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Short Communication

Plasmid-mediated resistance and virulence mechanisms in the private health sector in KwaZulu-Natal, South Africa: An investigation of methicillin resistant *Staphylococcus aureus* (MRSA) clinical isolates collected during a three month period

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ABSTRACT

Objectives: Due to the lack of information on the plasmid content of MRSA strains in South Africa (SA), this study investigated the resistance and virulence mechanisms of 27 clinical isolates from the private health care sector over a period of 3 months.
Methods: Plasmids were extracted and the presence of MRSA confirmed by the presence of *mec*A. The isolates were subjected to antimicrobial susceptibility testing and molecular characterization of common resistance encoding genes and frequently encountered virulence factors by PCR using plasmid DNA as the template. The genetic relatedness between the isolates was determined by pulsed field gel electrophoresis (PFGE).
Results: All isolates were plasmid positive, and displayed ampillicin, ciprofloxacin, gentamicin, rifampicin, tetracycline, erythromycin, and clindamycin resistance. They were all fully susceptible to daptomycin, linezolid, vancomycin, tigecycline and fusidic acid. Multidrug resistance (MDR) was found in 74.1% (20/27) of the MRSA isolates. The frequency of the resistance and virulence genes ranged from 100% to 0%. PFGE analysis revealed 10 pulsotypes, designated A–J, which showed correlation with resistance profile of the isolates in each group. Of note, 85.2% (23/27) of the isolates clustered into six major PFGE types giving an indication of similar circulating MRSA clones.
Conclusions: This study highlights the genetic diversity and resistance mechanisms in MRSA strains from the private health sector in SA hence the need for implementing effective infection control programs.

1. Introduction

MRSA is characterized by the presence of *mecA* that confers resistance to methicillin. This has far reaching consequences in the public health, economic and social sectors.[1] These strains also harbor mobile genetic elements (MGEs), including plasmids, pathogenicity islands, transposons, integrons and prophages, which comprise 15–25% of the genome. An understanding of these MGEs will broaden our knowledge on the genetic determinants of antibiotic resistance (AR).[2] Although research has been conducted on MRSA in SA, information on the plasmid content is largely unknown, a study of this nature is important understanding AR patterns, comparing the plasmid profiles will help in effective infection control. The aim of this study was to ascertain the genetic relatedness, and characterize the plasmid-encoded antibiotic resistance and virulence profile of clinical MRSA isolates collected obtained from a private laboratory in Durban, SA over a three month period.

2. Methods

A total of 27 consecutive non-repetitive MRSA isolates were obtained from June to August 2015, from a pathology laboratory that caters for the private healthcare sector. The isolates were

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Table 1
Clinical data, minimum inhibitory concentrations (MIC), and results of PCR for 27 MRSA isolates

