Characterization of meticillin-resistant *Staphylococcus aureus* isolates from hospitals in KwaZulu-Natal province, Republic of South Africa

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Epidemiological data based on phenotypic and molecular characterization of meticillin-resistant *Staphylococcus aureus* (MRSA) in sub-Saharan Africa are limited. This investigation studied 61 MRSA isolates obtained from 13 health-care institutions in KwaZulu-Natal (KZN) province, South Africa, from March 2001 to August 2003. More than 80 % of the isolates were resistant to at least four classes of antibiotics and six isolates were resistant to the aminoglycoside, macrolide-lincosamide and tetracycline groups of antibiotics, heavy metals and nucleic acid-binding compounds. PFGE of *Sma*I-digested genomic DNA revealed seven types, designated A–G. Type A was the main pulsotype (62.3 %) and was identified in 11 of the 13 health-care institutions, suggesting that it represented a major clone in health-care institutions in KZN province. Analysis of representative members of the three major pulsotypes by *spa*, multilocus sequence typing and *SCCmec* typing revealed the types t064-ST1173-*SCCmec* IV and t064-ST1338-*SCCmec* IV (PFGE type A, single-locus and double-locus variants of ST8), t037-ST239-*SCCmec* III (PFGE type F) and t045-ST5-*SCCmec* III (PFGE type G). The combination of various typing methods provided useful information on the geographical dissemination of MRSA clones in health-care institutions in KZN province. The observation of major clones circulating in health-care facilities in KZN province indicates that adequate infection control measures are urgently needed.

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INTRODUCTION

Meticillin-resistant *Staphylococcus aureus* (MRSA) was first reported from the UK in 1961 (Jevons, 1961) and is currently one of the most important nosocomial pathogens worldwide (Tiemersma *et al.*, 2004; Huang *et al.*, 2006; Sola *et al.*, 2006). Moreover, reports on community-associated MRSA (CA-MRSA), particularly USA300 (ST8-*SCCmec*IV, *pvl*⁺), outbreaks in hospitals are increasing and CA-MRSA threatens to replace hospital-associated MRSA in health-care facilities (Gonzalez *et al.*, 2006; Seybold *et al.*, 2006; Deurenberg *et al.*, 2007; Huang *et al.*, 2007; Maree *et al.*, 2007; Otter & French, 2008). The high morbidity and mortality rates of MRSA infections, the potential for intra- and inter-hospital dissemination, and the spread of

epidemic strains (Aires de Sousa *et al.*, 1998; Deplano *et al.*, 2000; Cosgrove *et al.*, 2003; Lodise & McKinnon, 2007) have led to an interest in tracking of strains to gain a better picture of the dynamics of clonal spread.

Phenotypic and genotypic data play an important role in understanding the epidemiology of MRSA and evaluating the effectiveness of infection control measures (Murchan *et al.*, 2003). Molecular typing approaches have been used to great advantage in identifying and monitoring the international spread of some unique MRSA strains (Oliveira *et al.*, 2002; Enright, 2003; Aires de Sousa & de Lencastre, 2004). Until recently, it was generally believed that the spread of MRSA resulted from the global dissemination of a few highly epidemic clones. However, a recent study by Nübel *et al.* (2008) has provided evidence that the population of MRSA in one of the clones (ST5) is geographically structured and that MRSA could have emerged very frequently in different parts of the world

Abbreviations: CA, community-associated; KZN, KwaZulu-Natal; MLST, multilocus sequence typing; MRSA, meticillin-resistant *Staphylococcus aureus*; *PVL*, Panton–Valentine leukocidin.

through independent imports of the meticillin-resistance determinant into their genomes (Nübel *et al.*, 2008). Similar investigations on other clonal lineages are ongoing. To gain more insight into the global rise and spread of MRSA, data from Africa will be useful in understanding clone distribution. To date, however, data on the molecular epidemiology of MRSA in Africa are scarce.

In a recent investigation, we reported the prevalence of MRSA in KwaZulu-Natal (KZN) province, South Africa, to be approximately 27 % (Shittu & Lin, 2006). However, there is a paucity of data on MRSA isolates from this province, and the mechanisms of MRSA emergence and spread are unknown. In this study, we apply a combination of phenotypic and molecular techniques to infer isolate inter-relatedness in order to provide health personnel and policy makers in KZN province with the baseline information needed to establish appropriate infection control programmes and health intervention strategies.

