CHARACTERISATION OF *STAPHYLOCOCCUS AUREUS* BLOODSTREAM ISOLATES FROM GAUTENG AND WESTERN CAPE PROVINCES, SOUTH AFRICA, 2016 AND 2017

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Executive summary

Staphylococcus aureus bacteraemia is one of the most prevalent bacterial infections in South African healthcare settings and according to international literature, it is the second most frequent pathogen isolated from patients with bacteraemia.[1] Of concern is the presence of methicillin-resistant *Staphylococcus aureus* (MRSA). The prevalence of MRSA differs globally; a surveillance study conducted in four South African provinces showed a MRSA prevalence of 40% in 2012. This decreased from 53% in 2010.[2] In the current study, a total of 374 (24%) MRSA isolates collected as part of a national surveillance programme from two provinces in South Africa were characterised phenotypically. Typing methods such as SCC*mec* and *spa*-typing provide important information about circulating clones of MRSA. All isolates were screened for methicillin resistance using real-time PCR and then typed using conventional typing methods to identify the *mec* element types and *spa*-types. Overall, resistance to antimicrobial agents was low - approximately 0-32% on average - and the MIC_{50} and MIC_{90} did not change for most of the antimicrobial agents over the two-year period except for the MIC_{90}s of rifampicin, which decreased from >2 in 2016 to <=0.5 in 2017 and vancomycin, which increased from 1 in 2016 to 2 in 2017. The most common SCC*mec* type was SCC*mec* type III (45%). The most common spa type was t037 (45%).

Introduction

Staphylococcus aureus infections are a significant cause of morbidity and mortality globally within healthcare settings and in the community.[3] Estimates of mortality of *S. aureus* bacteraemia from 15 studies conducted in Europe, the United States and Asia ranged from 5% to 64%.[4] A recent prospective observational study of patients over 13 years of age with *S. aureus* bacteraemia admitted to one referral hospital in South Africa showed a mortality of 47%.[5] The organism's ability to cause disease such as skin and soft tissue infections, bacteraemia, infective endocarditis, osteomyelitis and necrotising pneumonia is elevated by its ability to develop resistance to frequently-used antibiotics, e.g. methicillin, as well as virulence factors, e.g. those encoded by the staphylococcal cassette chromosome *mec* (SCC*mec*).[3,6]

Methicillin resistance is conferred by the exogenous gene *mecA* or its homologue *mecC*. These genes are located within a mobile genetic element, SCC*mec*[7], which is a large heterologous element consisting of a *mec* complex and a recombinase complex (*ccr*). The *mec* complex contains *mecA/mecC* and its regulators *mecI* and *mecRI*. The *ccr*

complex encodes a site-specific recombinase aiding in the mobility of the element.[8] Several SCC*mec* types exist (I, II, III, IVa, IVb, IVc, IVd, V, VI, VII, VIIII, IX, X and XI) depending on the combination of the class of the *mec* gene complex and the *ccr* allotype (http://www.sccmec.org/). Hospital-associated methicillin-resistant *S. aureus* (MRSA) infections are usually associated with SCC*mec* types I, II or III whereas community-associated MRSA infections are linked to smaller SCC*mec* types IV, V, VI or VII[6] although this may not strictly be the case; epidemiological data are required to make this conclusion.

Spa-typing is a single-locus typing technique that investigates DNA sequence of the protein A gene variable repeat region. This technique rapidly and accurately discriminates *S. aureus* strains[9] and investigates evolutionary relationships among isolates by studying routes of transmission to assess the source of infection. The aim of this study was to identify the common methicillin resistance determinant, SCC*mec* types and *spa*-types in South African MRSA isolates.

Materials and Methods

Bacterial Strains. Blood culture isolates from a surveillance study for the period 1 January 2016 to 31 December 2017 from sentinel centres in South Africa were analysed. Sites represented the Gauteng (n=841) and Western Cape (n=702) provinces. Ethical clearance was obtained from the University of the Witwatersrand's Human Research Committee.