Isolate No.	Clinical data					MIC (mg/l)[b]												PCR								
	Hos. code[c]	Source[a]	Ward type[a]	Sex	Age[a]	AP	CP	GT	ET	RF	TT	CM	DP	VM	LZ	FA	TG	*mecA*	*blaZ*	*ermC*	*aac-aph*	*tetK*	*hla*	*hld*	*eta*	*lukS/F-PV*
B11970	1	Blood	Neo ICU	F	NB	>512	0.5	32	8	≤0.25	2	≤0.25	1	1	2	≤0.25	≤0.25	+	+	+	+	-	+	+	-	-
P10781	15	Nasal	OPD	M	86	>512	256	64	32	512	256	≤0.25	0.5	1	2	≤0.25	≤0.25	+	+	+	+	-	+	+	-	-
P10747	2	CVP	ICU	F	66	>512	4	>64	64	512	128	≤0.25	0.5	0.5	1	≤0.25	≤0.25	+	+	+	+	-	+	+	-	-
S37938	-	-	-	-	-	>512	256	16	32	256	64	2	0.5	1	2	≤0.25	≤0.25	+	+	+	+	-	+	+	-	-
S18155	3	ETT	ICU	F	76	>512	256	64	64	256	128	≤0.25	0.25	0.5	2	≤0.25	≤0.25	+	+	-	+	-	+	-	-	-
B13178	5	Blood	LW	F	26	>512	256	>64	64	512	128	≤0.25	0.5	1	2	≤0.25	≤0.25	+	+	+	+	-	+	+	-	-
440260	-	-	-	-	-	>512	>512	>64	64	256	128	≤0.25	0.5	1	2	≤0.25	≤0.25	+	+	+	+	-	+	+	-	-
S18970	-	-	-	-	-	>512	256	64	32	512	64	≤0.25	0.5	0.5	2	≤0.25	≤0.25	+	+	-	+	-	+	+	-	-
P11520	6	Pus	OPD	M	62	512	>512	0.25	0.5	≤0.25	≤0.25	≤0.25	0.5	1	2	≤0.25	≤0.25	+	+	-	-	-	+	+	-	-
T5683	7	Nasal	OPD	F	43	>512	8	0.5	0.5	256	32	≤0.25	0.5	1	1	≤0.25	≤0.25	+	+	-	-	-	+	+	-	-
B15227	1	Blood	Neo ICU	F	NB	>512	4	64	8	≤0.25	≤0.25	≤0.25	1	1	1	≤0.25	≤0.25	+	+	+	+	-	+	+	-	-
P13563	-	-	-	M	49	>512	128	>64	0.5	128	16	≤0.25	0.5	1	2	≤0.25	≤0.25	+	+	-	+	-	+	+	-	-
S22589	4	Sputum	ICU	M	49	>512	128	>64	0.5	128	64	≤0.25	1	1	2	≤0.25	≤0.25	+	+	-	+	-	+	+	-	-
B15612	8	Blood	ICU	M	46	>512	128	>64	16	512	256	≤0.25	1	1	2	≤0.25	≤0.25	+	+	-	+	-	+	-	-	-
B15810	5	Pus	Surgical	M	41	>512	256	32	16	128	64	≤0.25	0.5	1	2	0.5	≤0.25	+	+	+	+	-	+	+	-	-
B15583	1	Blood	ICU	F	37	>512	16	>64	2	64	2	≤0.25	0.5	1	2	≤0.25	≤0.25	+	+	-	+	-	+	+	-	-
S24463	10	ETT	ICU	F	59	512	1	32	1	≤0.25	≤0.25	1	0.5	0.5	2	≤0.25	≤0.25	+	+	+	+	-	+	+	-	-
P15045	1	Wound	Surgical	F	47	>512	64	64	16	256	64	≤0.25	0.25	0.25	2	≤0.25	≤0.25	+	+	-	+	-	+	+	-	-
P15028	10	Eye	Nursery	F	NB	512	4	16	0.5	0.5	≤0.25	≤0.25	0.25	1	2	≤0.25	≤0.25	+	+	-	+	-	-	+	-	-
P14890	11	Wound	ICU	F	41	512	256	64	0.5	256	128	≤0.25	0.5	1	2	≤0.25	≤0.25	+	+	-	+	-	+	+	-	-
P15558	1	CVP	Medical	F	94	512	>512	0.12	1	256	≤0.25	>512	0.5	0.5	1	≤0.25	≤0.25	+	+	+	+	-	+	+	-	-
P15469	12	Humerus	General	F	68	128	64	1	0.5	≤0.25	0.5	≤0.25	0.25	0.5	2	≤0.25	≤0.25	+	+	-	+	-	+	+	-	-
P15490	13	Bone	General	M	63	>512	128	32	16	128	64	≤0.25	0.25	0.5	2	≤0.25	≤0.25	+	+	+	+	-	+	+	-	-
P15742	6	cheek	Trauma	M	29	256	0.5	0.5	16	≤0.25	64	≤0.25	0.25	0.5	2	≤0.25	≤0.25	+	+	+	+	-	+	+	-	-
P15825	14	Buttock	Paediatri	M	5	512	1	0.5	0.5	≤0.25	0.25	≤0.25	0.25	1	2	≤0.25	≤0.25	+	+	-	+	-	+	+	-	-
P15793	2	Head	Surgical	M	10	512	256	64	32	256	32	≤0.25	0.5	0.5	2	≤0.25	≤0.25	+	+	+	+	-	+	+	-	-
T8060	-	-	-	-	-	512	4	4	16	128	64	≤0.25	0.5	0.5	2	≤0.25	≤0.25	+	+	-	+	-	+	+	-	-

