



The association between pathogen factors and clinical outcomes in patients with *Staphylococcus aureus* bacteraemia in a tertiary hospital, Cape Town

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ABSTRACT

Background: *Staphylococcus aureus* is a serious pathogen, able to cause life-threatening infections such as bacteraemia. The association between *S. aureus* microbial characteristics and clinical outcomes is under-investigated in African settings. This study aimed to determine the molecular epidemiology and virulence characteristics of *S. aureus* isolates from bacteraemic patients at Tygerberg Hospital, South Africa, and to investigate the associations between pathogen characteristics and clinical outcomes.

Methods: This study included 199 *S. aureus* isolates collected from blood cultures between February 2015 and March 2017. Methicillin resistance was determined using disc diffusion and all resistant isolates were further characterized by staphylococcal cassette chromosome *mec* (*SCCmec*) typing. Genotyping was done using *spa* and *agr* typing, and *agr* functionality was assessed using the phenotypic δ -haemolysin assay. Logistic regression models were performed to describe the associations between strain characteristics and the clinical outcomes methicillin resistance, in-hospital mortality, and length of stay (LOS).

Results: Of the 199 *S. aureus* isolates collected, 27% were MRSA, and the overall crude in-hospital mortality rate was 29%. Seventy-three different *spa* types were identified, including seven new types. Agr I was the most common type, in 99 (49.7%) isolates, followed by agr II, III, and IV in 57 (28.6%), 37 (18.6%), and six (3%) isolates, respectively. Agr dysfunctionality was observed in 25 (13%) isolates, mostly belonging to *spa*-clonal complex (CC) 012. Methicillin resistance was significantly associated with hospital-acquired infection (odds ratio (OR) 4.77, 95% confidence interval (CI) 2.09–10.87). A significant increase in mortality was observed with increasing age (OR 7.48, 95% CI 2.82–19.8) and having a hospital-acquired infection (OR 2.26, 95% CI 1.12–4.55). *S. aureus* strains with a functional *agr* system showed an association with longer duration of stay (OR 1.66, 95% CI 0.93–2.99).

Conclusions: We report the lowest MRSA prevalence at Tygerberg Hospital for the past 10 years, and *agr* dysfunctionality was shown to be driven by a certain genotype, *spa*-CC012. Despite the limited available clinical data, the study provided insights into associations between *S. aureus* epidemiology and *agr*-related virulence characteristics, and clinical outcomes.

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Introduction

Staphylococcus aureus is an opportunistic pathogen responsible for a wide range of superficial as well as systemic toxin-associated

infections, such as abscesses and tissue invasion. It is a leading cause of hospital-acquired (HA), healthcare-associated (HCA), and community-acquired (CA) infections (Kaech et al., 2006; Perovic et al., 2015). Invasive *S. aureus* infections can manifest in many different ways, the most common manifestation being bacteraemia (Turnidge et al., 2009). More than 80% of *S. aureus* bacteraemia is endogenous in origin, which could be a result of the high carriage rates among individuals (Kaech et al., 2006; von Eiff et al., 2001). Bacteraemia caused by methicillin-resistant *S. aureus* (MRSA) is associated with high mortality rates due to their resistance to a wide range of antibiotics, including the last resort agents, creating a therapeutic challenge (Bassetti et al., 2017).

