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Molecular characteristics of *Staphylococcus aureus* isolated from a major hospital in Lebanon

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SUMMARY

Objectives: The occurrence and dissemination of methicillin-resistant *Staphylococcus aureus* (MRSA) in healthcare settings and the community and its risk of being introduced into hospitals are matters of great concern. The purpose of this study was to conduct a miniaturized epidemiological analysis of *S. aureus*-associated infections and to characterize the isolates by a variety of molecular typing techniques. Ongoing molecular surveillance is essential to prevent *S. aureus* strains from becoming endemic in the Lebanese healthcare setting.

Methods: A total of 132 *S. aureus* from different clinical specimens were isolated over a 6-month period. Characterization of the isolates was done by detection of the *mec*A gene, Panton–Valentine leukocidin determinant detection, staphylococcal chromosomal cassette (SCC*mec*) typing of MRSA, *S. aureus* protein A (*spa*) typing, multilocus sequence typing (MLST), pulsed-field gel electrophoresis (PFGE), and antibiogram analysis.

Results: MRSA represented 30% of the isolates, with PVL being detected in 54% of MRSA and 12% of methicillin-susceptible *S. aureus* (MSSA). A difference between MRSA and MSSA was observed in the *spa* types. Clustering SCC*mec* with MLST identified seven MRSA and 20 MSSA clones, with PVL-positive ST80-MRSA-IV being the dominant clone (7%), while PFGE revealed 32 groups with 80% cutoff similarity.

Conclusions: Although the results of this study are based on samples collected from one hospital, the high diversity observed along with the lack of any equivalence in the genetic backgrounds of the major MSSA and MRSA clones, emphasizes the urgent need for standardized surveillance combined with the application of well-validated typing methods to assess the occurrence of MRSA and subsequently to control its spread.

1. Introduction

Staphylococcus aureus is a major human pathogen that causes a broad range of serious community-acquired and nosocomial diseases in humans, from minor skin infections to severe infections such as septicemia.[1] The increasing prevalence of methicillin-resistant *S. aureus* (MRSA), and its ability to spread in the hospitals and the community, has posed a major challenge for infection control.[2] Today, although community-associated MRSA (CA-MRSA) imposes low biological cost since it carries a small, easily transferable type of staphylococcal cassette chromosome *mec* (SCC*mec*) and exhibits relative resistance to a limited number of antimicrobial agents, it continues to be a major public health crisis. The fast dissemination of CA-MRSA in the community and its ability to invade hospital settings thus replacing the traditional hospital-associated MRSA (HA-MRSA) strains, makes the epidemiological understanding of CA-MRSA even more complex.[1,3]

The Panton–Valentine leukocidin (PVL) toxin, a prophage-encoded bicomponent pore-forming protein, has been strongly linked epidemiologically to prevalent CA-MRSA strains, although its role in pathogenicity remains controversial, with a number of studies showing its association with primary skin infections and necrotizing pneumonia, while others mitigating its significance as a virulence factor.[1,4–7]

Knowledge of epidemic MRSA clones can help in the development of effective strategies to aid in controlling spread, optimizing

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treatment, and revealing the mode of pathogenicity.[8,9] Monitoring the evolutionary process of prevalent MRSA clones through current genotyping techniques is a crucial step to reveal relatedness among isolates, with sequencing of protein A (*spa* typing), SCC*mec* typing of MRSA, multilocus sequence typing (MLST), and pulsed-field gel electrophoresis (PFGE) being the most valuable typing tools.

In Lebanon, there are limited data on the prevalence of *S. aureus* obtained from both inpatient and outpatient populations, there is no information on the methicillin-susceptible *S. aureus* (MSSA)/MRSA population structure, and little attention has been paid to the molecular epidemiology of this pathogen. Previous findings from Lebanon have provided the first snapshot of the genetic population structure of *S. aureus* in the country.[10] Ongoing molecular surveillance is essential to prevent *S. aureus* strains from becoming endemic in the Lebanese healthcare setting. Accordingly, this study was conducted to better understand *S. aureus*-associated infections and to characterize representative isolates by a variety of molecular typing techniques.

