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Characterisation of *Staphylococcus aureus* bacteraemia at Tygerberg hospital

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To elucidate the local epidemiology of *Staphylococcus aureus* bacteraemia, we characterised blood culture isolates using molecular methods and prospectively collected clinical data to determine the occurrence of community-acquired, methicillin-resistant *S. aureus* (MRSA). Consecutive *S. aureus* blood culture isolates were collected over a one-year period from patients who were admitted to Tygerberg Academic Hospital in the Western Cape. A multiplex polymerase chain reaction (PCR) was used for the detection of *spa, mecA* and *lukS/F*-PV genes. Strain typing was performed using *spa* typing. Multiplex PCR for staphylococcal cassette chromosome *mec (SCCmec)* typing was also performed, as well as multilocus sequence typing (MLST) on selected isolates. Cases were categorised by clinical data as either hospital-acquired, healthcare-associated or community-acquired. One hundred and thirteen *S. aureus* isolates (30% MRSA) were collected from 104 cases of bacteraemia. According to clinical data, all community-acquired infections, 54% of hospital-acquired cases and the majority of healthcare-associated cases were due to methicillin-sensitive *S. aureus* (MSSA). Furthermore, all Panton-Valentine leukocidin (PVL)-positive isolates (15.9% of all *S. aureus*) were MSSA. MRSA strains were isolated from hospital-acquired cases (with a minority of healthcare-associated cases) and clustered mainly in *spa*-CC701 and CC012. SCC*mec* type IV was predominant. MLST clones included ST239-MRSA-III, ST36-MRSA-II and ST612-MRSA-IV. The predominant source for *S. aureus* bacteraemia was catheter-related infection (39%). Community-acquired S. aureus infections in our setting remain sensitive to methicillin and current treatment guidelines suffice. The majority of hospital-acquired and healthcare-associated infections were catheter-related. Prevention and treatment should be targeted accordingly.

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Introduction

The prevalence of community-acquired, methicillin-resistant *Staphylococcus aureus* (MRSA) infection in South Africa is unknown. Two recent studies described the molecular characterisation of MRSA in South Africa and identified MRSA clones such as ST5-MRSA-I, ST239-MRSA-III and ST36-MRSA-II, which are found worldwide, and ST612-MRSA-IV, which to date has only been described in South Africa and Australia.[1,2]. In this study, we characterised MRSA and MSSA isolates from bacteraemia episodes using molecular methods and prospectively collected demographic and clinical data on these patients to determine the prevalence of community-acquired MRSA according to the clinical definition.

Materials and method

Collection of isolates

Consecutive nonduplicate *S. aureus* blood culture isolates were prospectively collected from April 2008 to May 2009 from patients who were admitted to Tygerberg Academic Hospital in the Western Cape. Polymicrobial blood cultures were excluded from the study. Isolates were identified by conventional

laboratory methods and stored at -80^{0}C for further molecular typing. Antibiotic susceptibility testing was performed using the disc diffusion method and interpreted according to the Clinical and Laboratory Institute criteria.[3] Antibiotics that are routinely tested against *S. aureus* in our laboratory include penicillin, cefoxitin, gentamicin, erythromycin, clindamycin, rifampicin, fusidic acid, cotrimoxazole and vancomycin. Vancomycin minimum inhibitory concentrations were determined by Etest® (Biomérieux, France).

Definition of cases

A case was defined as an episode of *S. aureus* bacteraemia. An isolate for each episode (case) was included in the study when > 1 episode of *S. aureus* bacteraemia occurred (positive blood culture > 7 days after appropriate antimicrobial therapy was initiated and source control established).

Demographic and clinical data

Demographic and clinical data were prospectively obtained. Clinical information was attained at the time of telephonic reporting of the positive blood culture result. Further information was gathered through formatted interviews with the patient using a questionnaire that included specific questions, from the laboratory information system and from medical records.

Collected data included age, gender, ward, human immunodeficiency virus (HIV) status, other underlying medical conditions, previous hospitalisation and antimicrobial therapy. A record was also kept of dates of admission and blood culture collection, data on infective parameters, culture of *S. aureus* from other specimens, antimicrobial therapy and response to treatment.

The source of infection was determined according to the presence of clinical signs or other radiological investigations that indicated the primary site of infection with or without the culture of *S. aureus* from specimens that were collected from the primary site of infection, and without any other obvious source of infection.

The clinical outcome of each case was recorded as either discharged from hospital, transferred to another hospital or demised.

