A small proportion of community-associated methicillin-resistant *Staphylococcus aureus* bacteraemia, as compared to healthcare-associated cases, in two South African provinces

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Abstract

We compared the proportion of cases of CA-MRSA and HA-MRSA bacteraemia among patients at five hospitals in Gauteng and Western Cape provinces in South Africa then described the molecular characteristics and antimicrobial susceptibility trends. This was a cross-sectional study using data collected by enhanced surveillance for *Staphylococcus aureus* bacteraemia.

A total of 2511 cases of *S. aureus* bacteraemia were identified from January 2013 to January 2016. Among 1914 cases of *S. aureus*, 557 (29.1%) cases were identified as MRSA infection. Forty-four cases (44/1914 [2.3%] of all *S. aureus* cases) were considered CA-MRSA infection and 513/1914 (26.8% of all cases) had HA-MRSA infection; the majority were neonates. CA-MRSA constituted 7.9% (44/557) of all cases of MRSA infection. *S. aureus* isolates demonstrated significantly reduced susceptibility to the following classes of antimicrobial agents: macrolides, tetracyclines, aminoglycosides, and cotrimoxazole, in 2015 compared to 2013 ($p < 0.05$). Of the 557 MRSA isolates, 484 (87%) were typed for SCC*mec* element and *spa*-types: the most common SCC*mec* type was type III (n=236, 48.76%) followed by type IV (n=144, 29.76%). The most common *spa*-types were t037 (n=229, 47.31%) and t1257 (n=90, 18.60%). Of 28 isolates selected for MLST, the most common STs were ST239 and ST612 of clonal complex (CC8) (n=8 each) and a novel ST (ST4121) was obtained for one isolate. This study demonstrates that *S. aureus* bacteraemia is common in South African academic centres and characterized by HA-MRSA SCC*mec* III and IV types. A small proportion of CA-MRSA cases were caused by a few sequence types.

Keyword

Staphylococcus aureus bacteraemia*;* MRSA; Epidemiology of community vs. hospital MRSA; Surveillance for antimicrobial resistance

Introduction

Staphylococcus aureus is a major human pathogen that causes a wide range of clinical infections such as bacteraemia, endocarditis, arthritis, osteomyelitis, lung infections, skin and soft tissue and device-related infections (1). *S. aureus* is a commensal organism in about 30% of the human population (1). The epidemiology of *S. aureus* bacteraemia has been poorly described in low- and middle-income countries (LMICs) compared to high-income countries (HICs) (1, 2). While the case-fatality for *S. aureus* bacteraemia was approximately 80% in the pre-antibiotic era, this has remained persistently high (15%-50%) over the past several decades, partly owing to the emergence of methicillin-resistant *S. aureus* (MRSA) in the 1960s (1). Historically, MRSA isolates, which are

resistant to β-lactam antibiotics, were confined to healthcare facilities. However, in the mid-1990s, the emergence of community-associated MRSA (CA-MRSA) strains, which cause infections among patients with no previous exposure to the healthcare environment, resulted in a considerable shift to CA-MRSA-associated disease in HICs in North America, Asia, Europe and Australia, but less so in LMICs (2, 3, 4). The overall incidence of *S. aureus* bacteraemia has been stable over the past two decades, but MRSA rates have fluctuated and since 2005, HICs including the United States, Canada, United Kingdom, France and Australia have described significant reductions in MRSA bacteraemia through population-based active surveillance (1, 5). The incidence of CA-MRSA in LMICs is less well described and this highlights the need for surveillance in order to determine the burden of disease. This is not simple because CA-MRSA and HA-MRSA are not easily distinguished and case definitions for both involve numerous variables such as epidemiological and clinical features, which are required in conjunction with molecular characterization of a large staphylococcal cassette chromosome *mec* (SCC*mec*). SCC*mec* types I to III are traditionally assigned to hospital strains and types IV to VI to community strains but this distinction has become less clear in the last few years. The largest proportion of cases of *S. aureus* bacteraemia (both methicillin-susceptible and resistant) occurred among children aged less than one year in a previous report from South Africa (6). This report also showed a strong association between HIV-infection and MRSA bacteraemia (6).

To our knowledge, no South African studies have yet described the proportions of MRSA bacteraemia that are community versus healthcare-associated using epidemiological, clinical and molecular criteria. In this study, we compared the proportion of cases of CA-MRSA and HA-MRSA bacteraemia among hospitalised patients at five sentinel surveillance hospitals in Gauteng and Western Cape provinces in South Africa, evaluated factors associated with CA-MRSA versus HA-MRSA and compared in-hospital outcomes between the two groups. We also described the molecular characteristics and antimicrobial susceptibility trends over a three-year period, to guide clinicians in antimicrobial stewardship (AMS) programmes and to establish baseline antimicrobial resistance profiles of *S. aureus* isolates for guideline and policy formulation.

Methodology

Study design and population

This was a cross-sectional study using data collected by active, laboratory-based surveillance for *S. aureus* bacteraemia, through the GERMS-SA enhanced surveillance programme. Five tertiary public-sector hospitals, Helen Joseph Hospital [HJH] with 900 beds serving estimated population of 1 million, Steve Biko Academic Hospital at Tshwane District [SBAH] with 832 beds for 402 980 population, Charlotte Maxeke Johannesburg

Academic Hospital [CMJAH] with 1088 beds intended for 4.4 million people from Gauteng Province and Groote Schuur Hospital [GSH] with 893 beds serving population of 1.8 million from Western Cape, and Tygerberg Hospital [TBH] with 1384 beds serving population of 1.9 million from the Western Cape that started surveillance one year later on 1st January 2014.

Case definitions

A case was defined as a person of any age accessing public-sector healthcare at any one of the five hospitals during the period of surveillance with a blood culture specimen positive for *S. aureus.* All positive specimens within 21 days of the first positive specimen contributed to a single case while subsequent positive specimens were considered part of new cases. All cases of *S. aureus* bacteraemia, where *S. aureus* was found to be non-susceptible to oxacillin or cefoxitin, were classified as cases of MRSA infection.

Criteria to distinguish community- and hospital-associated MRSA

A number of criteria were used to distinguish CA from HA-MRSA bacteraemia. A case of CA-MRSA bacteraemia was defined as a patient with 1) MRSA isolated from a blood culture specimen ≤48 hours of admission to hospital and 2) no contact with a healthcare facility within one year prior to the current episode of MRSA infection (including prior dialysis, prior surgery and prior admission to a long-term care facility). A patient with MRSA isolated >48 hours after admission or with any prior healthcare contact was considered to be a case of HA-MRSA infection.

Data collection

GERMS-SA surveillance officers used standardised case report forms (CRFs) to collect demographic, clinical and treatment data from consenting patients with laboratory-confirmed *S. aureus* bacteraemia. Data were also obtained from medical and laboratory records. Isolates from these patients were submitted to the National Institute for Communicable Diseases (NICD) for confirmatory identification, antimicrobial susceptibility testing, confirmation of *mec*A gene and genotyping. Completeness of surveillance data was checked for the time period, 1 January 2013 to 31 January 2016, using the National Health Laboratory Service Corporate Data Warehouse, which houses information from routine laboratory testing and reporting.