2. Methods

2.1. Clinical isolates

Between May and October 2011 (6 months), a total of 132 consecutive non-duplicate *S. aureus* (designated HST 1–132) clinical samples representing all cultured *S. aureus* isolates recovered in the Clinical Microbiology Section of the American University of Beirut Medical Center (AUB-MC) were collected. AUB-MC provides tertiary services for over 300 000 patients annually with a 350-bed inpatient capacity, occupied by an expatriate population from all over Lebanon as well as neighboring countries. As such, the strain diversity in the hospital is likely to reflect the diversity in the region. All isolates were confirmed as *S. aureus* by growth on mannitol salt agar (MSA), Gram staining, and positive catalase reaction, as well as the ability to produce coagulase enzyme using the SLIDEX Staph Plus agglutination kit (bioMérieux, France).

2.2. Statistical analysis

Categorical comparisons were performed using the Chi-square test. A *p*-value of less than 0.05 was considered to be significant. The associations between MRSA and MSSA carriage along with patient demographics and characteristics were evaluated using the R statistical package (v. 3.0.1). Odds ratios (ORs) and their 95% confidence intervals (95% CIs) were also calculated. The functions used in R included "chisq.test()" from the package "stats" and "oddsratio.wald()" from the package "epitools".

2.3. DNA extraction

DNA was extracted using the Nucleospin Tissue genomic DNA kit (Macherey-Nagel, Germany) in accordance with the manufacturer's instructions.

2.4. Molecular approaches

Amplification of the 16S rRNA, PVL, and *mec*A genes was done as described previously.[11] A PVL-negative MRSA reference strain (N315) and a PVL-positive MSSA reference strain (ATCC 49775) were used. SCC*mec* elements of MRSA were typed using previously described PCR primers.[12] The conditions of the PCR were first optimized using the following reference strains: MRSA NCTC 10442 (SCC*mec* I), MRSA N315 (SCC*mec* II), MRSA 85/2082 (SCC*mec* III), MRSA JCSC 4744 (SCC*mec* IVa), MRSA JCSC 2172 (SCC*mec* IVb), MRSA JCSC 47882 (SCC*mec* IVc), and MRSA WIS (SCC*mec* V). Typing of the polymorphic X region of the *S. aureus* protein A (Spa) was carried out by amplifying the *spa* gene as described previously.[13,14] Thirty-six isolates representing all *spa* clonal clusters (*spa*-CCs) were MLST-typed. Amplification of seven housekeeping genes (carbamate kinase (*arcC*), shikimate dehydrogenase (*aroE*), glycerol kinase (*glpF*), guanylate kinase (*gmk*), phosphate acetyltransferase (*pta*), triose-phosphate isomerase (*tpi*), and acetyl coenzyme A acetyltransferase (*yqiL*)) by MLST was done according to published sequences.[15] Isolates subjected to MLST typing were also typed using PFGE. Genomic DNA was restricted with *Sma*I and the resulting fragments were separated by PFGE.[16]

2.5. Antibiotic susceptibility testing

All isolates were tested for antibiotic resistance by Kirby–Bauer disk diffusion method, in accordance with the standards recommended by the Clinical and Laboratory Standards Institute.[17] Resistance was tested against the following antibiotics: augmentin, cephalothin, ciprofloxacin, clindamycin, erythromycin, gentamicin, oxacillin, rifampin, teicoplanin, tetracycline, and trimethoprim–sulfamethoxazole. All disks were obtained from Oxoid, UK, and *S. aureus* ATCC 25923 was used as a quality control strain. Isolates showing resistance to erythromycin were further tested for inducible resistance, as described previously.[18]

3. Results and discussion

A total of 132 isolates recovered between May and October 2011 were characterized. Skin and soft tissue infections (SSTIs) accounted for 42% of clinical presentations, which is consistent with the high prevalence of SSTIs caused by *S. aureus*[1] (Table 1). Twenty-one MRSA isolates were PVL-positive (21/39; 54%), while 11 MSSA isolates were PVL-positive (11/93; 12%); the percentage of MRSA PVL-positive was significantly higher than that of the MSSA strains ($p = 8.79 \times 10^{-7}$). However, significant differences were not detected between MRSA and MSSA with respect to specimen origin, gender, or patient status (Table 1).