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The accessory gene regulator (*agr*) system regulates the expression of several virulence factors that contribute to *S. aureus* infections, especially those that are toxin-related. Four different *agr* types, I–IV, have been described based on sequence variation within the hypervariable region of the *agr* locus (*agrB*, *agrC*, *agrD*) (George and Muir, 2007). Some *S. aureus* strains have a dysfunctional *agr* system due to genetic changes within the locus, and in some cases, dysfunctionality may develop in vivo during infection (Gagnaire et al., 2012). *Agr* dysfunctionality results in failure to express the effector molecule RNAlII, which plays a critical role in the downstream regulatory process, influencing the production of virulence factors involved in invasive diseases (George and Muir, 2007). Previous studies have reported that *agr* types could be predictors of certain disease manifestations and clinical outcomes (Lim et al., 2013; Yarwood and Schlievert, 2003). For example, *agr* type I strains are more prevalent in HA infections and are associated with resistance to glycopeptides (Robinson et al., 2005; Sakoulas et al., 2002). *Agr* types III and IV have been associated with toxic shock syndrome and staphylococcal scalded skin syndrome, respectively (Robinson et al., 2005). It is therefore essential to perform strain typing and virulence profiling to track and manage the spread of infection (Earls et al., 2017). *S. aureus* strains with a dysfunctional *agr* system have shown a fitness advantage over functional strains, reflected in their ease of transmission and persistence at the site of infection (Chong et al., 2013; Sakoulas et al., 2009). *Agr* dysfunctional strains have also been linked to deleterious outcomes such as persistent bacteraemia, reduced susceptibility to vancomycin, and increased mortality (Fowler et al., 2004; Sakoulas et al., 2006; Schweizer et al., 2011).

In South Africa, limited data are available on the molecular epidemiology and virulence factors of *S. aureus* strains involved in *S. aureus* bacteraemia, and there is a lack of studies associating these microbial factors with clinical outcomes. Therefore, this study was performed to describe the *S. aureus* strains circulating at Tygerberg Hospital and to investigate associations between bacterial genetic background and virulence determinants and methicillin resistance, mortality, and length of stay (LOS) among bacteraemic patients.

Methods

This was a prospective study that included patients with laboratory-confirmed *S. aureus* blood stream infections at Tygerberg Hospital, South Africa between January 2015 and March 2017. Tygerberg Hospital is a 1384-bed tertiary academic hospital serving a population of approximately 1.9 million in the Western Cape, South Africa. Clinical and demographic data of patients included in the study were collected as part of the routine surveillance conducted by the Group for Enteric, Respiratory and Meningeal Disease Surveillance in South Africa (GERMS-SA).

Bacterial identification

Blood cultures were submitted to the National Health Laboratory Service (NHLS) microbiology laboratory at Tygerberg Hospital at the discretion of attending clinicians, and as part of the diagnostic investigation of patients presenting to the hospital. *S. aureus* was identified from positive blood cultures using standard microbiological methods: Gram morphology, catalase, mannitol fermentation, and DNase activity. Isolates with discrepant or inconclusive results were further identified using the Vitek 2 Advanced Expert System (AES; bioMérieux, France). Methicillin resistance was determined using cefoxitin disc diffusion, according to Clinical and Laboratory Standards Institute (CLSI) standards

(CLSI, 2015), and was confirmed using the Vitek 2 AES (bioMérieux, France). Isolates were stored at -80°C .

Molecular characterization

Genomic DNA was extracted following an in-house heat lysis protocol. All *S. aureus* isolates were genotyped using *spa*-typing targeting the variable region of the *spa* gene (Harmsen et al., 2003). Sequence analysis was performed using the Ridom StaphType software (Munster, Germany), and allocation of the *spa* clonal complexes (*spa*-CC) used the 'based upon repeat pattern' (BURP) algorithm that is implemented within the software (Harmsen et al., 2003; Strommenger et al., 2006). All MRSA were subjected to staphylococcal cassette chromosome *mec* (SCC*mec*) typing using a multiplex PCR (Milheirico et al., 2007). At least one isolate from the eight major *spa*-CC was selected for further characterization using multilocus sequence typing (MLST), as described previously (Enright et al., 2000).

Agr typing and functionality

The *agr* type was determined by multiplex PCR, as described previously (Lina et al., 2003). Functionality of the *agr* system was assessed using the phenotypic synergistic *agr* functionality assay (Sakoulas et al., 2002). Briefly, the test isolates were streaked out perpendicular to the β -haemolytic control strain *S. aureus* RN4220 on sheep blood agar plates (Green Point Media Lab, South Africa). *Agr* functionality was assigned based on the presence of δ -haemolysis detected as enhanced or complete haemolysis within the β -haemolysis zone of *S. aureus* RN4220. The strains NRS155 and NRS149 were used as *agr* dysfunctional and functional controls, respectively.