All *S. aureus* blood culture positive isolates were submitted. Any isolate received from the same patient within a 21-day period was considered a duplicate and was rejected. A new/recurrent case was accepted after the 21-day period. Based on the case definition, we received 1543 *S. aureus* isolates submitted on Dorset transport media (Diagnostic Media Products, National Health Laboratory Service). Each isolate was plated onto a 5% blood agar plate (Diagnostic Media Products, National Health Laboratory Service) followed by organism identification and antimicrobial susceptibility testing using automated systems. Organism identification was done using VITEK II (bioMèrieux, Marcy-l'Etoile France) and MALDI-TOF MS (Microflex, Bruker Daltonics, MA, USA) and antimicrobial susceptibility testing using the MicroScan Walkaway system (Siemens, Sacramento, CA, USA). Interpretation of susceptibility was performed according to the Clinical and Laboratory Standards Institute (CLSI) guidelines.[10]

Polymerase chain reaction (PCR) screening for *mecA* and *mecC* in MRSA isolates. DNA was extracted and used in the genotypic assays. The LightCycler 480 II (Roche Applied Science, Penzberg, Germany) instrument was used for the real-time PCR of *mecA* using the LightCycler 480 Probes Master kit (Roche Diagnostics, IN, USA) with previously published primers and probes.[11] The G-Storm (Somerton Biotechnology Centre, Somerton, UK) thermal cycler was used for the conventional PCR of *mecC* using the Qiagen Multiplex PCR kit (Qiagen, Nordrhein-Westfalen, Germany) with previously published primers.[12]

SCC*mec* Typing. All 374 *mecA*-positive MRSA isolates were typed by multiplex PCR using the Qiagen Multiplex PCR kit and previously published primers[13] to identify the current prevalent *mec* element types.

***Spa*-typing.** *Spa*-typing was performed on all 374 MRSA isolates. The *spa* gene was amplified using previously published primers[14] and the Amplitaq Gold DNA Polymerase kit (Applied Biosystems, CA, USA). Purified PCR products

(Qiagen Purification kit; Qiagen, Nordrhein-Westfalen, Germany) were sequenced. Sequences were assembled using CLC Bio main workbench (Qiagen, Hilden, Germany) and analysed using the Ridom StaphType software (Ridom GmbH, Würzburg, Germany).

Statistical analysis. Data analyses were performed using Stata version 14 (StataCorp LP, College Station, Texas, USA).

Results

Phenotypic. A total of 1543 isolates was received for the period 1 January 2016 to 31 December 2017. Of these 374 (24%) were MRSA. A majority of the isolates were received from the Gauteng Province (n=841, 55 %) followed by the Western Cape Province (n=702, 46%). Nine-hundred and fifty cases were males (62%). The gender for one case was unknown. Susceptibility to the following antimicrobial agents was high over the 2-year period: mupirocin, daptomycin, linezolid, teicoplanin, vancomycin, rifampicin, fosfomycin and fusidic acid ranged from 88% to 100% susceptible. The remaining agents showed relatively low resistance levels (ranging from 19% to 32% resistance) (Figure 1). The antimicrobial susceptibility profiles for most antibiotics were comparable for 2016 and 2017, with the exception of ciprofloxacin (p=0.012), tetracycline (p=0.04) and rifampicin (p=0.001), which showed significant increases in susceptibility in 2017. Overall, the MIC_{50} and MIC_{90} did not change for antimicrobial agents during the study period except for the MIC_{90}s for rifampicin and vancomycin (Table 1).

PCR Screening for *mecA* and *mecC* in MRSA isolates. All 374 phenotypically - and genotypically - confirmed MRSA isolates harboured the *mecA* gene. No isolate harboured the *mecC* gene.