METHODS

MRSA isolates. The 61 MRSA isolates investigated in this study have been described previously (Shittu & Lin, 2006). A total of 48 isolates (78.7 %) were recovered from wound samples, six (9.8 %) from sputum, two (3.3 %) from otitis media, and one isolate each (1.6 %) from a blood and a urine sample, an eye-related infection and an endotracheal aspirate. No clinical information was available for one isolate (Shittu & Lin, 2006).

Antibiotic susceptibility testing. In addition to the antibiotics tested in the previous study (gentamicin, kanamycin, streptomycin, neomycin, erythromycin, clindamycin, tetracycline and minocycline; Shittu & Lin, 2006), the isolates were tested against an additional set of six antibiotics. The antibiotics (Oxoid) comprised azithromycin (15 µg), fosfomycin (50 µg), linezolid (30 µg), oleandomycin (15 µg), tobramycin (10 µg) and quinupristin/dalfopristin (15 µg). Interpretative zone diameters not stated in the Clinical and Laboratory Standards Institute (formerly the National Committee for Clinical Laboratory Standards) guidelines (NCCLS, 2003) for resistance to the following antibiotics were considered to be as follows: oleandomycin ≤ 17 mm and fosfomycin ≤ 14 mm (Members of the SFM Antibiogram Committee, 2003). Testing of susceptibility to heavy metals (cadmium acetate, mercuric chloride) and nucleic acid-binding compounds (ethidium bromide and propamidine isethionate) was performed on the MRSA isolates as described previously (Emslie *et al.*, 1985). Discs were prepared in the laboratory with the indicated concentrations: cadmium acetate (50 µg), propamidine isethionate (50 µg), mercuric chloride (109 µg) and ethidium bromide (60 µg). Antibiotyping was based on their susceptibility pattern to selected antibiotics, representing various classes of antimicrobial agents, as reported previously (Shittu & Lin, 2006).

SCCmec typing. SCCmec types were determined by the multiplex PCR strategy reported by Oliveira & de Lencastre (2002). The following strains were used as controls: EMRSA-16 (SCCmec type II), EMRSA-1 (SCCmec type III) and K1814 (SCCmec type IV).

Detection of Pantón–Valentine leukocidin (PVL) genes. The PVL genes (*lukS-PV* and *lukF-PV*) were detected by PCR as described by Lina *et al.* (1999). A meticillin-susceptible *S. aureus* (*nuc*⁺, *pvl*⁺) isolate from Nigeria served as a positive control.

PFGE typing. PFGE typing of *Sma*I (Fermentas)-digested DNA was carried out by a modification of a protocol described previously by Bannerman *et al.* (1995). Electrophoresis was performed in 0.5 × TBE buffer [0.045 M Tris/HCl (pH 8), 0.045 M boric acid, 0.001 M disodium EDTA] by a contour-clamped homogeneous electric field method using a CHEF MAPPER system (Bio-Rad). The fragments were separated with a linear ramped pulse time of 6.8–63.8 s over a period of 23 h at 14 °C. The gels were stained with ethidium bromide (1 µg ml⁻¹; Sigma) for 1 h, visualized under UV light and photographed using a SynGene Bioimaging System.

The banding patterns were interpreted visually and the relatedness of the strains was determined according to the recommendation of Tenover *et al.* (1995). In addition, Gel Compar II software version 4.0 (Applied Maths) was used to calculate the Dice similarity indices and to construct a dendrogram after cluster analysis by the unweighted pair group method with arithmetic averages. Band position tolerance was set at 1.5 % and two strains belonged to the same cluster if their Dice similarity index was 80 % or more. Strains showing the same PFGE pattern were grouped as a pulsotype and assigned alphabetically (A, B, C, etc.). Numeric subcodes were used to represent up to three band difference (subtypes A1, B1, etc.).

spa typing and multilocus sequence typing (MLST). Isolates representing the major PFGE patterns were characterized by *spa* typing (Strommenger *et al.*, 2008) and MLST (Enright *et al.*, 2000) as described previously.