Clinical outcomes and definitions of variables

HA infections were defined as positive blood cultures taken more than 3 days after hospital admission. HCA infections were defined as positive blood cultures taken within 3 days of admission, with one or more of the following risk factors present: hospitalization, surgery, prior dialysis, or residence in a long-term care facility, within the year prior to the current hospital admission. CA infections were defined as positive blood cultures taken at hospital admission or within 3 days of admission without any of the above-mentioned risk factors (Perovic et al., 2017). Bacteraemia cases with undetermined source were classified as bacteraemia without focus. The patient outcomes that were assessed included crude in-hospital mortality and LOS. LOS was determined using the admission and final outcome (death or discharge) dates.

Statistical analysis

Logistic regression models for categorical variables were used to assess associations between the final outcomes of mortality and methicillin resistance, and the other patient and isolate covariates, using both univariable and multivariable analyses. All variables with *p*-values of <0.1 in the univariable analysis were included in the logistic regression model using stepwise elimination. Since LOS was a continuous variable, the two-sample Wilcoxon rank sum (Mann–Whitney) test and Cox proportional hazards model were used to assess associations between LOS and the other variables. The statistical analysis was done using Stata version 15 (StataCorp. LLC, College Station, TX, USA), and *p*-values of <0.05 were interpreted as significant.

Table 1
Clinical characteristics.

Characteristics	Total
Length of stay in days, median (IQR)	24 (11.5–46)
Final outcome (n = 193)	
Died	58 (29.1%)
Age group (n = 199)	
Neonates (<28 days)	21 (10.6%)
Children (≥28 days – <14 years)	32 (16.1%)
Adults (≥14 year – <50 years)	96 (48.2%)
Elderly (≥50 years)	50 (25.1%)
Sex (n = 193)	
Male	113 (56.8%)
Diagnosis (n = 186)	
Bacteraemia with focus	57 (28.6%)
Bacteraemia without focus	129 (64.8%)
Source of organism (n = 190)	
HA	105 (52.8%)
HCA	46 (23.1%)
CA	39 (19.6%)

IQR, interquartile range; HA, hospital-acquired infection; HCA, healthcare-associated infection; CA, community-acquired infection.

Results

Patient characteristics and clinical outcomes

Between January 2015 and March 2017, 473 *S. aureus* bacteraemia cases were documented by GERMS-SA at Tygerberg Hospital. Of these, 199 non-duplicate *S. aureus* isolates were collected from the NHLS microbiology laboratory. Clinical profiles were missing for six of these 199 patients and were further excluded from the statistical analyses. Table 1 summarizes the participants' characteristics. The median age of the patients was 30 years (interquartile range (IQR) 8.5–50 years), with the majority of the patients (48%) being adults. The median LOS was 24 days (IQR 11.5–46 days) and the overall crude mortality rate was 29%. More than 50% of the infections were HA, and bacteraemia without focus was the main diagnosis reported in this study (Table 1).

Table 2
Molecular characterization of the 199 *Staphylococcus aureus* isolates.

<i>spa</i> clonal complex (CC)	<i>spa</i> types	Isolates (n)	MLST ST (CC)	Methicillin resistance
CC 002	t002^a , t045 ^a , t071, t242, t509, t1154, t5213, t15306	42	5 (5) NT (5)	Mixed
CC 012	t012^a , t018, t021, t037 ^a , t318 ^a , t399, t1848	34	30 (30) 239 (30) 1865 (30)	Mixed
CC 701/2360	t190, t701 , t1257, t1476 ^a , t1971, t2360 , t4315	23	8 (5)	Mixed
CC 032/578	t032^a , t578 , t891, t1036	13	22 (22)	Mixed
CC 015/073	t015^a , t073 , t116, t331, t1078, t2171	10	508 (45)	MSSA
CC 084	t084^a , t085, t346, t14791, <u>t18222</u>	10	15 (15)	MSSA
CC NF 174	t127 ^a , t174 , <u>t18225</u> , <u>t18227</u>	7	1 (5)	MSSA
CC NF 9	t148 ^a , t2409	4	72 (5)	Mixed
CC NF 5916	t1490, t5916 , <u>t18226</u>	3	ND	Mixed
CC NF 10	t317 ^a , t6712 ^a	3	NT	MSSA
CC NF 11	t1597, t11970	2	ND	MSSA
CC NF 12	t258, t349	2	ND	MSSA
Singletons	t008, t091, t189, t223, t267, t269, t272, t355, t888, t1467, t2442, t2526, t2763, t6267, t10509, <u>t18223</u> , <u>t18224</u> , <u>t18228</u>	32		Mixed
Excluded ^b	t026, t2304, t9909,	4		MSSA
Non-typeable	N/A	10		MSSA