Definitions of clinical categories[4,5]

- *Hospital-acquired bacteraemia:* This was defined as a positive blood culture collected more than 48 hours after admission without evidence of a *S. aureus* infection at the time of admission. If the patient was transferred from another hospital, the duration was considered to begin from the date of the first hospital admission.
- *Healthcare-associated bacteraemia:* This was defined as a positive blood culture collected at the time or within 48 hours of hospital admission from patients who had been hospitalised or dialysed within the previous year or who were resident in a nursing home or long-term care facility.
- *Community-acquired bacteraemia:* This was defined as a positive blood culture collected within 48 hours of hospital admission from patients without a history of healthcare exposure.

Cases in which clinical information was lacking were not categorised according to the above criteria as the exact time of the onset of the infection could not be established. These cases were categorised as either "primary bacteraemia", where the source of infection was unknown, but infective parameters indicated infection, or "unknown" where clinical data were limited and clinical significance could not be established.

Strain typing and molecular characterisation

A multiplex polymerase chain reaction (PCR) was used for the simultaneous detection of the *spa, mecA* and Panton-Valentine leucocidin *lukF/S*-PV (PVL) genes according to a previously described protocol.[6] Strain typing was performed after sequencing of all isolates with positive *spa* PCR amplicons as previously published. *Spa* types were assigned using the Ridom StaphType® software package version 1.4 (Ridom Gmb, Würzburg, Germany) and *spa* clonal complexes (*spa*-CC) assigned using the BURP (Based Upon Repeat Pattern) algorithm of the software.

A multiplex PCR strategy was employed that allowed for the discrimination of staphylococcal cassette chromosome *mec* (SCC*mec)* types (I-V).[7] Multilocus sequence typing (MLST) was performed on selected isolates only as previously published.[8] MLST sequence types were assigned using the *S. aureus* database on the www.mlst.net website, and clonal complexes (CC) assigned using the based-upon-related-sequence-types (eBURST) algorithm that is available on the website. A representative isolate was randomly selected from each major *spa*-CC (≥ 4 *spa* types) as well as the most frequent *spa* types in the study. Where > 1 SCC*mec* type was found within a *spa*-CC, a representative isolate was randomly selected for each SCC*mec* type. Where only one isolate was available for a specific SCC*mec* type in the *spa*-CC, that isolate was selected.

Statistical analyses

Data were analysed using the Statistica® chi-square test. A p-value ≤ 0.05 was considered to be statistically significant.

Ethical approval

Ethical approval was obtained from the Health Research Ethics Committee of the Faculty of Health Sciences, Stellenbosch University on 8 October 2008 (Project number: N08/09/252).

Results

Description of cases and demographic data

One hundred and thirteen *S. aureus* isolates (34, 30% MRSA)

from 104 patients were included in the study. In five patients, more than one episodes of bacteraemia were clinically identified, therefore an isolate from each episode was included in the study.

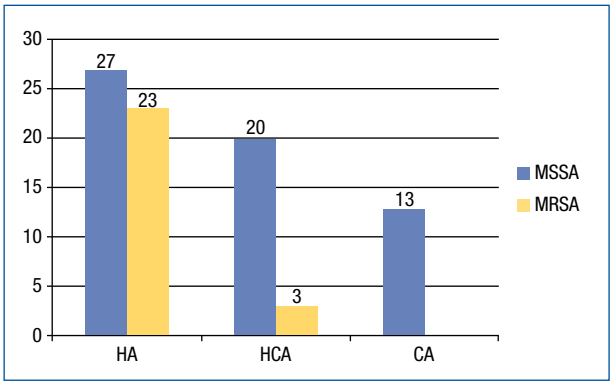
Table I lists the patients' demographic and clinical data.

Table I: Demographic and clinical data

Patient information	Number (%)
Demographics (n = 104 patients)	
Age range in years (median)	1-87 (23)
Gender	
Male	51 (49)
Female	53 (51)
Human immunodeficiency virus status	
Positive	5 (5)
Negative	49 (47)
Unknown	50 (48)
Clinical outcome (n = 102 patients)	
Discharged	63 (62%)
Transferred to another hospital	12 (12%)
Demised	27 (26%)
Source of infection (n = 113 cases)	
Catheter and prosthetic device-related infection	44 (39%)
Skin and soft tissue infection	20 (18%)
Primary bacteraemia	15 (13%)
Pneumonia	13 (11%)
Unknown	11 (10%)
Septic arthritis and osteomyelitis	5 (4%)
Infective endocarditis	3 (3%)
Other (pericarditis and urinary tract infection)	2 (2%)
Onset of infection (n = 86 cases)	
Hospital-acquired bacteraemia	50 (58%)
Healthcare-associated bacteraemia	23 (27%)
Community-acquired bacteraemia	13 (15%)