[a] ETT, Endotracheal tube; CVP, Central venous catheter; ICU, Intensive/High care unit; LW, Labour ward: OPD, outpatient department, NB, Newborn (day 0), -, No information.
[b] AP, ampicillin; CP, ciprofloxacin; GT, gentamicin; ET, erythromycin; RF, rifampicin; TT, tetracycline; CM, clindamycin; DP, daptomycin; VM, vancomycin; LZ, linezolid; FA, fusidic acid; TG, tigecycline.
[c] The numbers 1–15 indicates codes of the hospital centers where the MRSA isolates were collected.

identified using Vitek 2 (bioMerieux, Durham, NC, USA) and confirmed by matrix assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF/MS). The cefoxitin disc diffusion (CDD) test was used to identify putative MRSA, which was then confirmed by PCR detection of *mecA*. The MIC was determined for 12 antibiotics by the broth microdilution method. The Clinical and Laboratory Standards Institute (CLSI) Guideline[3] was used for interpreting the results, and *Staphylococcus aureus* ATCC 29213 was used as the control. Isolates resistant to β-lactams, and at least three classes of antibiotics, were defined as multidrug resistant (MDR). A plasmid DNA extraction kit (GeneJET Plasmid Miniprep kit, Thermoscentific) was used to purify the plasmid DNA from all 27 MRSA strains, according to the manufacturer's instructions. The presence of resistance genes conferring resistance to ampicillin-penicillin (*blaZ*), aminoglycoside (*aac (6′)–aph* (2″)), macrolide-lincosamide-streptogramins B [MLS_B] (*ermC*) and tetracycline (*tetK*) were determined using PCR. The virulence determinants encoding the bio-component Panton-Valentine leukocidin (*LukS/F-PV* gene), exfoliative toxin (*eta*), alpha and delta hemolysin genes (*hla and hld*) were also ascertained by PCR. PFGE was used to predict the genetic relatedness of the MRSA isolates. Clusters were defined using the criterion of a difference of ≤6 bands, as described by Tenover *et al*[4], and a similarity cut-off of ≥70.0%.

3. Results and discussion

The demographic data and resistance patterns of the isolates and MICs are summarized in Tables 1 and S1. Ampicillin showed no activity against MRSA isolates, while 85.2% (23/27) were resistant to ciprofloxacin, 74.1% (20/27) to gentamicin, 70.4% (19/27) to rifampicin, 66.7% (18/27) to tetracycline, 63.0% (17/27) to erythromycin, and 11.1% (3/27) to clindamycin. Multidrug resistance (MDR) was determined in 74.1% (20/27) of the MRSA isolates. Resistance rates in this study varied compared to those seen in another KZN study on 61 confirmed MRSA isolates by Shittu *et al*[5], particularly for gentamicin (74.1% vs. 96.7%), rifampicin (70.4% vs. 73.8%), tetracycline (66.7% vs. 90.2%) and erythromycin (63.0% v. 82.0%). Resistance to clindamycin of 11.1% was also much lower in this investigation than the rates of 82%[5] and 62.5%[6] reported in other studies conducted on MRSA isolates in KZN and SA. Only the ciprofloxacin resistance rate in our study was notably higher (85.2% v. 18%[5]), with its resistance on MRSA isolates in SA having been reported to 69.7% and 88.7% in the private sector.[6] Multidrug resistance rate was lower (74.1% vs. 87%) than those reported by Shittu *et al*[5] but similar to a study by Heysell *et al*[7] in KZN with a rate of 79% on 19 clinical MRSA. All MRSA isolates were susceptible to daptomycin, vancomycin, linezolid, fusidic acid and tigecycline, ampillicin. The susceptibility