MLST, multilocus sequence typing; ST, sequence type; NT, non-typeable by MLST; ND, not determined; MSSA, methicillin-susceptible *Staphylococcus aureus*. The founder *spa* types for each *spa*-CC are indicated in bold. New *spa* types are underlined.

^a *spa* types selected for MLST.

^b Excluded from the BURP clustering due to short number of repeats (<5 repeats).

Molecular epidemiology of *S. aureus* isolates from blood samples

The prevalence of MRSA in this cohort was 27.1% (n = 54). A total of 189 isolates (95%) were typeable by *spa*-typing, and 73 different *spa* types were identified, including seven new types (t18222–t18228) (Table 2). The BURP cluster analysis grouped the *spa* types into 12 *spa*-CCs (n = 153; 77%) and 32 singletons. Of the 12 *spa*-CCs identified, six contained only methicillin-susceptible *S. aureus* (MSSA) isolates (*spa*-CC 015/073, *spa*-CC 084, *spa*-CC 174, *spa*-CC NF10, *spa*-CC NF11, *spa*-CC NF12) and six (*spa*-CC002, *spa*-CC012, *spa*-CC701/2360, *spa*-CC032/578, *spa*-CC5916, *spa*-CCNF9) consisted of both MRSA and MSSA isolates. *spa* type t045 (*spa*-CC002) was the most abundant, accounting for 11% (n = 22) of the typed strains, most of which were methicillin-resistant (20/22, 91%). At least one isolate from each of the larger *spa*-CC was selected for further typing using MLST. The identified sequence types (STs) belonged to the most common MLST CCs, namely, 5, 15, 22, 30, and 45 (Table 2). SCCmec typing was done on all MRSA strains and only three isolates were non-typeable using the protocol of Milheiro et al. SCCmec type IV and a putative novel SCCmec variant (NV) were each identified in 19 (35.2%) isolates. The NV had a similar genetic structure to SCCmec type I, with an additional *ccrC* gene (unpublished data). SCCmec type III (n = 7; 13%) was the third most prevalent, followed by SCCmec type II (n = 6; 11.1%).

The MRSA clone t045-ST5-MRSA-NV accounted for 19 (35.2%) of the MRSA isolates, followed by t037-ST239-MRSA-III and t032-ST22-MRSA-IV, each accounting for seven (13%) MRSA isolates. Clones t1257-MRSA-IV and t012-ST30-MRSA-II accounted for five (9.3%) and four (7.4%) of the MRSA isolates, respectively. The dominant MSSA clone was t318-ST1865 (n = 14/154; 9.7%), followed by t002-ST5 (n = 13/154; 9%).

Agr typing and functionality

Agr typing was successful for all 199 isolates and *agr* I was the most common type, identified in 99 (49.7%) isolates, followed by *agr* II in 57 (28.6%) isolates and type III in 37 (18.6%) isolates. Only six isolates (3%) were identified as *agr* IV. No difference was observed in the distribution of *agr* types by methicillin resistance

Table 3
Associations between agr type and functionality status and both clinical and microbial characteristics.