Site of primary infection and categorisation according to clinical onset

Catheter and prosthetic device-related bacteraemia was the most common primary site of infection that was identified. Most of these cases resulted from intravascular catheter infections. Furthermore, 86 of the 113 bacteraemia episodes could be clinically categorised as hospital-acquired, healthcare-associated or community-acquired, as shown in Figure 1. A significant finding was that all 13 community-acquired infections were due to MSSA and that no community-acquired MRSA was detected in our study. Community acquired-infections included pneumonia, skin and soft tissue infections, septic arthritis or osteomyelitis and infective endocarditis. Of the MRSA infections, 23 (88%) were hospital-acquired. A minority of MRSA infections were healthcare-associated (3, 11.5%). Healthcare-associated bacteraemia occurred in patients with chronic renal failure on haemodialysis or peritoneal dialysis and who developed catheter-related infection. The majority of healthcare-associated infections were due to MSSA (20, 87%).



CA: community-acquired, HA: hospital-acquired, HCA: healthcare-associated, MRSA: methicillin-resistant *Staphylococcus aureus*, MSSA: methicillin-sensitive *Staphylococcus aureus*

Figure 1: Comparison of methicillin resistant Staphylococcus aureus and methicillin-sensitive Staphylococcus aureus hospital-acquired, healthcare-associated and community-acquired infections (chi-square (df = 2) = 18.6, p-value = 0.00009)

Strain characteristics

mecA was detected in 33 of 34 MRSA isolates, PVL genes in 18 of the 113 *S. aureus* isolates (15.9%) and *spa* in 101 isolates (89%). PVL was detected in 18 of the 79 MSSA strains (22.7%), whereas none of the MRSA strains tested positive for PVL. Forty-nine *spa* types were identified, including five novel types. Table II shows the most frequent *spa* types, each representing ≥ 5 isolates in this study. Apart from the singleton, t891, these common *spa* types represent the major *spa*-CC in this study. These strains were isolated from patients from a variety of wards, but the numbers of isolates per ward were too low to determine if there was any association with specific wards.

Table II: Most frequent spa types detected in the study (n = 101)

Spa type	Number of isolates (%)
t037	9 (9%)
t891	7 (7%)
t1257	6 (6%)
t002	6 (%)
t015	6 (%)
t021	5 (5%)

An unweighted pair group method using average linkages dendogram shows the clustering of *spa* types into *spa*-CC (Figure 2). Two *spa* types were excluded from cluster analyses as they contained < 5 repeats, which is insufficient to deduct evolutionary history. Assessment of the four major *spa*-CC, representing 70 (62%) of all isolates, displayed that MRSA strains (n = 30) clustered predominantly in *spa*-CC701