RESULTS AND DISCUSSION

All of the isolates were susceptible to quinupristin/dalfopristin, fosfomycin and linezolid. However, more than 90 % of the MRSA isolates were resistant to gentamicin, kanamycin and tobramycin, and more than 80 % were resistant to antibiotics in the macrolide-lincosamide group. Resistotyping revealed that 46 and 82 % of the MRSA isolates were resistant to mercuric chloride and cadmium acetate, respectively; however, approximately 70 % were susceptible to propamidine isethionate and ethidium bromide (Table 1). Moreover, 14 MRSA isolates were resistant to the heavy metal and nucleic acid-binding compounds. Overall, six MRSA isolates obtained from clinical samples in health-care institutions in Durban, Pietermaritzburg and Empangeni were resistant to the aminoglycoside, macrolide-lincosamide and tetracycline groups of antibiotics, heavy metals and nucleic acid-binding compounds.

SCCmec typing showed that SCCmec IV was identified in 38 strains (62.3 %), followed by type III in 11 strains (18.0 %) and type IIIa in five strains (8.2 %). Two isolates belonged to types II and IIIb and one isolate belonged to type I. Two isolates were non-typable (Table 1).

The PFGE profiles and the dendrogram of the MRSA strains representing the various pulsotypes and the distribution of pulsotypes in health-care institutions within KZN province are shown in Fig. 1(a, b). PFGE analysis grouped the 61 isolates into seven pulsotypes: pulsotypes A (38/61 strains; 62.3 %), F (10/61; 16.4 %), G (6/61; 9.8 %) and B (4/61; 6.5 %); and C, D and E, each represented by

Table 1. Relationship between antimicrobial resistance, PFGE and SCCmec typing of MRSA isolates

All of the MRSA isolates were susceptible to quinupristin/dalfopristin, fosfomycin and linezolid.

Antimicrobial agent	No. of resistant isolates (%)	PFGE pulsotype							SCCmec type						
		A (n=38)	B (n=4)	C (n=1)	D (n=1)	E (n=1)	F (n=10)	G (n=6)	I (n=1; 1.6 %)	II (n=2; 3.3 %)	III (n=11; 18.0 %)	IIIa (n=5; 8.2 %)	IIIb (n=2; 3.3 %)	IV (n=38; 62.3 %)	Non-type (n=2; 3.3 %)
Gentamicin*	59 (96.7)	36	4	1	1	1	10	6	1	1	11	5	2	37	2
Kanamycin*	59 (96.7)	36	4	1	1	1	10	6	1	1	11	5	2	37	2
Streptomycin*	19 (31.1)	3	0	0	0	0	10	6	0	0	11	4	1	3	0
Neomycin*	19 (31.1)	3	0	0	0	0	10	6	0	0	11	4	1	3	0
Tobramycin	58 (95.1)	36	4	0	1	1	10	6	1	1	11	5	2	36	2
Erythromycin*	50 (82.0)	28	3	1	1	1	10	6	1	1	11	5	2	28	2
Oleandomycin	50 (82.0)	28	3	1	1	1	10	6	1	1	11	5	2	28	2
Azithromycin	50 (82.0)	28	3	1	1	1	10	6	1	1	11	5	2	28	2
Clindamycin*	50 (82.0)	28	3	1	1	1	10	6	1	1	11	5	2	28	2
Tetracycline*	55 (90.2)	38	4	1	1	1	10	0	1	2	5	5	2	38	2
Minocycline*	55 (90.2)	38	4	1	1	1	10	0	1	2	5	5	2	38	2
Ethidium bromide	19 (31.1)	7	0	0	0	0	6	6	0	0	9	2	1	7	0
Propamidine isethionate	18 (29.5)	5	1	0	0	0	6	6	0	0	9	2	1	6	0
Mercuric chloride	28 (45.9)	12	0	0	0	0	10	6	0	0	11	4	1	12	0
Cadmium acetate	50 (82.0)	31	4	1	1	0	7	6	1	1	9	4	2	31	2

*Data reported previously by Shittu & Lin (2006).

Fig. 1. (a) Dendrogram of representative MRSA types in KZN province, South Africa. (b) Map of KZN province and the PFGE types at the various locations (illustrated in different colours). The numbers indicate the health-care institutions.

single strains. PFGE types A and F were subdivided into nine and two subtypes, respectively.