SCCmec typing. The most common SCC*mec* type identified was SCC*mec* type III (n=168, 45%) followed by types IV (n=110, 29%), II (n=32, 9%) and one each for types V and VI (0.3%). Unidentified banding patterns were obtained for 62 isolates (17%) (Figure 2). No type I SCC*mec* element was observed. Most of the isolates representing SCC*mec* type II (n=25, 7%) and type IV (n=79, 21%) were from Western Cape Province and most of the type III isolates were from Gauteng Province (n= 141, 38%). Forty-two (11%) of the unidentified banding patterns were seen in Western Cape Province. One isolate (0.3%) each of types V and VI were from Gauteng Province.

***Spa*-typing.** *Spa*-typing of 374 of the isolates revealed 40 different *spa*-types, seven of which were novel and have not as yet been assigned. One isolate was nontypeable as it produced no *spa*-type. The five most common *spa*-types were t037 (n=168, 45%), t1257 (n=61, 16%), t045 (n=41, 11%), t012 (n=2, 8%) and t01971 (n=14, 4%) which accounted for 83% of the isolates tested (Figure 3). The remaining *spa*-types represented a minimum of one to a maximum of seven isolates. Of the five most common *spa*-types, the majority of the t1257 (n=36, 10%), t045 (n=31, 8%), t012 (n=19, 5%) and t01971 (n=14, 4%) *spa*-types were seen in Western Cape Province and t037 (n=137, 37%) was observed in Gauteng Province.