Statistical analysis

Demographic and clinical characteristics of cases were summarised and compared using Chi squared/ Fisher's exact tests for categorical data and Student's T-test/ Wilcoxon ranked sum tests for continuous data. We calculated proportions by dividing the number of CA-MRSA and HA-MRSA cases by the total number of *S. aureus* bacteraemia cases, as well as, by the number of cases with MRSA bacteraemia. Multivariable logistic regression analysis was performed to evaluate factors associated with CA-MRSA versus HA-MRSA and to compare in-hospital outcome between the two groups. Exposure variables (including age, sex, hospital, province, clinical diagnosis, body temperature at diagnosis, mental status, mechanical ventilation, cardiac arrest, prior MRSA, prior surgery, prior dialysis, previous admission to a long-term care facility, HIV-infection, participation in contact sports, living or working in crowded facilities, and pre-existing medical and surgical conditions) were independently evaluated as risk factors for MRSA-type and mortality by univariate analysis. Variables with p-values<0.2 were included by forward stepwise manual addition into two multivariable models. A p-value of <0.05 in the multivariable models was considered as statistically significant.

Microbiology

Phenotypic methods

S. aureus isolates were submitted by diagnostic laboratories on Dorset transport medium (Diagnostic Media Products, NHLS, Sandringham). Organism identification was confirmed using the Vitek 2 GP card (bioMerieux, France). Susceptibility testing was performed on the MicroScan Walkaway system (Siemens Healthcare Diagnostics, USA) using the Positive MIC Panel Type 33. Categorical results and susceptibility profiles of most tested antimicrobial agents were based on the Clinical and Laboratory Standards Institute (CLSI) interpretative criteria (7); for fosfomycin interpretation of MICs was performed by using EUCAST interpretative criteria 2016 recommendations (8) and mupirocin MICs interpretation was based on manufacturer recommendations. The MIC_{50} and MIC_{90} (minimum inhibitory concentrations needed to inhibit the growth of 50% and 90% of organisms, respectively) were determined for all tested agents.

Molecular methods

DNA extraction

Pure bacterial colonies were re-suspended in 400 µl TE buffer. This was vortexed briefly and heated at 95°C for 25 min to allow bacterial cell lysis to release the DNA. Centrifugation followed at 12000 rpm for 3 min to pellet the cellular debris. The supernatant was then aliquoted and stored at -70°C for further investigations.

Polymerase chain reaction (PCR) screening for mecA and mecC genes in MRSA isolates

The Light Cycler 480 II instrument (Roche Applied Science, Germany) was used for the real-time PCR amplification of the methicillin resistance determinant, *mecA* and the species-specific gene, *nuc* which were amplified in a multiplex assay using the Light Cycler 480 Probes Master kit (Roche Diagnostics, IN, USA) with previously published primers and probes (9). In the absence of *mecA*, the G-Storm (Somerton Biotechnology Centre, UK) thermal cycler was used for conventional PCR-based amplification of the methicillin resistance determinant, *mecC* using the Qiagen Multiplex PCR kit (Qiagen, Germany) with previously published primers (10).

SCCmec typing

All *mecA*-positive MRSA isolates were typed by multiplex PCR using the Qiagen Multiplex PCR kit (Qiagen, Germany) and previously published primers (11).

Spa-typing

The *spa* gene was amplified using previously published primers (12) and the Amplitaq 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^{TM} software (Ridom GmbH, Würzburg, Germany).

Multilocus sequence typing (MLST)

For our hospital-associated infection (n=513) group, four isolates belonging to each of the five most common *spa*-types (t037, t1257, t012, t045 and t064) were selected for MLST. For our community-associated infection (n=44) group, the following *spa*-types were observed: t037, t1257 t064 and t032. Two isolates belonging to the t037 and

t1257 *spa*-types were selected for MLST; and one isolate each was observed and sequenced for the t064 and t032 *spa*-types. Since t032 was observed in the community-associated infection group, we also included two isolates belonging to this sequence type in our hospital-associated infection group. Primers amplifying 7 reference genes were used (13). Amplification was performed using the Amplitaq Gold DNA Polymerase kit (Applied Biosystems, CA, USA). Purified PCR products were sequenced (Inqaba Biotech, South Africa). Sequences were assembled using CLC Bio main workbench (Qiagen, Germany) and analysed using the online database (https://pubmlst.org/).

Results

Community-associated MRSA and hospital-associated MRSA

A total of 2511 cases of *S. aureus* bacteraemia were identified from January 2013 through to January 2016, including 597 cases with no corresponding isolates (missing or non-viable) detected by review of the laboratory information system. This accounted for 2414 discreet patients, as 76 patients had more than one episode of infection. Among 1914 cases of *S. aureus* bacteraemia with a viable isolate, confirmed molecular identification and full susceptibility profile, 557 (29.1%, 95% confidence interval [CI] 27.1-31.2) cases were classified as MRSA infection (Figure 1). Forty-four cases (44/1914 [2.3%] of all *S. aureus* cases) were considered CA-MRSA infection and 513/1914 (26.8% of all cases) had HA-MRSA infection. CA-MRSA constituted 7.9% (44/557) of all cases of MRSA infection.