Four MRSA isolates representing the three major pulsotypes (two isolates of pulsotype A, one isolate each of pulsotypes G and F) were analysed by *spa* typing and MLST. The two isolates assigned to pulsotype A comprised two related clones, namely *spa* type t064, ST1173 and SCCmec IV, and t064, ST1338 and SCCmec IV. MLST sequence types ST1173 (allelic profile 3-3-1-1-4-131-3) and ST1338 (3-3-1-1-4-131-83) are single-locus and double-locus variants of ST8 (3-3-1-1-4-4-3), a clone that has been observed previously in Europe, Australia and North America (www.mlst.net). Furthermore, the MRSA isolates in pulsotype F were *spa* type t037, ST239 and SCCmec III, whilst those of pulsotype G were *spa* type t045, ST5 and SCCmec III.

This study has defined the clonal types of MRSA isolates in health-care institutions in KZN province, South Africa. The majority of the isolates studied belonged to pulsotype A with SCCmec IV. Generally, SCCmec IV strains are not multiresistant and carry only the *mecA* gene (Ito *et al.*, 2003; Hanssen & Ericson Sollid, 2006). However, studies have indicated that some SCCmec IV strains are resistant to agents other than β -lactams and aminoglycosides (Donnio *et al.*, 2004; Kim *et al.*, 2006; Laplana *et al.*, 2007). A similar feature was observed in this study in which all of the SCCmec IV strains were resistant to tetracycline and minocycline, and more than 95% were resistant to gentamicin, kanamycin and tobramycin. Furthermore, 73.7% of the MRSA isolates in SCCmec type IV were resistant to the macrolide-lincosamide group of antibiotics. These data suggest that some multiresistant strains with

Table 2. Correlation between antibiotyping, PFGE and SCCmec typing of 61 MRSA strains from South Africa

Antibiotype* (no. of strains)	PFGE type (no. of strains)	SCCmec types (no. of strains)
1. PEN, ERY, GM, TET, MU5, TS, CIP, CHL (4)	F1 (3)	IIIa (3)
	F1 (1)	IIIb (1)
2. PEN, ERY, GM, TET, MU5, TS, CIP (6)	F1 (5)	III (4), IIIa (1)
	F2 (1)	III (1)
3. PEN, ERY, GM, TET, TS, RF, CHL (4)	A1 (3)	IV (2), II (1)
	A6 (1)	IV (1)
4. PEN, ERY, GM, TET, TS, RF, MU (3)	A3 (1)	IV (1)
	A5 (1)	IIIa (1)
	A8 (1)	IV (1)
5. PEN, ERY, GM, TET, TS, RF, CIP (1)	A5 (1)	IV (1)
6. PEN, ERY, GM, TET, TS, RF (25)	A1 (10)	I (1)
		IV (7)
		IIIb (1)
		NT (1)
	A2 (1)	IV (1)
	A3 (2)	IV (1)
		NT (1)
	A4 (1)	IV (1)
	A5 (1)	IV (1)
	A6 (1)	IV (1)
	A8 (1)	IV (1)
	A9 (2)	IV (2)
	B (3)	IV (3)
	C (1)	IV (1)
	D (1)	IV (1)
	E (1)	IV (1)
7. PEN, GM, TET, TS, RF, CHL (2)	A3 (1)	IV (1)
	A8 (1)	IV (1)
8. PEN, GM, RF, TET, MU5, TS (2)	A8 (2)	IV (2)
9. PEN, GM, RF, TET, TS (5)	A1 (1)	IV (1)
	A6 (3)	IV (3)
	B (1)	IV (1)
10. PEN, ERY, GM, RF, TET (1)	A3 (1)	IV (1)
11. PEN, ERY, GM (6)	G (6)	III (6)
12. PEN, RF, TET (2)	A5 (1)	II (1)
	A7 (1)	IV (1)

NT, Non-typable.

*PEN, Penicillin; ERY, erythromycin; GM, gentamicin; TET, tetracycline; MU5, low-level mupirocin resistance; TS, trimethoprim; CIP, ciprofloxacin; RF, rifampicin; CHL, chloramphenicol.