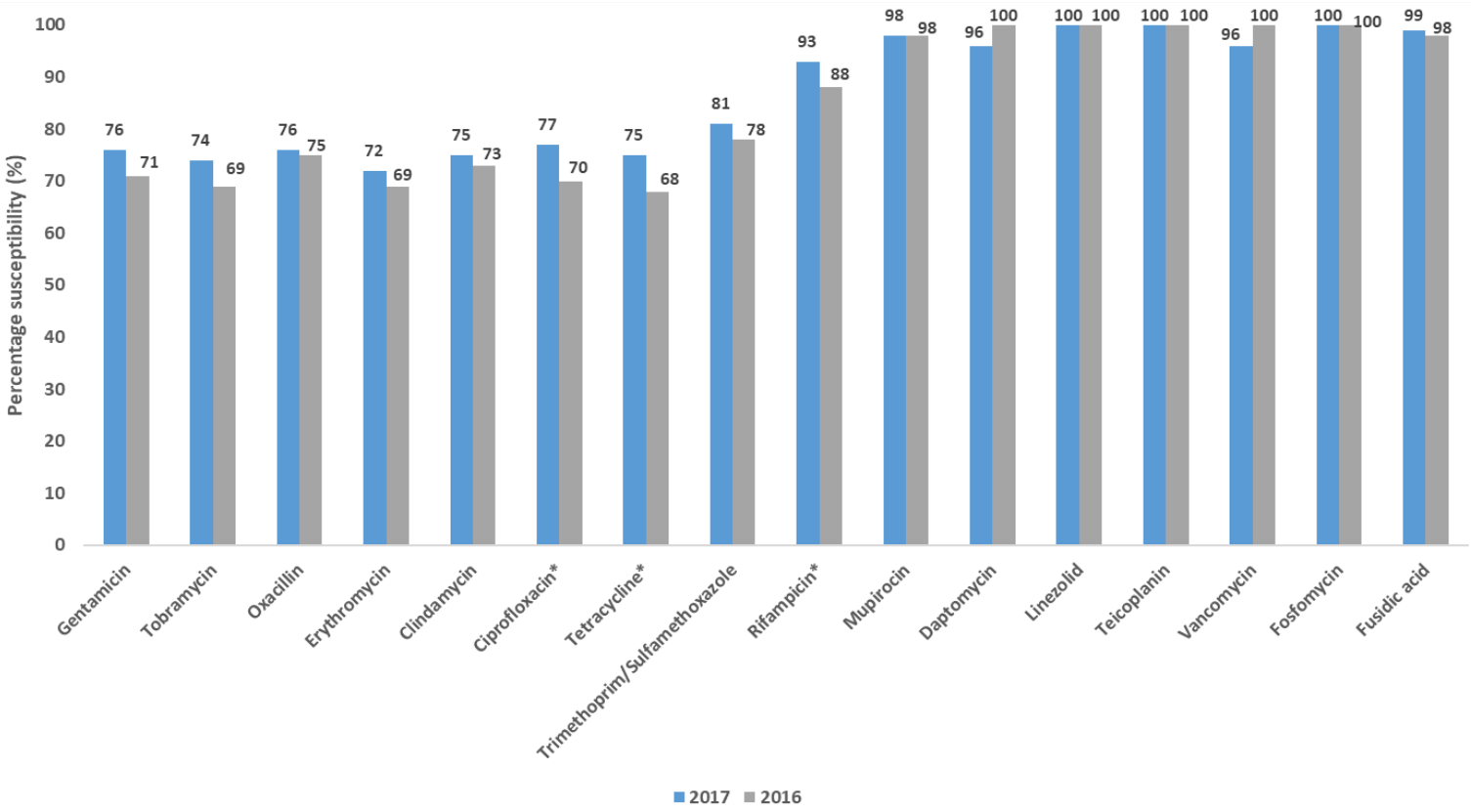

Figure 1. *Staphylococcus aureus* percentage susceptibilities to antimicrobial agents from Gauteng and Western Cape provinces, South Africa, 2016-2017 (n=1543).

* The antimicrobial susceptibility profiles for ciprofloxacin (p=0.012), tetracycline (p=0.04) and rifampicin (p=0.001) showed significant increases in susceptibility in 2017.

Table 1. *Staphylococcus aureus* minimum inhibitory concentrations (MIC_{50} and MIC_{90}) by antimicrobial agent using the Microscan breakpoint panel (PM33), South Africa 2016 and 2017.

Antibiotic	MIC_{50}		MIC_{90}		MIC interpretive breakpoints (µg/ml) based on CLSI guidelines (2017)	
	2016	2017	2016	2017	S	R
Gentamicin	<=1	<=1	>8	>8	<=4	>=16
Tobramycin	<=1	<=1	>8	>8	<=4	>=16
Oxacillin	<=0.25	<=0.25	>2	>2	<=2	>=4
Erythromycin	<=0.5	<=0.5	>4	>4	<=0.5	>=8
Clindamycin	<=0.25	<=0.25	<=0.25	<=0.25	<=0.5	>=4
Ciprofloxacin	<=1	<=1	>2	>2	<=1	>=4
Tetracycline	<=1	<=1	>8	>8	<=4	>=16
Trimethoprim/Sulfamethoxazole	<=2/38	<=2/38	>4/76	>4/76	<=2/38	>4/76
Rifampicin	<=0.5	<=0.5	>2	<=0.5	<=1	>=4
Mupirocin	<=256	<=256	<=256	<=256	<=4	>=256
Daptomycin	<=1	<=1	<=1	<=1	<=1	_
Linezolid	2	2	2	2	<=4	>=8
Teicoplanin	<=1	<=1	<=1	<=1	<=8	>=32
Vancomycin	1	1	1	2	<=2	>=16
Fosfomycin*	<=32	<=32	<=32	<=32	<=32	>=32
Fusidic acid**	<=2	<=2	<=2	<=2	<=2	>=32

MIC_{50} - minimal inhibitory concentration needed to inhibit 50% organism growth

MIC_{90} - minimal inhibitory concentration needed to inhibit 90% organism growth

S - susceptible

R - resistant

*Based on European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines.

**Based on Comite de Antibiogramme de la Societe Francaise de Microbiologie (CA-SFM, 2008).

For 2017, results for linezolid were missing for 3 isolates and results for vancomycin were missing for 1 isolate.