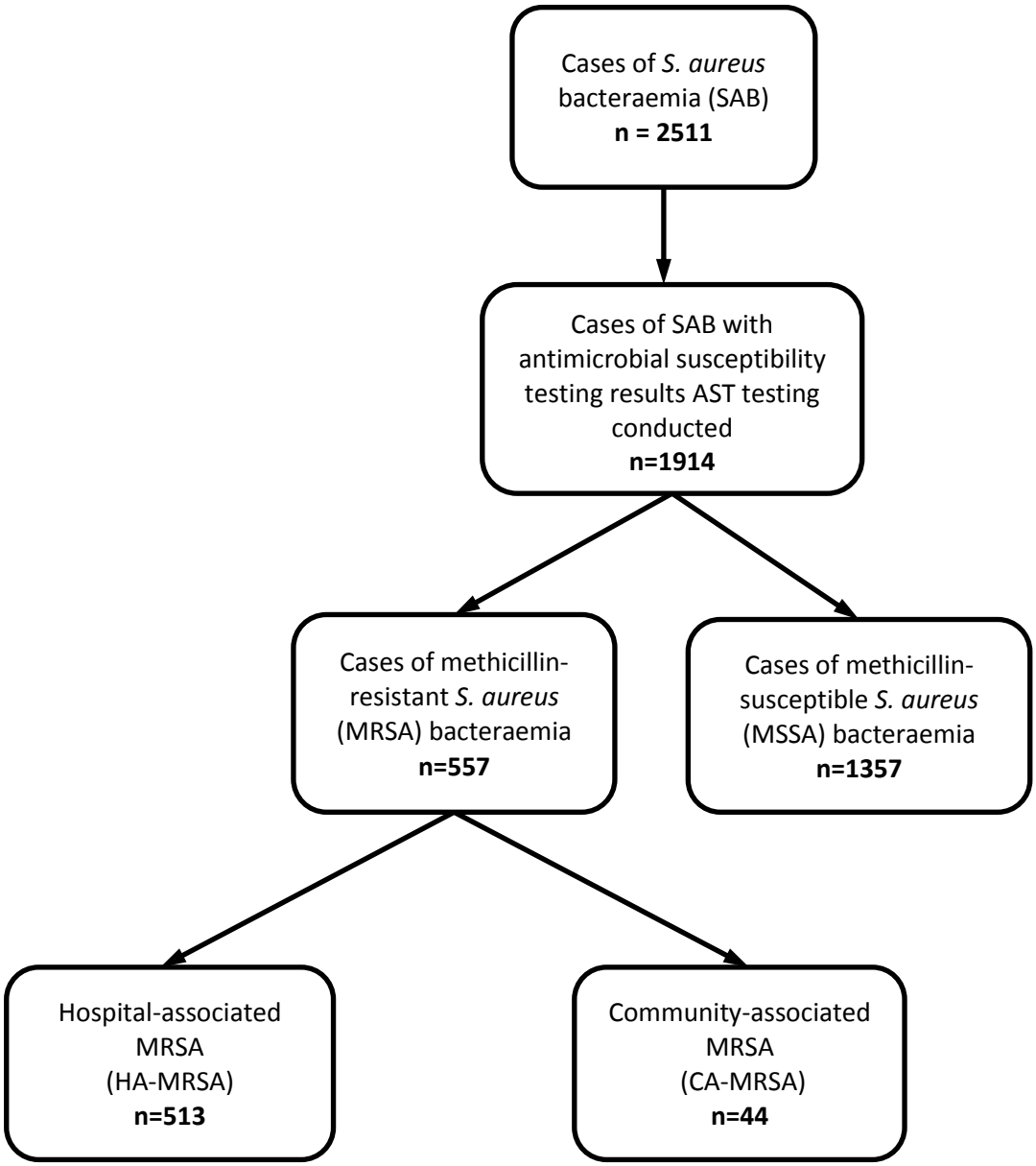

Figure 1: Flowchart of cases of *Staphylococcus aureus* bacteraemia among patients at five sentinel hospitals in South Africa, January 2013 to January 2016. AST=antimicrobial susceptibility testing

Table 1: Demographic and clinical characteristics of patients with hospital-associated MRSA (HA-MRSA) and community-associated MRSA (CA-MRSA) at five sentinel hospitals in South Africa, January 2013 to January 2016 (n=557).

Patient characteristics	Type of MRSA (n=557)						
	HA-MRSA (n=513)		CA-MRSA (n=44)		Total		p-value
	n	%	n	%	n	%	
Male sex	310	60.7	25	56.8	335	60.4	0.633
Age group (years)							
≤28 days	160	31.3	11	25.0	171	30.8	
29 days-1 year	79	15.4	6	13.6	85	15.3	
1-9	30	5.9	1	2.3	31	5.6	
10-19	18	3.5	1	2.3	19	3.4	
20-29	37	7.2	4	9.1	41	7.4	
30-39	52	10.2	7	15.9	59	10.6	
40-49	43	8.4	6	13.6	49	8.8	
50-59	47	9.2	6	13.6	53	9.5	
≥60	46	9.0	2	4.6	48	8.6	0.634
Province							
Gauteng	308	60.0	28	63.6	336	60.3	
Western Cape	205	40.0	16	36.4	221	39.7	0.749
Clinical syndrome							
Bacteraemia without focus	291	63.0	28	63.6	319	63.0	
Joint infection	6	1.3	0	0.0	6	1.2	
Pneumonia	69	14.9	10	22.7	79	15.6	
Meningitis	4	0.9	2	4.6	6	1.2	
Skin or soft tissue infection	73	15.8	1	2.3	74	14.6	
Other	19	4.1	3	6.8	22	4.4	**0.017**
Mental status/ GCS							
GCS 15 / Alert	119	22.8	16	69.6	135	76.7	
GCS 13-14 / Disorientated	18	11.8	4	17.4	22	12.5	
GCS 9-12 / Stuporous	8	5.2	1	4.4	9	5.1	
GCS 3-8 / Coma	8	5.2	2	8.7	10	5.7	0.628
In-hospital outcome							
Survived	261	58.5	27	61.4	288	58.8	
Died	185	41.5	17	38.6	202	41.2	0.751
Predisposing factors							
Prior MRSA infection	27	6.0	1	2.3	28	5.7	0.497
Prior dialysis	16	3.5	0	0.0	16	3.2	0.382
Prior surgery	133	29.0	1	2.3	134	26.7	**<0.001**
Resident in a long-term care facility	22	4.8	0	0.0	22	4.4	0.242
HIV-infection	65	25.2	10	52.6	75	27.1	**0.015**
Pre-existing conditions§	28	6.7	1	3.6	29	6.5	1.000

§ Pre-existing conditions include pulmonary, cardiac, renal, hepatic, neurological conditions, head injury, anaemia, connective tissue disorders, diabetes mellitus, primary immune disorders, alcohol use, smoking, prematurity, burns, malnutrition and malignancy

Denominators vary owing to missing data

∞ GSC-Glasgow Coma Score

Demographic and clinical characteristics of cases with emphasize on CA and HA MRSA

Among all patients with MRSA infection, 60.4% were male (335/555) (Table 1). The median age of all patients with MRSA infection was 3 years (interquartile range (IQR): 0.05-42.44). The majority of both CA-MRSA and HA-MRSA cases with age data available were <1 year of age [CA-MRSA: 17/44 (38.6%); HA-MRSA: 239/512 (46.7%)], with most cases ≤28 days [CA-MRSA: 11/44 (25%); HA-MRSA: 160/512 (31.3%)]. Among cases with CA-MRSA, almost 16% were aged 30-39 years, but only 4.6% of cases were aged ≥60 years or older. Among cases of HA-MRSA, 10.2% were aged 30-39 and 9% ≥60 years (Table 1 and Figure 2). The ratio of CA-MRSA to HA-MRSA cases was similar for Gauteng and Western Cape provinces (p=0.75). Bacteraemia without a known focus was the most common clinical syndrome in both CA-MRSA (28/44, 63.6%) and HA-MRSA cases (291/462, 63%). Pneumonia accounted for 22.7% among CA-MRSA (10/44) versus 14.9% among HA-MRSA (69/462) cases. Among 277 cases with known HIV-infection status, 10 of 19 case patients with CA-MRSA were HIV-infected (52.6%), compared to 65 of 258 (25.2%) among those with HA-MRSA infection (p=0.015). The ratio of MRSA cases to MSSA cases increased from 27% (59/216) in 2013 to 32% (242/748) in 2015 but this was not a significant change (p=0.37). Additionally, the ratio of CA-MRSA to HA-MRSA cases did not change significantly over the three-year period (p=0.29).