Characteristic	Number	Agr functionality, n (%)		p-Value ^a	Agr types, n (%)				p-Value ^a
		Functional	Dysfunctional		I	II	III	IV	
Methicillin resistance	54	44 (81)	10 (19)	0.12	27 (50)	20 (37)	7 (13)	0	0.15
Mortality	58	51 (88)	7 (12)	0.89	34 (59)	16 (28)	7 (12)	1 (1)	0.30
Bacteraemia				0.08					0.34
With focus	57	54 (93)	3 (7)		32 (56)	12 (21)	12 (21)	1 (2)	
Without focus	129	111 (86)	18 (14)		59 (46)	43 (33)	23 (18)	4 (3)	
Source of infection				0.78					0.21
HA	105	92 (88)	13 (12)		52 (50)	37 (35)	13 (12)	3 (3)	
HCA	46	42 (91)	4 (9)		24 (52)	9 (20)	11 (23)	2 (5)	
CA	39	34 (87)	5 (13)		17 (44)	10 (26)	11 (28)	1 (2)	
Main <i>spa</i> -CCs				0.02					<0.01
CC002	42	7 (88)	5 (12)		0	42 (100)	0	0	
CC012	34	22 (65)	12 (35)		8 (24)	0	26 (76)	0	
CC701/2360	23	23 (100)	0		23 (100)	0	0	0	
Singletons	32	29 (91)	3 (9)		25 (78)	3 (10)	2 (6)	2 (6)	
Other <i>spa</i> -CC	54	49 (91)	5 (9)		32 (59)	10 (19)	9 (17)	3 (5)	

HA, hospital-acquired infection; HCA, healthcare-associated infection; CA, community-acquired infection; CC, clonal complex. Significant associations are indicated in bold.

^a Chi-square test.

or other clinical characteristics evaluated (Table 3). However, *spa*-CC701/2360 and CC002 were made up of only agr I and II, respectively. CC012 was mostly represented by agr III, with only 24% of the isolates belonging to agr I (Table 3).

Based on the phenotypic synergistic haemolysis test, 25 (13%) of the isolates had a dysfunctional agr system, of which 10 (40%) were MRSA. The prevalence of agr dysfunctionality was highest among agr type IV (33.3%; $n = 2/6$) isolates, followed by agr type III (32.4%; $n = 12/37$), II (8.8%; $n = 5/57$), and I (6.1%; $n = 6/99$). An association was observed between agr dysfunctionality and the *spa*-CCs ($p = 0.02$). This was further interrogated by univariable logistic regression, and *spa*-CC012 was the cluster driving this association, with odds ratio (OR) 4.03 (95% confidence interval (CI) 1.25–12.9; $p = 0.01$) compared to *spa*-CC002. The ORs for singletons and 'other *spa*-CCs' were 0.7 (95% CI 0.16–3.4) and 0.58 (95% CI 0.56–2.1), respectively. Agr dysfunctionality was twice as common amongst patients diagnosed with bacteraemia without focus compared to those with a known focus, although this finding was not statistically significant (Table 3).

Associations between pathogen characteristics and clinical outcomes

Possible associations between *S. aureus* characteristics and the clinical outcomes methicillin resistance, mortality, and LOS were assessed (Table 4). Univariable analysis showed that neonates were at higher risk of infection with a methicillin-resistant strain than the older age groups. Methicillin resistance was significantly associated with HA infection compared to CA infection (OR 4.77, 95% CI 2.09–10.87). A significant association was observed between *spa*-CC002 and methicillin resistance, relative to all other *spa*-CCs (except 012 and 701/2360) and singletons. A significant increase in mortality was observed with increasing age and having a HA infection. However, HCA infections were associated with a longer LOS (OR 1.97, 95% CI 1.13–3.42) and HA infections with a shorter LOS (OR 0.48, 95% CI 0.30–0.76) compared to CA infections. *S. aureus* strains with a functional agr system showed an association with longer duration of stay, but this did not reach statistical significance (Table 4).

Discussion

This study provided a comprehensive epidemiological investigation of *S. aureus* from bacteraemic patients at Tygerberg Hospital, both at the molecular and the clinical level, making it one of few studies to explore associations between *S. aureus*

microbial characteristics and clinical outcomes within Southern Africa.