SCCmec type IV may have acquired resistance to non- β -lactam antibiotics in order to be able to survive in the hospital environment (Aires de Sousa & de Lencastre, 2003) or through exposure to the antibiotics (Okuma *et al.*, 2002). All of the MRSA isolates in this group with the SCCmec IV element were PVL-negative, a feature that has been reported in nosocomial MRSA isolates in previous studies (Cuevas *et al.*, 2007; Aires de Sousa *et al.*, 2008). The ability of pulsotype A (ST1173, ST1338) to spread over distances within KZN province is suggested by our results as shown in Fig. 1(b). It was observed in three hospitals in Durban, and in health-care institutions in Pietermaritzburg,

Newcastle, Greytown, Kokstad, Port Shepstone, Empangeni, Scottburgh and Eshowe. These findings suggest that inter-hospital spread of this clone occurs frequently and that it is a major clone circulating in health-care institutions in the KZN province of South Africa.

The second major clone, t037-ST239-SCCmec III (PFGE type F), was identified in three of the four health-care institutions in Durban and in health-care facilities in Pietermaritzburg and Empangeni. Some degree of correlation between antibiotyping and the molecular typing methods was observed in the characterization of this clone.

The MRSA isolates in antibiotypes 1 and 2 were grouped in this clone and SCCmec type III was identified in strains grouped in the two antibiotypes. Moreover, resistance to ciprofloxacin and susceptibility to rifampicin was a unique feature in the multiresistant MRSA clone, and all of the isolates exhibited low-level resistance to mupirocin (Table 2). The t037-ST239-SCCmec III clone (called the Brazilian/Hungarian clone based on PFGE) has been reported to be widely disseminated in Brazilian hospitals (Teixeira *et al.*, 1995) and is widespread in many countries in South America (Corso *et al.*, 1998), Europe (Aires de Sousa *et al.*, 1998) and Asia (Feil *et al.*, 2008). Moreover, geographical stratification of this clone was reported recently (Smyth *et al.*, 2008). Our investigation appears to be the first on the detection of this clone in Africa.

The third major clone, t045-ST5-SCCmec III (PFGE type G), is similar to the New York/Japan clone (ST5-SCCmec II) and the paediatric clone (ST5-SCCmec IV). In contrast, however, the clone in this study carried SCCmec III. It was observed in two hospitals in Durban and Pietermaritzburg and in a health-care facility in Eshowe and Scottburg. A study by Nübel *et al.* (2008) indicated that the ST5 clone is associated with at least six types of SCCmec, and investigation of sequence diversity within ST5 revealed that ST5 MRSA clones have emerged many times by multiple independent introductions of SCCmec into methicillin-susceptible ST5. The study also observed that MRSA in ST5 from South Africa and Kenya formed a unique sublineage (termed 'ST5-D') and was not closely related to MRSA from other continents which shared identical *spa* types (t045). Furthermore, the closest relatives of MRSA from South Africa were methicillin-susceptible, *lukS/F*-positive *S. aureus* from Kenya. These observations suggest that long-distance spread of MRSA may not have occurred between different countries in Africa, but rather that there has been a widespread occurrence of MSSA clones which may become resistant through multiple independent imports of SCCmec.

The MRSA isolates grouped in ST5 were not multiresistant (Table 2), but we observed that they were characteristically resistant to heavy metals/nucleic acid-binding compounds and susceptible to tetracycline and minocycline. Characterization based on antibiotic susceptibility testing has been regarded as a timely and inexpensive tool for MRSA phenotyping and for identifying specific clones (Amorim *et al.*, 2007; Nimmo *et al.*, 2008). The susceptibility of MRSA to trimethoprim-sulfamethoxazole and spectinomycin has been useful in differentiating the Iberian and Brazilian clones (Aires de Sousa *et al.*, 1998), and susceptibility of MRSA isolates to ciprofloxacin has been an important phenotypic marker of CA-MRSA in a London teaching hospital (Otter & French, 2008). Although antibiotyping showed that multidrug-resistant MRSA isolates were detected in the different pulsotypes and that the dominant antibiotype was not discriminatory for MRSA as it was detected in PFGE types A-E (Table 2), the antibiotic-resistance markers identified in MRSA in

types F and G could be useful in monitoring the spread of such clones and alerting clinical microbiologists to the detection of new clones as and when they arise.

This study examined only a small number of isolates but the combination of various typing tools, especially *spa* and MLST, has provided useful information on the existence and geographical distribution of MRSA clones in KZN province, South Africa. The identification of widely disseminated clones in health-care institutions in KZN province indicates that urgent measures are needed to curtail their emergence, spread and establishment in this province. More studies are needed to investigate the clonal evolution of MRSA over time and the emergence of CA-MRSA in the hospital environment in South Africa.

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