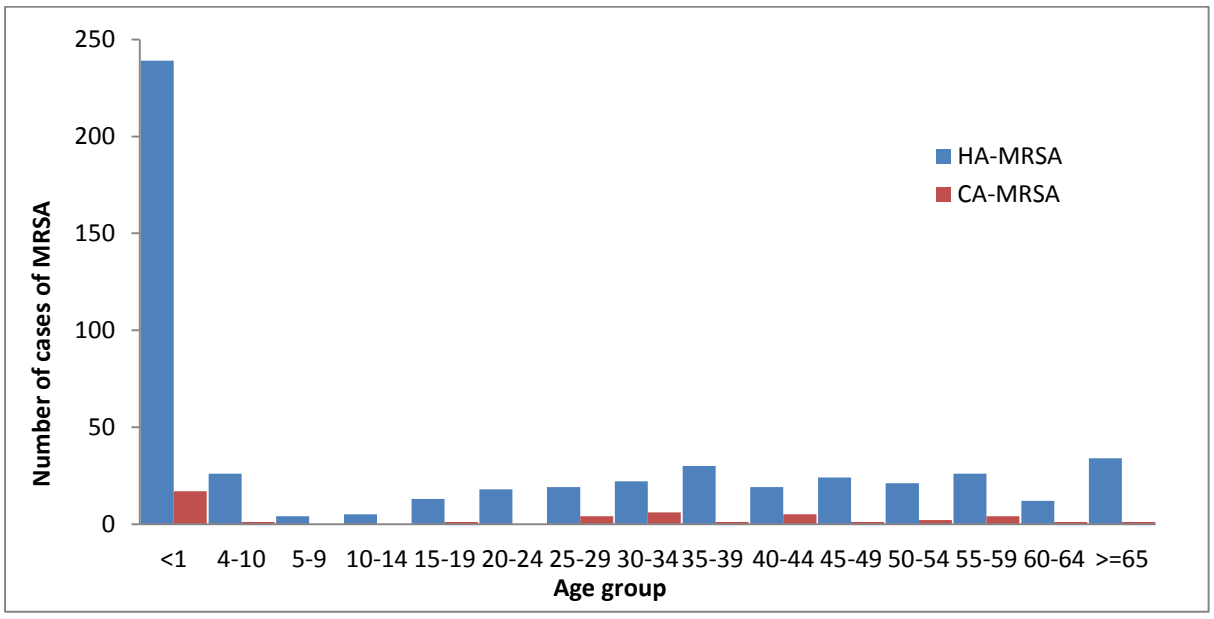

Figure 2: Distribution of cases of methicillin-resistant *Staphylococcus aureus* (MRSA) bacteraemia by age group at five sentinel hospitals, South Africa, January 2013 to January 2016. HA-MRSA = Hospital-associated methicillin-resistant *Staphylococcus aureus*; CA-MRSA = Community-associated methicillin-resistant *Staphylococcus aureus*

Antimicrobial susceptibility results for all S. aureus isolates

All *S. aureus* blood culture isolates demonstrated significantly reduced susceptibility to the following classes of antimicrobial agents: β- lactams, macrolides, tetracyclines, aminoglycosides, and cotrimoxazole, in 2015 compared to 2013 ($p < 0.05$) but not compared to 2013 (Figure 3). The MIC_{50} and MIC_{90} values of all antimicrobials were unchanged over the three-year period (Table 2). Figure 4 compares susceptibilities to antimicrobial agents and these were not significantly different for HA-MRSA versus CA-MRSA isolates, except for a higher proportion of HA-MRSA isolates resistant to rifampicin. We recorded 72 isolates with vancomycin MIC of 2; 17 patients died including 10 with MRSA and 48 recovered including 38 patients with MRSA; majority had SCC*mec* type III.

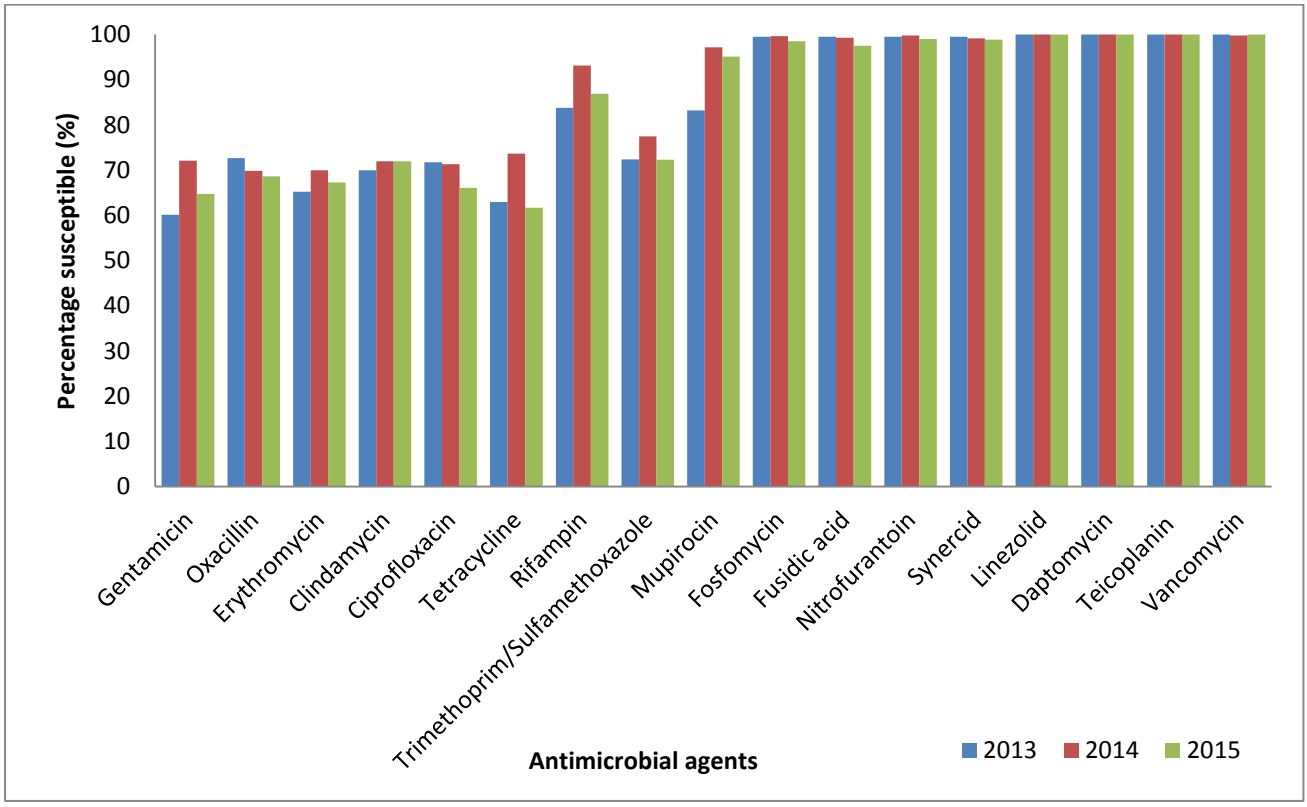

Figure 3: Antimicrobial susceptibility testing results for 1623 isolates from surveillance sites for 2013 to 2015

Table 2: MIC_{50} and MIC_{90} on 1623 *S. aureus* isolates from surveillance sites