The 27% MRSA prevalence reported from bacteraemic patients in this study is lower than the 43% reported from similar patients at Tygerberg Hospital during 2010 to 2012 (Karayem, 2014). A steady decrease in the MRSA prevalence in the public sector hospitals in the Western Cape is evident, with MRSA rates of 37%, 33%, and 24% in 2012, 2015, and 2017, respectively (Crowther-Gibson et al., 2015, 2016; Perovic et al., 2015). This notable decrease in the MRSA prevalence is possibly due to regular monitoring through surveillance and proper management of antibiotic use within the public healthcare settings in South Africa (Boyles et al., 2013; Crowther-Gibson et al., 2015, 2016). Compared to public hospitals, data on the prevalence of MRSA from the private sector in the Western Cape are scarce; however, the two existing studies have reported 36% national MRSA prevalence from bacteraemic patients during the year 2006 (Brink et al., 2007) and 20% from a wide range of clinical samples from Cape Town centres during 2013 (Wasserman et al., 2014). These findings might also reflect a decrease in the MRSA prevalence in the private sector in South Africa; however, more surveillance studies are required to confirm this speculation. Overall, the prevalence of MRSA across the African continent is lower than 50%, with Egypt (52–82%) and Algeria (35–75%) being the exceptions (Falagas et al., 2013).

As expected, more strain diversity was observed amongst methicillin-susceptible isolates as determined by *spa*-typing, which is in agreement with previous reports (Grundmann et al., 2014; Miko et al., 2013; Park et al., 2017; Pérez-Montarelo et al., 2018). On the other hand, globally predominant MRSA clones were circulating in our setting. The clone t045-ST5-MRSA, which was isolated during an outbreak in the neonatal and paediatric wards, accounted for 35% of the isolates and it carried a putative novel SCCmec type (unpublished data). This clone has been described previously across South Africa, but has been linked to various SCCmec types, supporting the ease of transmissibility and recombination of this mobile genetic element through horizontal gene transfer (Essa et al., 2009; Jansen van Rensburg et al., 2011; Perovic et al., 2015; Shittu et al., 2009). In addition, we noted the presence of the pandemic clone t037-ST239-MRSA-III (CC5), also known as the Brazilian/Hungarian clone, which seems to be endemic across South African hospitals (Abdulgader et al., 2015). Although not selected for MLST, the strain type t1257-MRSA-IV has been associated with the local clone ST612, which has been shown to be dominant in other hospitals in South Africa since 2008, but it was infrequent in this study (Jansen van Rensburg et al., 2011; Perovic et al., 2015).

Table 4Univariable and multivariable analyses of associations between *Staphylococcus aureus* characteristics and clinical outcomes.

Variable	Mortality OR (95% CI)		MRSA OR (95% CI)		Length of stay ^a						
	Univariable	p-Value	Multivariable	p-Value	Univariable	p-Value	Multivariable	p-Value			
Age group (vs. neonates)								0.002			
Children	0.98 (0.24–4.00)	0.978			0.25 (0.08–0.81)	0.021		–			
Adults	2.55 (0.80–8.17)	0.115	2.28 (0.92–5.62)	0.073	0.26 (0.10–0.70)	0.008		–			
Elderly	5.41 (1.59–18.39)	0.007	7.48 (2.82–19.89)	<0.001	0.16 (0.05–0.51)	0.002		–			
Sex					0.023			0.901			
Male	1.12 (0.61–2.04)	0.717			0.62 (0.33–1.18)	0.145		–			
Diagnosis					1.81 (0.85–3.87)	0.124		0.169			
Bacteraemia without focus	1.76 (0.87–3.55)	0.114						–			
Place of onset of infection (vs. CA)								<0.001			
HA	1.28 (0.59–2.77)	0.530	2.26 (1.12–4.55)	0.023	12.8 (2.94–56.09)	0.001	4.77 (2.09–10.87)	<0.001	–	0.48 (0.30–0.76)	0.002
HCA	0.71 (0.27–1.80)	0.466			3.32 (0.65–17.03)	0.150		–	1.97 (1.13–3.42)	0.017	
spa-CC (vs. spa-CC002)								0.045			
spa-CC012	1.11 (0.44–2.84)	0.821			0.68 (0.27–1.71)	0.413		–			
spa-CC701/2360	1.65 (0.59–4.64)	0.342			0.48 (0.16–1.41)	0.182		–			
Singletons	0.94 (0.36–2.47)	0.905			0.07 (0.02–0.35)	0.001	0.12 (0.03–0.57)	0.007	–		
Other spa-CCs	0.98 (0.44–2.19)	0.964			0.24 (0.10–0.56)	0.001	0.36 (0.16–0.79)	0.01	–		
Agr type (vs. Agr I)								0.01			
II	0.73 (0.37–1.44)	0.368			1.44 (0.72–2.91)	0.307		–			
III	0.50 (0.22–1.15)	0.103			0.62 (0.24–1.58)	0.319		–			
IV	0.68 (0.12–3.88)	0.663			1.00	–		–			
Phenotypic agr assay								0.451			
Functional	0.72 (0.31–1.67)	0.452			0.51 (0.21–1.21)	0.127		–	1.66 (0.93– 2.99)	0.089	