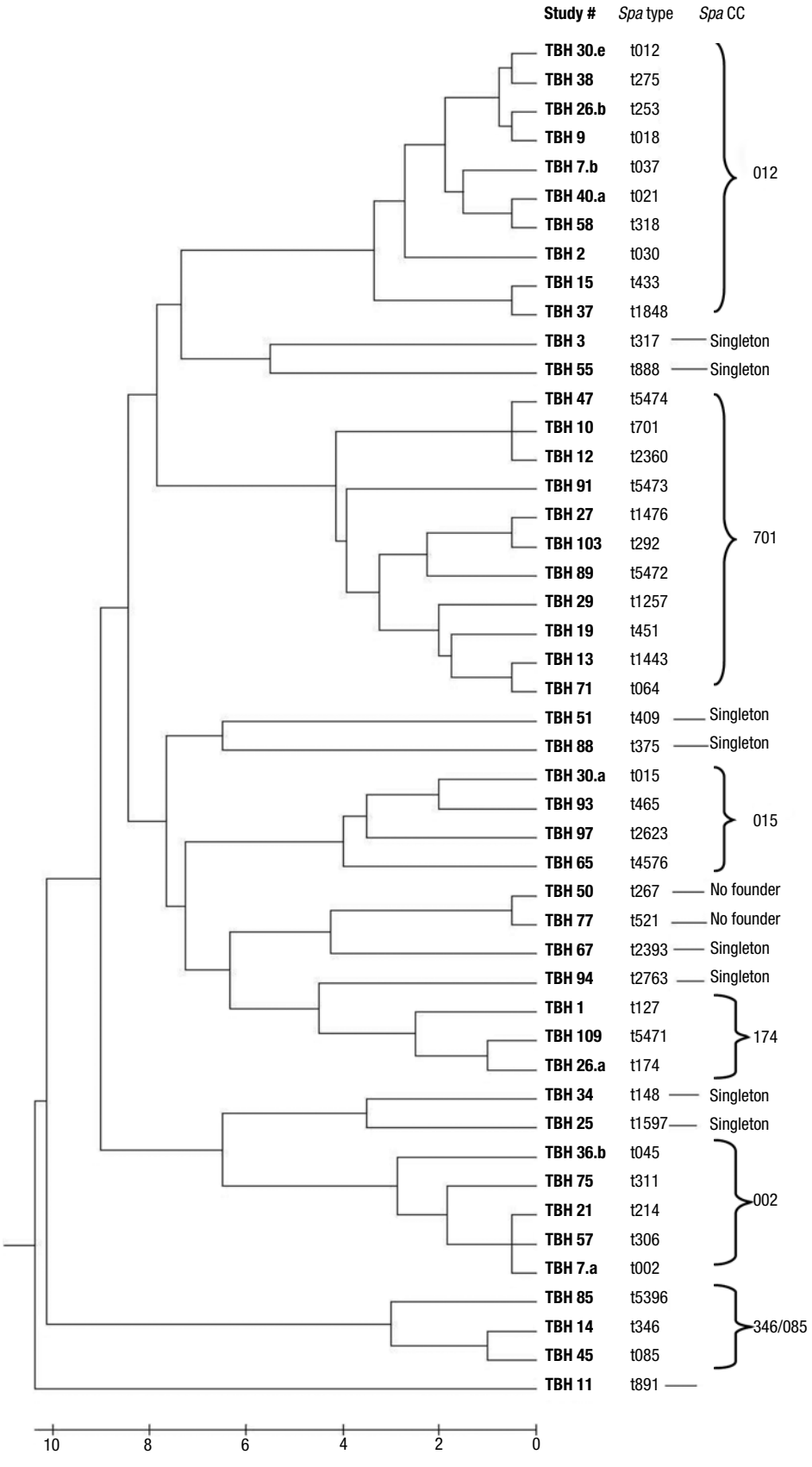


Figure 2: An unweighted pair group method using average linkages dendogram of *spa* types clustering into *spa*-CC (Only one representative strain per *spa* type is shown)

Table III: The multilocus sequence typing clonal lineages and antimicrobial resistance profiles of selected isolates

Isolate number	MLST		Methicillin susceptibility	SCC*mec*	*Spa* typing		PVL	Antimicrobial resistance profile
	ST	CC			*spa*	*Spa*-CC		
TBH107	239	8	MRSA	III	t037	012	Neg	PEN, FOX, GEN, ERY, CLI, COTR
TBH69	239	8	MRSA	V	t037	012	Neg	PEN, FOX, GEN, ERY, CLI, COTR
TBH9	612	8	MRSA	IV	t018	012	Neg	PEN, FOX, ERY, CLI
TBH13	612	8	MRSA	IV	t1443	701	Neg	PEN, FOX, GEN, RIF, COTR
TBH86	36	30	MRSA	II	t021	012	Neg	PEN, FOX, GEN, ERY, CLI
TBH17	2090	45	MRSA	IV	t015	015	Neg	PEN, FOX, GEN, ERY, CLI, RIF, COTR
TBH57	650	5	MSSA	N/A	t306	002	Neg	PEN
TBH11	217	22	MSSA	N/A	t891	Singleton	Pos	PEN, GEN

CC: clonal complex, CLI: clindamycin, COTR: co-trimoxazole, ERY: erythromycin, FOX: cefoxitin, GEN: gentamicin, MLST: multilocus sequence typing, MRSA: methicillin resistant *Staphylococcus aureus*, N/A: not applicable, Neg: negative, PEN: penicillin, Pos: positive, PVL: Panton-Valentine leucocidin, RIF: rifampicin, SCC*mec*: staphylococcal cassette chromosome *mec*, ST: sequence type

(13, 43%) and *spa*-CC012 (16, 53%), compared to *spa*-CC015 (1, 3%) and *spa*-CC002 (0, 0%). MSSA showed a diverse genetic background and was identified in all *spa*-CC, as well as numerous singletons (p-value = 0.0016 for the association between major *spa*-CC and MRSA/MSSA).

SCC*mec* typing of MRSA isolates showed type IV to be the dominant type (16, 47%) followed by SCC*mec* III, II and V, with 8 (23%), 6 (18%) and 2 (6%), respectively. Type IV MRSA constituted multi-resistant phenotypes, with resistance to ≥ 3 non-beta-lactam antibiotics for 15 isolates and resistance to two non-beta-lactam antibiotics in one isolate.