Antibiotics	2013		2014		2015	
	MIC50	MIC90	MIC50	MIC90	MIC50	MIC90
Gentamicin	≤4	>8	≤1	8	≤1	>8
Oxacillin	≤0.25	≤0.25	≤0.25	≤0.25	≤0.25	≤0.25
Erythromycin	≤0.5	>4	≤0.5	>4	≤0.5	>4
Clindamycin	≤0.25	≤0.25	≤0.25	≤0.25	≤0.25	≤0.25
Ciprofloxacin	≤0.5	>2	≤0.5	>2	≤0.5	>2
Tetracycline	≤4	>8	≤4	>8	≤4	>8
Rifampin	≤1	>2	≤0.5	≤1	≤0.5	>2
Trimethoprim/Sulfamethoxazole	≤2/38	>4/76	≤2/38	>4/76	≤2/38	>4/76
Mupirocin	≤256	≤256	≤256	≤256	≤256	≤256
Fosfomycin	≤32	≤32	≤32	≤32	≤32	≤32
Fusidic acid	≤2	≤2	≤2	≤2	≤2	≤2
Nitrofurantoin	≤32	≤32	≤32	≤32	≤32	≤32
Quinupristin-dalfopristin	≤1	≤1	≤1	≤1	≤1	≤1
Linezolid	≤2	≤2	≤2	≤2	≤2	≤2
Daptomycin	≤1	≤1	≤1	≤1	≤1	≤1
Teicoplanin	≤1	≤1	≤1	≤1	≤1	≤1
Vancomycin	1	1	1	1	1	1

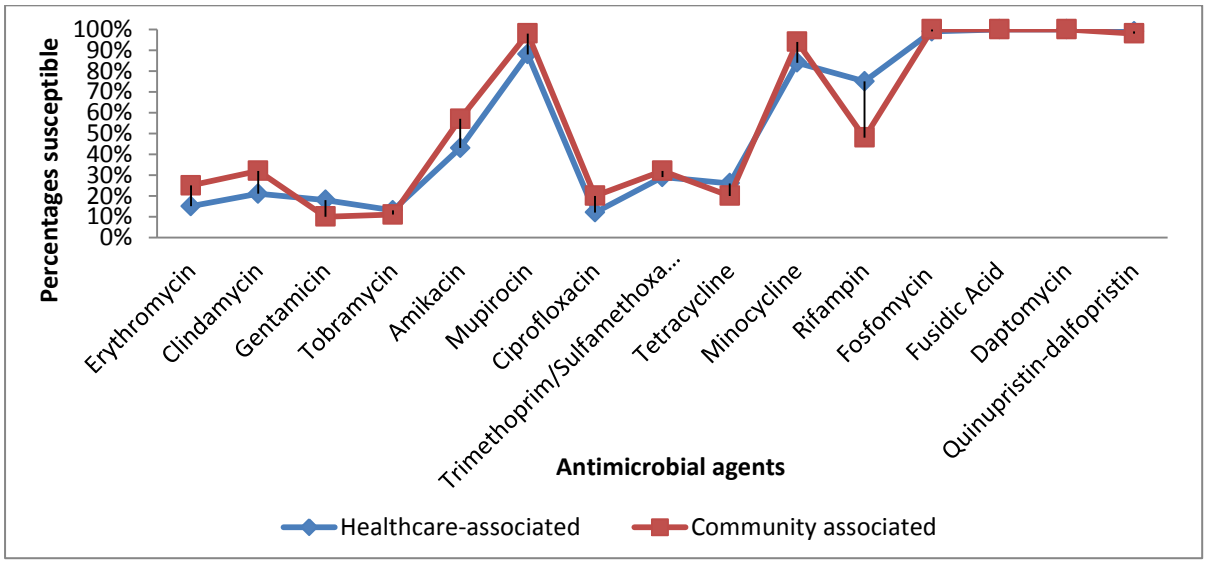

Figure 4: Antimicrobial susceptibility patterns of 557 MRSA isolates stratified by hospital or community origin

PCR screening for mecA and mecC in MRSA isolates

We analysed the available 484 isolates resistant to oxacillin and confirmed *mec*A in 483, except one MRSA isolate was negative for both *mecA* and *mecC*.

SCCmec typing

Of the 557 MRSA isolates, 484 (87%) were typed: the most common SCC*mec* type was SCC*mec* type III (n=236, 48.76%) followed by types IV (n=144, 29.76%), II (n=42, 8.68%), VI (n=4, 0.83%), V (n=3, 0.62%) and type I (n=1, 0.21%); the rest of isolates could not be typed (Figure 5. Unknown typing patterns were identified for 52 isolates (10.74%). Multiple banding patterns were observed and require further investigation. Two isolates produced no amplicons and therefore no resultant SCC*mec* type. Overall, SCC*mec* typing results showed a difference between the two provinces; a predominance of type III (n=206/484, 43%) was observed in Gauteng and a predominance of type IV (n=77/484, 16%) was seen in the Western Cape. Among the type IV isolates, the majority were of hospital origin unexpectedly and showed the similar susceptibility to classes of antibiotics other than the β-lactams (Figures 4 and 5).

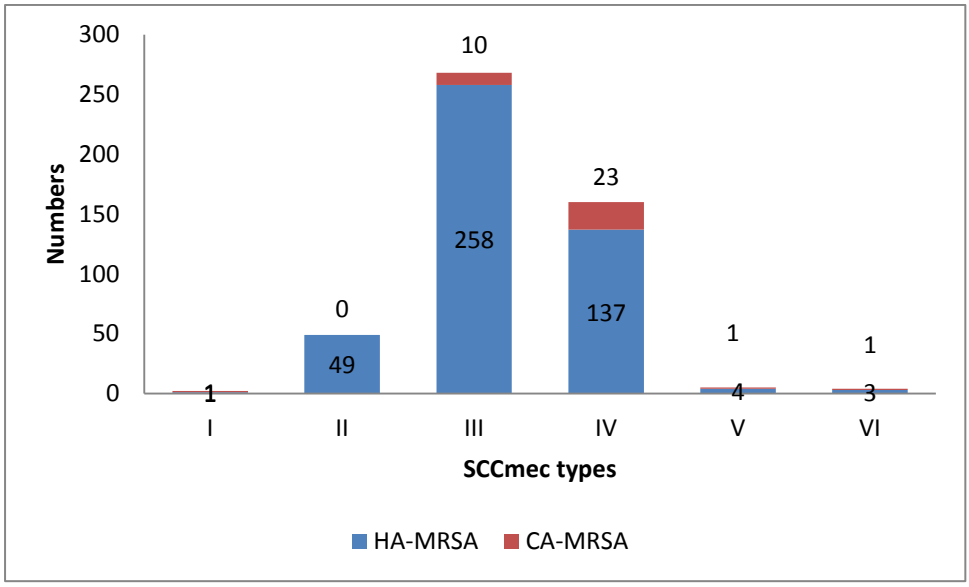

Figure 5: Distribution of 484 SCC*mec* types among HA and CA MRSA

Spa-typing

Spa-typing of 484 oxacillin resistant isolates revealed 49 different *spa*-types, 15 of which were novel and have not as yet been assigned. Two isolates could not be typed. The five most common *spa*-types were t037 (n=229, 47.31%), t1257 (n=90, 18.60%), t045 (n=44, 9.09%), t012 (n=32, 6.61%) and t064 (n=10, 2.07%) which accounted for 84% of the isolates tested. The majority (n=214, 44.21%) of the SCC*mec* type III isolates belonged to *spa*-type t037; the majority (n=80, 16.53%) of the SCC*mec* type IV isolates belonged to *spa*-type t1257 and the majority (n=27, 5.58%) of the SCC*mec* type II isolates belonged to *spa*-type t012. The majority (n=35, 7.23%) of