MRSA represented 30% of the isolates collected in this study, which is significantly lower than the percentage reported previously (93/130; 72%) in a study from Lebanon, where a number of randomly collected isolates from the same hospital (AUB-MC) were partially characterized.[10] It is important to note that an infection control and prevention program (ICPP) serves the AUB-MC to limit MRSA infections.[19] Practices in this program include, but are not limited to, standard precautions (hand hygiene, use of gloves and gowns, appropriate handling of patient care equipment and laundry) and contact precautions (patient placed in a single-patient room when available, limiting patient transport outside the room, use of disposable non-critical patient-care equipment when necessary, frequent cleansing and disinfection of the rooms). The occurrence of MRSA among *S. aureus* varies according to the geographical region, with a low frequency (~1%) in some countries in Europe (e.g., the Netherlands, Denmark, and Sweden) and a high frequency (>60%) in countries such as the USA and Japan.[20–22]

SCC*mec* typing revealed the prevalence of the mobile genetic element SCC*mec* type IV (33/39; 85%), commonly known to be associated with CA-MRSA infections.[23–25] All MRSA isolates harboring the SCC*mec* IV cassette were positive for the PVL gene, while five (13%) harboring the SCC*mec* V cassette were PVL-negative. It is noteworthy that one MRSA recovered from the sputum of a 64-year-old female in the intensive care unit, showed resistance to almost all tested antibiotics and harbored SCC*mec* III; SCC*mec* III is known to be associated with HA-MRSA.[25–27] Similar results were obtained in the study conducted by Tokajian et al. in

Table 1
Patient demographics and characteristics of MRSA and MSSA isolates

Aspect	MRSA ($n = 39$)		MSSA ($n = 93$)		p-Value[a]	OR (95% CI)
	n[b]	%	n	%		
Specimen origin						
Wound/cyst/abscess	17	44	39	42	0.8579	1
Respiratory	12	31	33	35		1.19 (0.50–2.86)
Other[c]	10	26	21	23		0.91 (0.35–2.35)
Sex						
Male	26	67	58	62	0.7869	1
Female	13	33	35	38		0.828 (0.37–1.82)
Patient						
Inpatient	22	56	49	53	0.8415	1.162 (0.54–2.46)
Outpatient	17	44	44	47		1
PVL						
Positive	21	54	11	12	8.79×10^{-7}	8.696 (3.57–21.18)
Negative	18	46	82	88		1

MRSA, methicillin-resistant *Staphylococcus aureus*; MSSA, methicillin-susceptible *Staphylococcus aureus*; OR, odds ratio; CI, confidence interval; PVL, Panton–Valentine leukocidin gene.
[a] Chi-square.
[b] Numbers are rounded up.
[c] Others: aspirates, ear, joint fluid, urine, blood, catheter.

Lebanon, in which SCC*mec* IV was detected in 88% of the MRSA isolates.[10] PVL along with SCC*mec* type IV are markers for CA-MRSA infections in Europe and the USA,[28–30] and having PVL-positive MRSA with type IV SCC*mec* is in agreement with previous studies.[10,31–33] The detection of PVL-negative MRSA carrying type V SCC*mec* is also in concordance with several other studies conducted in Lebanon and elsewhere.[10,33–36]

The isolates investigated in this study ($n = 132$) were assigned to 71 different *spa* types, with the most common being t021 (6%), t044 (5%), and t267 (5%). Fifty-three different *spa* types were identified in the MSSA isolates ($n = 93$) compared to 25 in the MRSA isolates ($n = 39$). Using the 'based upon repeat pattern' (BURP) algorithm, *spa* types were clustered into 40 different groups, with 15 groups comprising more than one *spa* type and 25 so-called singletons (Figure 1). The most prevalent *spa* type within the MRSA population was *spa* t044 (5/39; 13%), which is in agreement with other studies from within the region (Jordan and Lebanon),[10,33] as well as in Europe.[37] However, *spa* t021 (7/93; 8%) was the predominant type within the MSSA isolates, and this differs from previous findings.[10,33] *spa* t021 has been detected in