OR, odds ratio; CI, confidence interval; MRSA, methicillin-resistant *Staphylococcus aureus*; CA, community-acquired infection; HA, hospital-acquired infection; HCA, healthcare-associated infection.

^a The analysis of length of stay was done by two-sample Wilcoxon rank sum test for continuous variables and the Kruskal–Wallis equality of populations rank test for the univariable analysis, and by Cox proportional hazards regression for the multivariable analysis. Significant associations from the multivariable analyses are highlighted in bold.

Interestingly, ST612 has only been described in hospitals in South Africa and Australia, suggesting possible introductions through travel between these two countries (Jansen van Rensburg et al., 2011; Murphy et al., 2019).

Agr type I was the most predominant (50%), followed by types II (29%) and III (19%), while agr type IV was rare in our setting. This distribution may be driven by the genotypes circulating within the hospital, as the agr locus is strongly linked to the bacterial genetic background and may reflect the molecular epidemiology of *S. aureus* within a setting (Lina et al., 2003). This was well demonstrated by the study data, since all of the isolates representing spa-CC701/2360 and CC002 belonged to agr types II and III, respectively. However, we noted an exception: spa-CC012 consisted of 75% agr type III and 25% agr type II. Evolution studies proved that MLST CC30 is the genetic background for spa-CC012, which belongs to agr type III (Monecke et al., 2011). However, 25% of this cluster (spa-CC012) was represented by spa type t037 (ST239), which has evolved by recombination of a 557-kb fragment from the chromosome of ST30 into a ST8 (CC5) background that belongs to agr type I (David, 2019; Deurenberg and Stobberingh, 2008).

Animal models have demonstrated the importance of a functional agr system for the pathogenesis of *S. aureus*; however,

the isolation of *S. aureus* with low-level agr expression, or sometimes a defective agr system from or during clinical infections, questions its role in pathogenesis in humans (Gagnaire et al., 2012; Gong et al., 2014). In this study, only 13% of the isolates had a dysfunctional agr system and this seemed to be driven by a single cluster (spa-CC012). The prevalence of agr dysfunctionality reported amongst bacteraemic patients in previous studies has varied between 10% and 32% (Butterfield et al., 2011; Gagnaire et al., 2012; Schweizer et al., 2011). In the United States, 22% agr dysfunctionality was reported in severely ill patients (as measured by acute physiology score) with *S. aureus* bacteraemia (Schweizer et al., 2011). Another study by Butterfield and co-workers, also in the United States, reported a prevalence of 32% agr dysfunctionality; however, the study only targeted patients with MRSA bacteraemia, which explains the higher prevalence, as agr dysfunctionality has previously been associated with MRSA (Butterfield et al., 2011). Comparing these findings across studies should be done with caution, since factors such as age, severity of illness, and previous exposure to antibiotics or healthcare settings, could impact the prevalence of agr dysfunctionality. Agr dysfunctionality was more abundant among the isolates belonging to spa-CC012, in contrast to a study by Shopsin et al., who reported that agr functionality is not clone-specific in isolates from healthy

carriers (Shopsin et al., 2008). It is notable that our collection reflects mostly nosocomial isolates; therefore the predominance of certain strains due to selection pressure in hospital settings is to be expected (Stefani et al., 2012). Agr dysfunctionality was not associated with MRSA, contradicting studies from France and Korea (Gagnaire et al., 2012; Jang et al., 2012). This could be explained by the high predominance of SCCmec type IV in this study, since agr dysfunctionality has been reported to be less frequent amongst isolates carrying this element compared to SCCmec types I–III (Jang et al., 2012; Kang et al., 2015).