the unknown SCC*mec* type isolates belonged to *spa*-type t045. SCC*mec* types I, V and VI accounted for only a few isolates. *Spa*-CC-064 (17% of all *spa*-types) was the largest clonal complex followed by *spa*-CC-012 (15% of all *spa*-types) and *spa*-CC-032/022 (9% of all *spa*-types). *Spa*-CC-064 contained isolates displaying predominantly the SCC*mec* type IV element. *Spa*-CC-037 contained isolates that displayed predominantly SCC*mec* types III as well as types II, IV and unknown typing patterns (Table 4). *Spa*-CC-064 and *spa*-CC-037 were identified in both Gauteng and the Western Cape (6, 7). Table 3 shows the six most common *spa*-types and correlating SCC*mec* types for hospital- and community-associated infections. All six *spa*-types and all represented SCC*mec* types were observed for hospital-associated infections. For community-associated infections, SCC*mec* type III-*spa*-type t037 was observed for nine isolates; SCC*mec* type IV-*spa*-type t1257 was observed in 13 isolates and SCC*mec* type IV-*spa*-type t064 and -t032 was observed in one isolate each.

Table 3: SCC*mec* types and *spa* types observed for Hospital-associated and Community-associated Infections

	II				III					IV					V	
	t037	t1257	t012	t064	t037	t1257	t012	t045	t032	t037	t1257	t012	t064	t032	t045	Total
HA	4	2	28	1	227	6	3	7	1	8	71	1	8	7	3	377
CA					9						13		1	1		24

Those representing the 6 most common spa types overall are included in this table. Untypable SCCmec types were excluded from this analysis.
HA: Hospital-associated infection
CA: Community-associated infection

Multilocus sequence typing (MLST)

The most common STs observed were ST239 and ST612 both belonging to the clonal complex 8 (CC8) (n=8 each followed by ST612 (CC8) (n=5, ST36 (CC30) and ST5 (CC5) (n=4 each) and ST22 (CC22) (n=3) One isolate produced a novel ST (ST4121). The allelic profile was very similar to 13 existing STs on the MLST database but differed by one allele (*aroe*). The 13 STs included the following: ST22, 44, 854, 927, 928, 970, 1037, 1645, 2037, 2892, 2893, 2894, 3211. The isolates and their corresponding SCC*mec* types, *spa*-types, sequence types (ST) and clonal complexes (CC) can be seen in Table 4.

Table 4: Molecular characterisation of 28 MRSA isolates

Sample ID	SCC*mec* type	*Spa*-type	ST	CC	Type of infection	Year
7775	IV	t064	612	8	HA	2013
8307	II	t012	36	30	HA	2014
8538	IV	t012	22	22	HA	2014
8544	III	t012	612	8	HA	2014
8204	Unknown	t037	239	8	HA	2014
8203	III	t037	239	8	HA	2014
8371	IV	t037	239	8	HA	2014
8401	II	t037	36	30	HA	2014
8366	III	t045	36	30	HA	2014
8325	II	t064	36	30	HA	2014
8451	IV	t064	612	8	HA	2014
8534	IV	t064	612	8	HA	2014
8362	II	t1257	239	8	HA	2014
8398	III	t1257	239	8	HA	2014
8462	IV	t1257	612	8	HA	2014
9518	III	t012	239	8	HA	2015
9843	IV	t032	22	22	HA	2015
9210	IV	t032	4121	Not assigned	HA	2015
9192	V	t045	5	5	HA	2015
9335	V	t045	5	5	HA	2015
9336	Unknown	t045	5	5	HA	2015
9509	Unknown	t1257	5	5	HA	2015
7730	IV	t064	612	8	CA	2013
8243	III	t037	239	8	CA	2014
9588	IV	t032	22	22	CA	2015
8853	III	t037	239	8	CA	2015
9018	IV	t1257	612	8	CA	2015
9316	IV	t1257	612	8	CA	2015

HA: Hospital-associated infection
CA: Community-associated infection
* novel ST. ST similar to the following 13 STs on the MLST database (http://saureus.beta.mlst.net/), however the *aroe* allele differed: ST22, 44, 854, 927, 928, 970, 1037, 1645, 2037, 2892, 2893, 2894, 3211. The CC is not assigned.

Predisposing factors and clinical outcome

Among all patients with MRSA infection, HIV-infected patients were three times more likely to have CA-MRSA than HA-MRSA, after adjustment for age and sex (aOR: 3.3; 95% CI: 1.1-9.3). Fever (body temperature ≥37.5^{0}C) at diagnosis of bacteraemia was associated with HA-MRSA (aOR: 0.2; 95% CI: 0.05-0.7) rather than CA-MRSA, while other underlying conditions such as diabetes mellitus and kidney disease were not associated with CA-MRSA (Table 4). Crude in-hospital mortality was 41.2% among 490 MRSA cases with known clinical outcome. Seventeen of 44 cases with CA-MRSA died (38.6%), compared to 41.5% of cases with HA-MRSA (185/446).

There was no clear association between the type of MRSA infection (CA-MRSA or HA-MRSA) and clinical outcome. However, increasing age, having suffered cardiac arrest on the day of specimen collection (aOR: 23.03; 95% CI: 2.88-183.93), being mechanically ventilated at the time of specimen collection (aOR: 1.89; 95% CI: 1.21-2.95), and prior MRSA infection (aOR: 3.44; 95% CI: 1.38-8.53) were significantly associated with mortality among patients with MRSA infection (Table 5).

Table 5: Univariate and multivariable logistic regression analysis of predisposing factors associated with CA-MRSA and HA-MRSA bacteraemia

Characteristics	**CA-MRSA (n=44) n/N (%)**	**HA-MRSA (n=513) n/N (%)**	**Univariate analysis**		**Multivariable analysis**	
			OR (95% CI)	**p-value**	**aOR (95% CI)**	**p-value**
Sex						
Male	25/44 (56.8)	310/511 (60.7)	reference		reference	
Female	19/44 (43.2)	201/511 (39.3)	1.17 (0.6-2.2)	0.617	1.3 (0.4-3.6)	0.605
Age						
Median age in years (IQR)	26.5 (0.09-42.8)	2.4 (0.05-42.3)	1.0 (0.99-1.01)	0.414	1.0 (0.9-1.0)	0.409
Province						
Gauteng	28/44 (63.6)	308/513 (60.0)	reference			
Western Cape	16/44 (36.4)	205/513 (40.)	0.85 (0.4-1.63)	0.64		
Clinical syndrome*						
Bacteraemia	28/44 (63.6)	291/462 (63.0)	reference			
Joint infection	0/44 (0)	6/462 (1.3)	1	-		
Pneumonia	10/44 (22.7)	69/462 (14.9)	1.5 (0.6-3.3)	0.296		
Meningitis	2/44 (4.6)	4/462 (0.9)	5.2 (0.9-29.7)	0.064		
Skin or soft tissue infection	1/44 (2.3)	73/462 (15.8)	0.1 (0.01-1.1)	0.057		
Other	3/44 (6.8)	19/462 (4.1)	1.6 (0.4-5.9)	0.447		
Clinical signs						
Fever at diagnosis (temperature ≥37.5°C)						
No	33/43 (76.7)	250/416 (60.1)	reference		reference	
Yes	10/43 (23.3)	166/416 (39.9)	**0.46 (0.21-0.96)**	**0.036**	**0.19 (0.05-0.70)**	**0.012**
Mental status/ GCS						
GCS 15 / Alert	16/23 (69.6)	119/153 (77.8)	reference			
GCS 13-14 / Disorientated	4/23 (17.4)	18/153 (11.8)	1.7 (0.4-5.6)	0.413		
GCS 9-12 / Stuporous	1/23 (4.4)	8/153 (5.2)	0.93 (0.10-7.93)	0.947		
GCS 3-8 / Coma	2/23 (8.7)	8/153 (5.2)	1.9 (0.3-9.6)	0.457		
Predisposing factors						
Participation in contact sports						
No	42/42 (100)	445/451 (98.7)	reference			
Yes	0/42 (0)	6/451 (1.3)	1	-		
Living or working in crowded facilities						