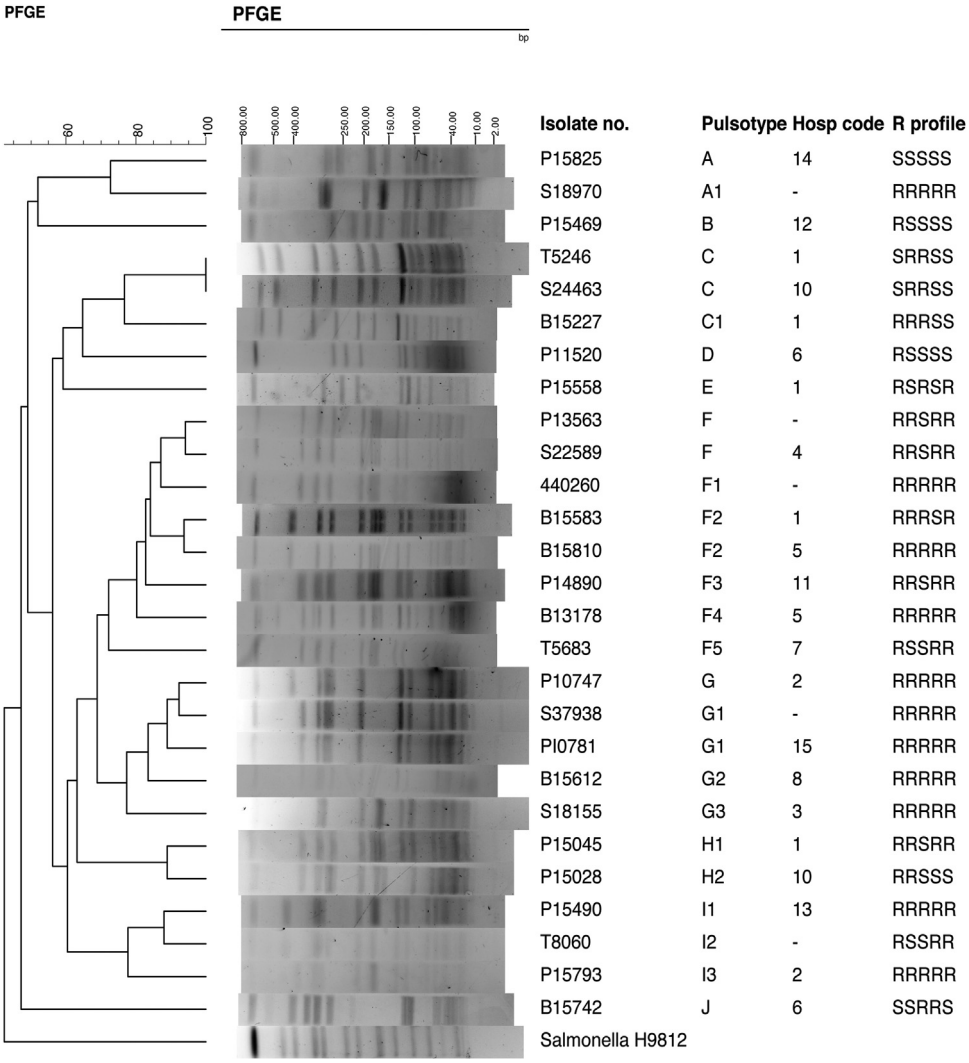

Figure 1. PFGE *Sma*I genotypic types generated from clinical MRSA isolates from private sector in KZN. Pretested *Salmonella* serotype *Braenderup* strain H9812 was used as the reference control strain. The R and S indicate resistance or susceptibility for ciprofloxacin, gentamicin, erythromycin, tetracycline and rifampicin respectively. The alphabets A–J shows the main pulsotype and subtype of each isolate. The numbers 1–15 indicates codes of the hospital centers where the MRSA isolates were collected.

patterns of the isolates in this study were comparable to studies conducted on MRSA in South Africa[5,6] which were totally susceptible to daptomycin, vancomycin, linezolid, fusidic acid and tigecycline. The susceptibility of MRSA to these antibiotics observed in this study confirms their use as treatment options for infections in SA.

