = 1, 0.2%); t324 (n = 1, 0.2%); t379 (n = 1, 0.2%); t4268 (n = 1, 0.2%); t432 (n = 1, 0.2%); t4864 (n = 1, 0.2%); t5691 (n = 1, 0.2%); t758 (n = 1, 0.2%); t951 (n = 1, 0.2%); novel spa types: txAI, txcX and txAI (n = 2, 0.4%, each), txAI, txAE, txAG, txAJ, txAL, txAM, txAN, txAS, txAO, txBA, txBB, txB4, txB5 (n = 1, 0.2%, each) |
| SCCmec type V (n = 3) | t1476, t045, t037 (n = 1, 33.3%, each) |
| SCCmec type VI (n = 5) | t8183 (n = 2, 80%; t174 (n = 1, 20%); t223 (n = 1, 20%); novel spa type txAI (n = 1, 20%) |

n = 9; t1257, n = 10; t012, n = 9; t064, n = 9; t045, n = 8; t032, n = 3). The predominating circulating clones based on common spa types are seen in Table 6. Although only data for 48 isolates are present, the circulating clones are relatively diverse. As MLST was only done on a few selected isolates we could not confidently establish the circulating clones that are representative of entire surveillance population. We can therefore not comment on the evolution of MRSA clones in our setting.

Table 6 Predominating circulating clones

| ST (CC) | SCCmec type | Spa type | No. of isolates |
|---------|-------------|----------|-----------------|
| 5 (S)   | III         | t045     | 1               |
| 5 (S)   | V           | t045     | 2               |
| 5 (S)   | Unknown     | t045     | 4               |
| 5 (S)   | Unknown     | t1257    | 1               |
| 22 (22) | IV          | t012     | 1               |
| 22 (22) | IV          | t032     | 2               |
| 4121 (22) | IV         | t032    | 1               |
| 36 (30) | II          | t012     | 5               |
| 36 (30) | II          | t037     | 1               |
| 36 (30) | II          | t064     | 1               |
| 36 (30) | III         | t045     | 1               |
| 239 (8) | III         | t1257    | 1               |
| 239 (8) | III         | t012     | 1               |
| 239 (8) | III         | t037     | 6               |
| 239 (8) | IV          | t037     | 1               |
| 239 (8) | Unknown     | t012     | 1               |
| 239 (8) | Unknown     | t037     | 1               |
| 612 (8) | III         | t012     | 1               |
| 612 (8) | IV          | t064     | 8               |
| 612 (8) | IV          | t1257    | 6               |
| Unknown | IV          | t1257    | 1               |

*ST is unknown due to new allele for the pta gene; at position 277 the nucleotide adenine (A) is present and not the expected nucleotide, guanine (G)*
spa type in our study was t037. This is in keeping with a review conducted in 2018 of European, Asian, American, Australian and African studies from 2007 onwards including 18 studies from Africa which showed that the most prevalent spa type was t037 [5]. The review also showed that t084 and t064 were common in Africa. In contrast to our study, t064 was present in a small number of isolates (n = 49) and t084 was not observed at all. Interestingly this review also showed that the most prevalent spa type in America was t008, which was reported only in America and Canada. Our current study has shown the occurrence of t008 in three isolates from Gauteng and the Western Cape provinces.

Isolates harbouring SCCmec type III and IV elements were the most resistant as evidenced by the large number of non-susceptible phenotypes to majority of the antimicrobial agents (Table 1). A 2014 study in Iran showed similar findings; they further molecularly characterised resistance genes and found that their type III isolates contained different resistance genes [37]. In contrast, an Indian study in 2016 showed more phenotypic resistance to non-beta-lactam antibiotics in their type I isolates [38].

A 2017 Chinese study on 120 MRSA isolates showed differences to the current study; 100% of their spa type t037 isolates were resistant to clindamycin, erythromycin, ciprofloxacin, gentamicin, tetracycline and trimethoprim/sulfamethoxazole whereas only 6% of our isolates were resistant to clindamycin and 45 to 47% were resistant to the remaining antibiotics. However, in keeping with the study from China, none of our t037 isolates were resistant to rifampin and vancomycin (Table 4) [39]. Another Chinese study with 106 t037 isolates showed predominant resistance to clindamycin, erythromycin, ciprofloxacin, gentamicin, tetracycline, trimethoprim/sulfamethoxazole and chloramphenicol [40]. Of six Nigerian t037 isolates, all were resistant to clindamycin, erythromycin, ciprofloxacin, gentamicin, tetracycline and trimethoprim/sulfamethoxazole in addition to penicillin, oxacillin and moxifloxacin [41]; in the current study, almost 50% (47–48%) of the t037 isolates were resistant to penicillin, oxacillin and moxifloxacin.

The study of circulating clones and clonal evolution is important because it is used to assess the relationship between clonal types, disease symptoms, antibiotic choice and clinical outcomes [42]. Clones are bacterial strains that have descended from a common ancestor and through point mutations, recombination, acquisition and deletion of mobile genetic elements they diversify resulting in wide-ranging genotypes and phenotypes [43]. In order to establish circulating clones and clonal evolution, multiple molecular tools should be employed; the combination of ST, SCCmec type and spa type would ideally be preferred. However, as MLST is more costly, we were not able to perform this technique on all isolates. Studies have shown that SCCmec typing is not a very discriminatory method and that spa typing alone was not able to clearly predict ST or PFGE type but when combined with BURP analysis producing spa CCs, it is sufficient for describing the clonal structure of S. aureus [6, 10]. Although useful, it should be noted that spa typing takes only one gene into consideration in relation to the entire genome and therefore does not reflect mutational events occurring throughout the genome [5]. Nevertheless, spa typing is extremely useful and we have coupled it with SCCmec typing and sequence typing to a lesser extent, to provide information on the circulating S. aureus strains in our population.