No	40/42 (95.2)	427/449 (95.1)	reference			
Yes	2/42 (4.8)	22/449 (4.9)	0.97 (0.22-4.28)	0.968		
Prior MRSA infection						
No	43/44 (97.7)	425/452 (94.0)	reference			
Yes	1/44 (2.3)	27/452 (6.0)	0.37 (0.04-2.77)	0.33		
Prior dialysis						
No	44/44 (100)	443/459 (96.5)	reference			
Yes	0/44 (0)	16/459 (3.5)	1	-		
Resident in a long-term care facility						
No	42/42 (100)	433/455 (95.2)	reference			
Yes	0/42 (0)	22/455 (4.8)	1	-		
Pre-existing conditions§						
HIV infection						
No	9/19 (47.4)	193/258 (74.8)	reference		reference	
Yes	10/19 (52.6)	65/258 (25.2)	**3.3 (1.2-8.5)**	**0.013**	**3.3 (1.1-9.3)**	**0.026**
Kidney disease						
No	22/28 (78.6)	385/416 (92.6)	reference			
Yes	6/28 (21.4)	31/416 (7.5)	**3.4 (1.2-9.0)**	**0.014**		
Diabetes mellitus						
No	23/28 (82.1)	386/416 (92.8)	reference			
Yes	5/28 (17.9)	30/416 (7.2)	**2.8 (1.0-7.8)**	**0.052**		

∞GCS-Glasgow Coma Score

Note: An odds ratio (OR) >1 suggests an association with CA-MRSA, while an OR<1 suggests an association with HA-MRSA. Denominators differ due to missing data for certain variables

Table 6: Univariate and multivariable logistic regression analysis of factors associated with mortality among patients with CA-MRSA and HA-MRSA bacteraemia.

	Died (n=202)	**Univariate analysis**		**Multivariable analysis**	
Characteristics	**n/N (%)**	**OR (95% CI)**	**p-value**	**aOR (95% CI)**	**p-value**
MRSA type					
HA-MRSA	185/446 (41.5)	reference		reference	
CA-MRSA	17/44 (38.6)	0.89 (0.47-1.68)	0.715	0.86 (0.42-1.73)	0.661
Sex					
Male	118/290 (40.7)	reference			
Female	83/199 (41.7)	1.04 (0.72-1.50)	0.822		
Age group (years)					
<1	72/230 (31.3)	reference		reference	
1-9	9/28 (32.1)	1.04 (0.44-2.41)	0.928	0.88 (0.33-2.33)	0.793
10-19	6/13 (46.2)	1.88 (0.61-5.80)	0.271	2.1 (0.66-6.60)	0.204
20-29	11/34 (32.4)	1.05 (0.48-2.27)	0.902	1.09 (0.48-2.46)	0.833
30-39	25/53 (47.2)	**1.96 (1.06-3.60)**	**0.03**	**1.97 (1.02-3.81)**	**0.043**
40-49	19/37 (51.4)	**2.32 (1.14-4.68)**	**0.019**	**2.53 (1.21-5.29)**	**0.013**
50-59	29/49 (59.2)	**3.18 (1.68-6.0)**	**<0.001**	**3.14 (1.61-6.13)**	**0.001**
≥60	30/45 (66.7)	**4.39 (2.22-8.66)**	**<0.001**	**5.15 (2.50-10.60)**	**<0.001**
Province					

Gauteng	125/297 (42.1)	reference			
Western Cape	77/193 (39.9)	0.91 (0.63-1.32)	0.63		
Clinical diagnosis					
Bacteraemia	126/311 (40.5)	reference			
Joint infection	1/6 (16.7)	0.29 (0.03-2.55)	0.266		
Pneumonia	34/78 (43.6)	1.13 (0.68-1.88)	0.622		
Meningitis	3/5 (60)	2.20 (0.36-13.37)	0.391		
Skin or soft tissue infection	26/68 (38.2)	0.91 (0.53-1.56)	0.728		
Other	12/21 (57.1)	1.96 (0.80-4.79)	0.141		
Cardiac arrest					
No	185/472 (39.2)	reference		reference	
Yes	13/14 (92.9)	**20.17 (2.61-155.47)**	**0.004**	**23.03 (2.88-183.93)**	**0.003**
Mechanical ventilation					
No	134/352 (38.1)	reference		reference	
Yes	64.131 (48.9)	**1.55 (1.03-2.33)**	**0.033**	**1.89 (1.21-2.95)**	**0.005**
Clinical signs					
Mental status/ GCS					
GCS 15 / Alert	52/132 (39.4)	reference			
GCS 13-14 / Disorientated	12/21 (57.1)	**2.1 (0.80-5.22)**	**0.131**		
GCS 9-12 / Stuporous	5/9 (55.6)	1.92 (0.49-7.50)	0.346		
GCS 3-8 / Coma	7/10 (70)	**3.59 (0.88-14.52)**	**0.073**		
Temperature at diagnosis (°C) (median, IQR)	37 (36.5-37.9)	0.96 (0.81-1.14)	0.614		
Predisposing factors					
Prior MRSA infection					
No	178/455 (39.1)	reference		reference	
Yes	17/25 (68.0)	**3.31 (1.39-7.83)**	**0.006**	**3.44 (1.38-8.53)**	**0.008**
Prior dialysis					
No	191/470 (40.6)	reference			
Yes	7/15 (46.7)	1.28 (0.45-3.59)	0.641		
Prior surgery					
No	139/356 (39.0)	reference			
Yes	59/129 (45.7)	**1.32 (0.87-1.98)**	**0.186**		
Resident in a long-term care facility					
No	181/459 (39.4)	reference			
Yes	13/21 (61.9)	**2.50 (1.01-6.14)**	**0.046**		
Pre-existing conditions					
No	30/60 (50)	reference			
Yes	172/429 (40.1)	**0.67 (0.38-1.15)**	**0.146**		
HIV infection					
No	79/196 (40.3)	reference			
Yes	33/70 (47.1)	1.3 (0.7-2.3)	0.321		

Note: denominators differ due to missing data for certain variables

Discussion

To our knowledge, this is one of the largest epidemiological studies to describe *S. aureus* bacteraemia in South Africa; we analysed data from 1914 patients and compared cases of HA-MRSA and CA-MRSA infection. Overall, among cases of *S. aureus* bacteraemia, more than a quarter were due to MRSA, of which only 8% were categorised as CA-MRSA. This prevalence estimate is much lower than reported from studies in the United States, where the prevalence of CA-MRSA varied from 35% to 80% and suggests that the epidemiology of MRSA infection varies by region (5). Our findings might also differ from other regions due to different criteria used to define cases of CA-MRSA infection. In contrast to a recent review by Tong et al. and reports from sub-Saharan Africa and North America of cases among adults, most cases of bloodstream MRSA infection in South Africa were diagnosed among infants and young children in Gauteng province (42%) but not in Western Cape (10%) perhaps due to overcrowding in paediatric wards (1, 5, 6).