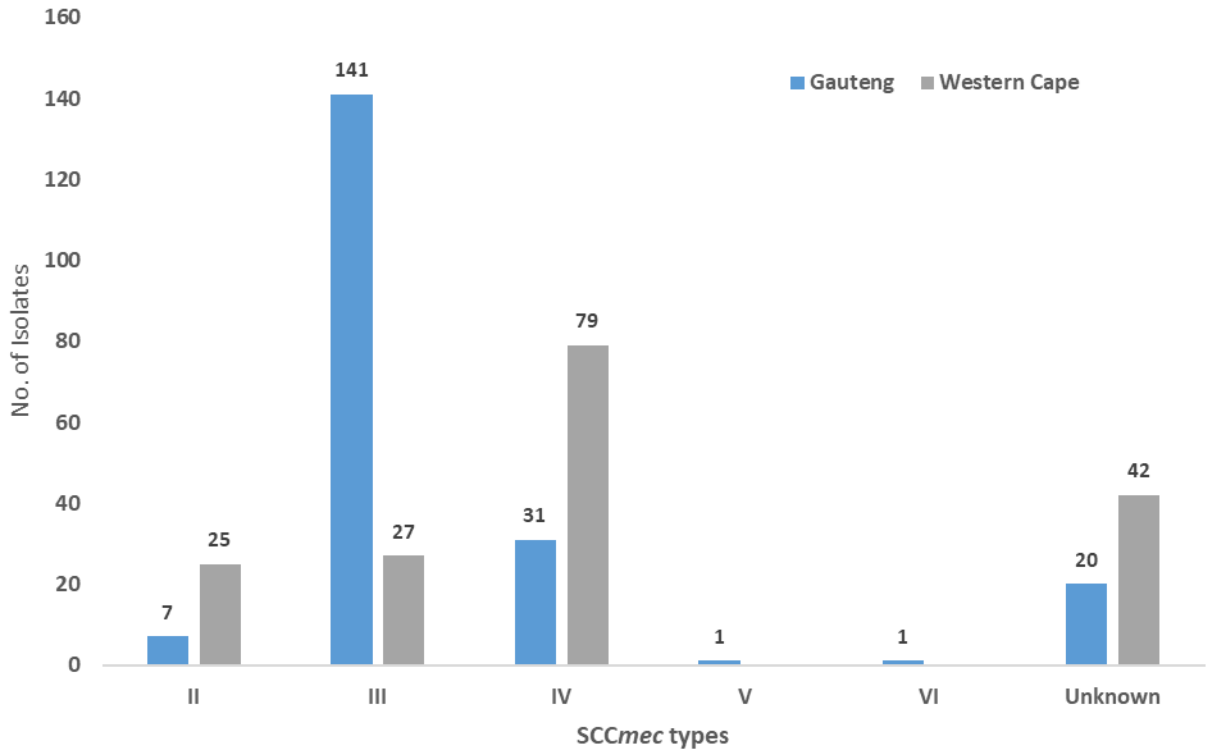

Figure 2. SCC*mec* type elements for 374 methicillin-resistant *Staphylococcus aureus* isolates by province, South Africa, 2016 and 2017.