A review manuscript by Asadollahi et al., in 2018 [5] showed that from five African studies, t037 was most associated with SCCmec type III (106 isolates) and least associated with type V (one isolate). Our study showed similar findings; t037 was mostly associated with SCCmec type III (656 isolates) and least associated with type V (one isolate). In another study of German, French, Japanese and Finnish isolates in 2007, majority of the t037 isolates (n = 8) were also associated with SCCmec type III [44]. This was also seen in seven isolates from a 2014 Iranian study but two t037 isolates were also associated with SCCmec type IV and one was associated with SCCmec type I [37].

The Asadollahi et al., review manuscript further showed that t037 was associated with ST239 and t064 was associated with ST8 [5]. In our study, t037 was mainly associated with ST239 but one isolate was associated with ST36. The isolates belonging to t064 were mainly associated with ST612 and one isolate was associated with ST36. The review further showed that t032 was always associated with ST22 irrespective of the continent in which it was observed; one of the t032 isolates in our study also showed this finding whereas the second t032 isolate was associated with ST4122. Both ST22 and ST4121 belong to MLST CC22. As MLST was only performed on a few selected isolates, the results could have potentially differed if ST data was available for more isolates.

Other publications have used ST and the SCCmec element to define clonal types [45, 46]. In the current study, the Brazilian/Hungarian clone (ST239-MRSA-III) accounted for eight out of the 48 (17%) isolates typed. This is also a common MRSA strain in New Zealand, where the most common associated spa type is t037. Alternative clone names include EMRSA-1, EMRSA-4, EMRSA-11, Por/Bra, Vienna, AUS-2 EMRSA and AUS-3 EMRSA (http://esr.cri.nz/assets/HEALTH-CONTENT/ Images-and-PDFs/MRSAdescriptions.pdf), [45]. Of the eight isolates in the current study, six were spa type t037. This clone has also been observed in Finland,
Molecular typing is extremely useful in studying MSSA through the excision of the SCCmec element. Our study included ST5-MRSA-III (United Kingdom [45]). Another clonal type observed in t064. This clone was also seen in Finland and the current study; however none were associated with prescriptions.pdf. MRSA-II (EMRSA-16) also common in New Zealand from 1994 [40]. The presence of this clone was commonly found over a 15 year period in a study in China. Therefore, this element and consequently the loss of methicillin resistance. Therefore, it is possible for a clone to evolve from MSSA into MRSA through the acquisition of the SCCmec element or from MRSA to MSSA through the excision of the SCCmec element [50]. Molecular typing is extremely useful in studying genetic diversity and a study on a collection of isolates from 19 countries in Europe, the United Kingdom, The United States and Latin America has shown that MRSA and MSSA differ with regards to the diversity of their genetic backgrounds as MSSA has shown to be more diverse [10]. A limitation of the current study is that molecular typing was performed on MRSA isolates only; results for MSSA is therefore lacking and we cannot make any remarks on this matter. To add to genetic diversity, clones responsible for causing HA infections and CA infections may differ and the recombination between HA and CA clones does occur [50]. A detailed investigation taking into consideration aspects like virulence factors such as surface proteins, invasins, biochemical properties, membrane-damaging toxins, exotoxins e.g. Panton-Valentine Leukocidin (PVL), biofilm production, antimicrobial resistance genes and clinical syndromes [42, 43, 50, 51] would be beneficial.

Conclusion
This study reports a large dataset of isolates collected from various provinces in South Africa from 2010 to 2017. A variety of spa types were observed in this study; this is in keeping with other reports showing the presence of multiple spa types in the MRSA population. Moreover, data from Africa is not abundant. It is evident that MRSA clones are diverse; they disseminate both rapidly and efficiently and it is important to understand why particular clones dominate in a specific geographical location in order to develop effective strategies to control the spread of S. aureus infections.

Abbreviations
HA: Healthcare-associated; CA: Community-associated; MSSA: Methicillin-susceptible S. aureus; MRSA: Methicillin-resistant S. aureus; PFGE: Pulsed-field Gel Electrophoresis; MLST: Multilocus Sequence Typing; spa: Staphylococcal protein A; SCCmec: Staphylococcal Cassette Chromosome mec; BURST: Based Upon Related Sequence Type; ST: Sequence type; ccr: Cassette chromosome recombinase; DMP: Diagnostic Media Products; NHLS: National Health Laboratory Service; NICD: National Institute for Communicable Diseases; AST: Antimicrobial susceptibility testing; CLSI: Clinical and Laboratory Standards Institute; PCR: Polymerase Chain reaction

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Authors’ contributions
ASM conceived and designed the experiments, performed the experiments, analysed the data and wrote the manuscript. OP was responsible for the study, its overall design and coordination and in editing of the manuscript. RM performed laboratory experiments and assisted in the editing of the manuscript. ML assisted in laboratory experiments and in the editing of the manuscript. All authors read and approved the final manuscript.

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