The ratio of MRSA to MSSA cases was stable over the study period. The prevalence of *S. aureus* bacteraemia over the three-year study period was also largely unchanged. This is different to the trend observed in the USA (2). In this study, we also showed no evidence of replacement of HA-MRSA strains with CA-MRSA, unlike in HICs, where a majority of MRSA infections now originate in the community (5). In the last few decades, the incidence of CA-MRSA has reportedly increased in HICs, while in contrast, South Africa's epidemiology with a high proportion of HA-MRSA infection is similar to that reported from African region, study from Soweto and southern Europe (14,15). These variations may be explained by factors such as risk profile of hospitalised patients. We found no difference in the epidemiology of CA-MRSA in two provinces with relatively well-resourced healthcare systems.

Interestingly, we found a dominance of SCC*mec* types III in Gauteng and IV in the Western Cape. Type III in Gauteng province was associated mostly with *spa* type t037 and type IV with the *spa* type t1257. These were previously reported as dominant in these provinces (6). The *spa*-type CC 012 in these two provinces was similar to a study from Tygerberg Academic Hospital (16). When the six most common *spa*-types (t037, t1257, t012, t045, t064 and t032) and SCC*mec* types were correlated, SCC*mec* types II, III, IV and V and all six *spa*-types were linked to hospital-associated infections. SCC*mec* types III and IV and *spa*-types t037, t1257, t064 and t032 were observed in community-associated infections. It should be noted that the majority of our isolates were regarded as hospital-associated infections (n=513) according to the definition used. The remaining 44 isolates were community-associated infections. Regarding *spa*-types, we confirmed that the majority of t037 (47%) and t1257 (17%), belonged mostly to the MLST CC8 clonal complex unlikely in Breurec study where CC8 was the

sporadic clone and majority belong to CC239 related to Brazilian/Hungarian clone (17). This was observed in isolates belonging to both HA- and CA-infections. Of the six isolates that were *spa*-type t037, one isolate was ST36 (CC30) as expected and the remaining five isolates belonged to ST239 (CC8). The latter is an unusual occurrence which may be supported by a previous 2016 study which has shown that genetic exchange and recombination can occur resulting in isolates exhibiting *spa*-types which are usually known from another core genome as indicated by MLST. (18,19). One isolate produced a novel ST. The allelic profile for *aroe* differed when compared to all other existing MLST profiles and a new ST was assigned. The commonest sequence types identified in this MRSA study were ST5, ST36, ST612, ST239, with a predominance of SCC*mec* types III and IV. This is similar to the other parts of the world, such as Europe and Australia. Similar to other African countries, ST239 related to Brazilian/Hungarian clone was common in our hospital settings (17,19). There was no difference in molecular characteristics between the hospital- and community-associated infection groups. Furthermore, no evolution of clonal types was observed from 2013 to 2015.

HIV-infected persons were more likely to have CA-MRSA than HA-MRSA. This association might be explained by overall increase in this group of incidence of *S. aureus* bacteraemia (1,20). In addition, fever at diagnosis was associated with HA-MRSA. These predisposing factors could potentially be used to construct a clinical algorithm for predicting whether a patient has CA-MRSA or HA-MRSA infection. In this study we demonstrated that SCC*mec* type IV MRSA infections were common among patients in healthcare facilities which makes it difficult to establish clear and objective differences between CA-MRSA and HA-MRSA strains (2). The high crude in-hospital mortality of patients with both CA-MRSA and HA-MRSA infection was not a surprising finding and risk factors for mortality identified in the multivariable analysis such as old age, heart conditions, patients on mechanical ventilation, previous MRSA infections, patients in long term care facilities are in keeping with previous studies (20,21, 22). No outcome difference was found between CA-MRSA versus HA-MRSA infections in our study like indicated in previous research where NICU patient characteristics were similar (23). Regarding susceptibility pattern over the study period *S. aureus* did not reveal significant increase in antimicrobial resistance. There were non-significant differences of susceptibility to other antimicrobial agents between CA- and HA-MRSA. CA-MRSA isolates showed more susceptibility to erythromycin and clindamycin but not rifampin which may be related to use for treatment and prevention of tuberculosis (15). This finding is in keeping with reports of community-associated infections from the USA and Australia showing that CA-MRSA isolates were more susceptible to macrolides, aminoglycosides and fluoroquinolones (2). A study from Portugal showed a

decrease in multidrug-resistance profile in MRSA over a 20-year period (15) and a meta-analysis by Matthew and Falagas et.al. (4) showed varied susceptibility to various antibiotics. In hospitalized South African paediatric patients with bacteraemia high prevalence of MRSA was found in HIV-infected children (20). We found no resistance to the following classes of antibiotics: glycopeptides, oxazolidines, quinupristin/dalfopristin and daptomycin in both groups.

Our study has a number of limitations. Firstly, the presence of SCC*mec* types IV, V and VI, as well as a non-multidrug resistance (non-MDR) antimicrobial phenotype (isolates not resistant to two or more classes of non-β-lactam antibiotics) were considered for inclusion in the case definition. However, these criteria were not included in the final definition as the number of cases with all four criteria was too low. This may have resulted in misclassification of CA-MRSA cases as HA-MRSA. Secondly, the majority of our patients had bacteraemia with no origin, perhaps due to incomplete clinical data to indicate origin of bacteraemia. Another limitation of our study was the inclusion of only academic centres in urban areas. Therefore, the results may not be representative of all healthcare facilities in South Africa (24).

Conclusions

In conclusion, this study demonstrates that *S. aureus* bacteraemia is common in South African academic centres and HA-MRSA is still the dominant cause of MRSA bacteraemia. A small proportion of CA-MRSA cases were caused by a few sequence types. The dominance of SCC*mec* type III existed however presence of SCC*mec* type IV have become evident that this replacement is emerging in hospitals. We found no resistance to glycopeptides, oxazolidines, quinupristin/dalfopristin and daptomycin in both MSSA and MRSA. Our resistance profile of CA-MRSA isolates suggests that the macrolide group of agents should be used with caution or should be directed by results from susceptibility testing for treatment of localized infections. We recommend continuous surveillance of *S. aureus* bacteraemia to monitor trends of MRSA.

Conflict of Interest: There is no conflict of interest from authors on this manuscript as no external funding was received for the surveillance of *Staphylococcus aureus* bacteraemia.

Ethics approval: This study was approved by the University of the Witwatersrand Human Research Ethics Committee (Medical),); clearance certificate number: M160667.

Informed consent: Informed Consent Form (ICF) was given to each patient enrolled in the study with description of the procedure, confidentiality and declaration with signatures of participant, surveillance officer and witnesses.

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