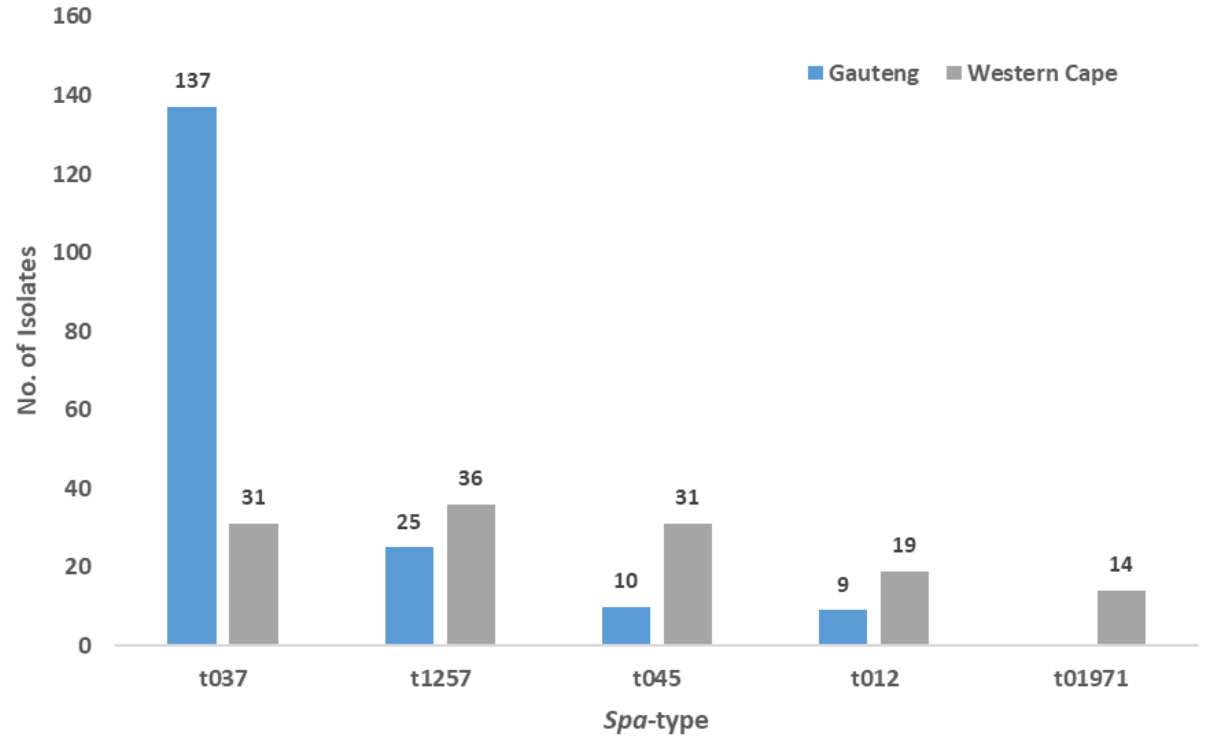

Figure 3. *Spa*-types for 312 methicillin-resistant *Staphylococcus aureus* isolates by province, South Africa, 2016 and 2017.

Discussion

This study investigated *S. aureus* isolates obtained over a 2-year surveillance study period. Overall resistance levels were low for all antimicrobial agents and the MIC_{50} and MIC_{90} were stable. The population structure of MRSA causing bacteraemia was described in 374 surveillance isolates using a combination of molecular typing methods and confirmed with *mec*A. Strains carrying *mecC* were not present. It should be noted that only human samples were included with no isolates from livestock, which has often been associated with *mecC*. The majority of the isolates were classified as SCC*mec* type III, most of which were from Gauteng Province. Based on conventions[6], it is speculated that the majority of the *S. aureus* bacteraemia infections are hospital-associated but epidemiological data are required to make this conclusion. The most common *spa*-type identified was t1037; this is consistent with previous findings from various studies in South Africa[2,15-17] and indicates that there has not been much evolution of circulating MRSA types. The majority of these were from Gauteng Province. It should be noted that most of the isolates were received from Gauteng. When considering the molecular results more variability was seen in isolates from Western Cape Province as compared to Gauteng Province, perhaps indicating a genetically diverse MRSA population in Western Cape Province and a more conserved MRSA population in Gauteng Province.

In conclusion, this report demonstrates the common and established circulating SCC*mec* element types and *spa*-types in two provinces. When compared to previous South African findings, these have not changed over the past eight years showing no evolution of MRSA clonal types.

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