The structural component of *mecA* encodes the penicillin-binding protein 2a (PBP2a) that establishes resistance to methicillin, other semisynthetic penicillinase-resistant beta-lactams that are frequently co-carried with genes conferring resistance to aminoglycosides, macrolide-lincosamide-streptogramin B [MLS_B] and spectinomycin.[8] All the isolated plasmids of the MRSA isolates contained the *mecA* and *blaZ* resistance genes, showing the correlation between MICs and the presence of genes encoding resistance against beta-lactams. The gentamicin resistance gene *aac (6′)–aph* (2″) was identified in 25 (92.6%) of the isolates, which varied from the phenotypic resistance profile of 74.1%, indicating that gene carriage does not necessarily translate into the resistance phenotype. The *ermC* gene responsible for macrolide-lincosamide-streptogramins B [MLS_B] resistance was amplified in 48.2% (13/27) of the MRSA isolates, while it was not found in those that were susceptible to both erythromycin and clindamycin. The 23.5% (4/17) with phenotypic resistance to MLS_B that did not contain the *ermC* gene indicates the occurrence of other resistance mechanism, *ermA, ermB and msrA*, which was not investigated in this study but have been previously reported.[9] This confirms that the incidence of MLS_B phenotypes and genotypes vary according to country, patterns of infections and drug use.[9] Although there was high tetracycline resistance, the *tet*K gene was not detected, indicating that this may be due to different mechanisms and not mediated by active drug efflux, as *tet*K resistance has so far not been reported in clinical MRSA studies in South Africa.

The prevalence of virulence factors in all isolated plasmids showed a similar trend, with the *hla* and *hld* being the most abundant genes, with frequencies of 96.3% (26/27) and 92.6% (25/27) respectively. Comparatively, this was similar to other studies conducted from Uganda[10] and United States,[11] with either *hla* being more frequent than the *hld* genes, or both showing 100% co-dominance. The prevalence rate of *eta* in our study was 0%, however, the prevalence of *eta* differed among studies, which could be associated with a variety of geographical and health conditions.[12] *LukS/F-PV* was not detected in any of the 27 clinical MRSA isolates, which was comparable to a study conducted in South Africa on 320 clinical MRSA isolates with only one positive LuKS/F-PV gene being detected.[13]

The PFGE profiles and the dendrogram of the MRSA isolates are shown in Figure 1. PFGE analysis grouped the 27 isolates into 10 pulsotypes designated A-J, displaying 70.0% similarity, and correlating with their resistance profile and the genetic determinants tested in this study (Fig. 1). Of note, 85.2% of the isolates were clustered into six major PFGE types: pulsotypes F (8/27 strains; 29.6%), G (5/27; 18.5%), C, I (3/27; 11.1%) and A, H (2/27; 7.4%). Pulse types B, D, E and J were each represented by single isolates. Although the sample size was too small to show a definite correlation, the assertion of similar circulating clones in the province was supported by our study, as the PFGE analysis revealed some form of association between pulsotypes and the centers of sample collection. Centers 1 and 10 were found to contain pulsotypes C and H, while identical pulsotypes F and G were spread across nine of the 15 centers, intimating the possibility of similar clones of MRSA within the health care centers in the province as predicted by Shittu *et al*[14] and Moodley *et al*[13] in their study in KZN and SA respectively.

To the best of our knowledge this is the first study characterizing the plasmid-mediated resistance and virulence genes of clinical MRSA isolates in the private sector in Durban. The study provides a private sector perspective of antibotic resistance patterns and genetic relatedness of MRSA highlighting the need for implementing efficient and effective infection control programs.

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Ethical approval: Permission to carry out this study was granted by the Biomedical Research Ethics Committee (BREC) (**REF/No: BE394/15**) of the University of KwaZulu-Natal (UKZN).

Conflict of interest: Professor SY Essack is a member of the Global Respiratory Infection Partnership sponsored by Reckitt and Benckiser.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ijid.2016.03.019.

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