A highlight of this study is the assessment of possible associations between the pathogen-related characteristics and clinical data. This is critical, since it facilitates the identification of institution-specific risk factors, which can be used to develop processes to monitor dissemination of virulent pathogens and guide empirical antibiotic therapy decisions (Butterfield et al., 2011). This study assessed the possible predictors for three main clinical outcomes: crude mortality, LOS, and methicillin resistance. Older age and HA infections were the only independent risk factors for mortality. This is not surprising, since older patients are considered a high risk group with possibly impaired immune systems and underlying comorbidities (Albur et al., 2012; McGarry et al., 2004). In keeping with this, Malani et al. suggested that with every decade increase in age, the odds of death within 6 months due to *S. aureus* blood stream infection doubles (Malani et al., 2008). HA infections are mostly associated with higher antibiotic resistance rates, which may result in treatment failure leading to death (Ignacio Barrasa-Villar et al., 2017). This is also supported by the present study results, since HA infections were significantly associated with methicillin resistance, which is usually linked to resistance to a wider range of other antibiotics (Ignacio Barrasa-Villar et al., 2017; Malani et al., 2008). In addition, methicillin resistance was associated with *spa*-CC002, which is evident in the reduced odds of being methicillin-resistant amongst the singletons and the 'other' *spa*-CCs compared to *spa*-CC002. This association was driven by the clone t045-MRSA-NV, representing more than a third of the total methicillin-resistant isolates, which was dominant during a neonatal outbreak at Tygerberg Hospital during the study period.

Interestingly, no significant associations were found between any of the other pathogen-related characteristics and clinical outcomes studied, except for methicillin resistance. Although not reaching statistical significance, patients who had bacteraemia of unknown source were more likely to be infected with a dysfunctional isolate compared to patients with a definitive diagnosis. Agr dysfunctionality has been reported as an independent risk factor for bacteraemia and in-hospital mortality in patients with persistent MRSA bacteraemia in the United States and South Korea (Kang et al., 2017; Schweizer et al., 2011). However, these studies were conducted in settings with higher rates of agr dysfunctionality (22% and 32%). We further noted a trend towards a shorter LOS for patients infected with agr dysfunctional strains. Although a few studies suggested that agr dysfunctionality has been associated with worse clinical outcomes such as treatment failure and increased mortality, the body of literature remains conflicted regarding the effect of agr dysfunctionality on clinical outcomes (Gomes-Fernandes et al., 2017; McDanel et al., 2015; Schweizer et al., 2011). The association between agr dysfunctionality and shorter LOS remains to be investigated; however, the small proportion of agr dysfunctional isolates in this study in addition to the overall lengthy duration of hospital stay in our setting (median of 24 days) might have skewed these data.

Limitations of this study are mainly related to the incomplete clinical data collected from patients. Also, we were not able to analyse the effect of prior antibiotic exposure on agr dysfunctionality,

which has been shown to be a predictor for infection by dysfunctional isolates; patients who received a prior β -lactam or fluoroquinolone were twice as likely to be infected with an agr dysfunctional versus functional isolate (Butterfield et al., 2011).

In conclusion, we report the lowest MRSA prevalence at Tygerberg Hospital for the past 10 years, and agr dysfunctionality was shown to be more predominant amongst the *spa*-CC012 cluster. Despite the limited available clinical data, the study provided insights into the association between *S. aureus* epidemiology and agr-related virulence characteristics, and clinical outcomes.

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Ethical approval

The work has been approved by the Health Research Ethics Committee of Stellenbosch University (Reference number N14/06/065).

Conflict of interest

The authors have none to declare.

Author contributions

AW and MNF conceptualized the study design, AvR performed the experiments. SA, AvR, AW, and MNF contributed to data analysis and interpretation. SA drafted the initial manuscript and all authors contributed to and approved the final manuscript.

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