The MLST clonal lineages and antimicrobial resistance profiles of selected strains are shown in Table III. The singleton, t891, was also selected for MLST because it was the second most frequent *spa* type in the study, following t037 which represented the major *spa*-CC012.

Clinical outcome of cases

Clinical outcome was obtained for 102 of the 104 patients (Table I) of which 27 patients (26%) demised. Of the patients that survived, two patients presented with both a MSSA and a MRSA episode. Therefore, both episodes for each patient were included in the MSSA and MRSA groups respectively to compare the fatality ratios of the patients with MRSA vs. those with MSSA. Ten (31%) of the 32 MRSA patients demised, compared to 17 (24%) of the 72 MSSA patients. These numbers did not achieve statistical significance (p-value = 0.412). None of the MRSA case fatalities were due to PVL-positive strains. In the MSSA group, 17 patients demised (3 PVL-positive, 17.6%) and 55 patients survived (15 PVL-positive, 27%). The number of PVL-positive cases was too low to determine statistical significance between the cases of survival and fatality.

Discussion

This study provides data on the molecular characterisation of *S. aureus* bloodstream isolates that are linked to prospective clinical data at Tygerberg Academic Hospital.

In our setting, 30% of *S. aureus* bloodstream isolates were identified as MRSA. Of note is that none of the MRSA cases were community-acquired. This is in contrast to the emergence of community-acquired MRSA that has been reported from other countries.[4] Therefore, in our setting, cloxacillin or other beta-lactam agents are still suitable to treat patients with suspected community-acquired staphylococcal infections. Furthermore, the virulence factor, PVL, has been associated with community acquired-MRSA, but in this study, PVL was only detected in MSSA isolates. Only three of the PVL-positive cases demised. Similar findings have been reported by Nickerson et al from Thailand.[9] That study included a larger proportion of MSSA isolates, predominantly community-acquired skin and soft tissue infections, but all PVL-positive isolates were MSSA and constituted 49% of all isolates. In addition, in that study, PVL-positive isolates were strongly associated with survival compared to PVL-negative isolates. In our study, overall mortality was 26%. Mortality that was associated with MRSA bacteraemia was higher than that linked to MSSA bacteraemia, but this finding was not found to be statistically significant. In the study by Nickerson et al,[9] overall bacteraemia-associated patient mortality was 53% and was linked to MRSA. No community-acquired MRSA infections were detected in that setting as well.

The spectrum of primary sites of infection that are associated with bacteraemia reflects the local epidemiology of *S. aureus* at our institution. The majority of infections were hospital-acquired and related to intravascular catheters, particularly in renal patients receiving haemodialysis and in patients in the intensive care units. A wide range of *spa* types were identified in these cases and no specific strain was associated with intravascular catheter infections. A high number of hospital-acquired and healthcare-associated infections were due to MSSA. This may reflect the endogenous route of infection in carriers. Exogenous acquisition may also occur because of lack of adherence by staff to infection prevention measures, such as hand hygiene. Overcrowded units and staff shortages are common problems in our resource-constrained setting.

Hospital-acquired strains were mainly found to cluster in *spa*-CC 701 and *spa*-CC-012. MLST typing of selected strains

identified clones that are found globally, such as ST239-MRSA-III and ST36-MRSA-II. In accordance with another recent study from Cape Town that focused on MRSA only, the ST612-MRSA-IV clone was identified as a dominant MRSA clone.[2] This clone has only been described in South Africa and Australia.[2] Interestingly, t037, t021 and t018 clustered together in *spa*-CC012, although from different MLST clones, CC8 and CC30, and SCC*mec* types. Robinson and Enright reported that chromosomal replacement in the MRSA isolates of CC8 with a large genetic element containing the *spa* gene from ST30 (CC30) resulted in ST239 (CC8).[10] Furthermore, a novel MLST sequence type, ST2090-MRSA-IV, was identified from a strain characterised as t015 (*spa*-CC015) and was the only MRSA isolate among six that were characterised as t015. This may reflect the local emergence of a new MRSA sequence type. This strain was isolated from a one-year-old HIV-negative infant after > 1 month of hospitalisation.

A possible limitation of the study was the limited clinical data, particularly pertaining to the group of cases classified as "unknown", as we were unable to rule out isolates that may have been contaminants in this group.

In conclusion, we identified no community-acquired MRSA in our setting. Cloxacillin is still the agent of choice for treating community-acquired *S. aureus* infection. The majority of isolates were derived from cases defined as hospital-acquired and the major source was intravascular catheter-related infection. This information will be useful when targeting infection control and prevention practices to reduce *S. aureus* bacteraemia in our setting.

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