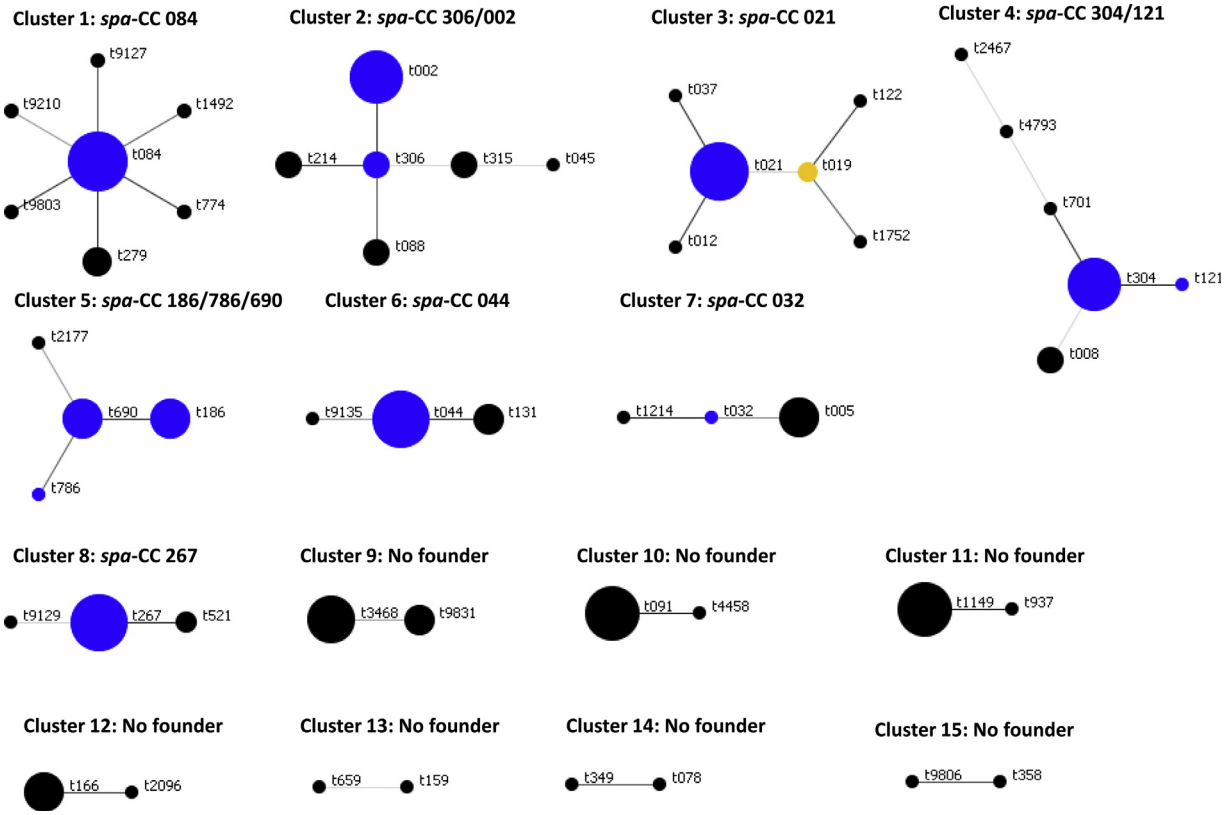


Figure 1. Population snapshot based on BURP analysis of 132 isolates. BURP grouping using default parameters resulted in 15 *spa*-CCs with eight having group founders, 25 singletons, and four excluded *spa* types. Each dot represents a unique *spa* type. The diameter of the dot is proportional to the quantity of the corresponding *spa* type. Blue dots represent group founders, defined as the *spa* type(s) with the highest founder score within a CC, while yellow dots represents major subgroup founders.

Table 2
Molecular characteristics of the nine novel *spa* types detected in this study

spa type	Number found among isolates	*mec*A	PVL	Ridom repeat succession	*spa*-CC
t9804	1	MSSA	Neg	08–16–34–02–43–34–16–16–34–02–16	Singletons
t9805	2	MSSA	Pos	26–22–10–17–20–17–12	Singletons
		MSSA	Neg		
t9806	1	MSSA	Neg	7–17–12–23–02–12–23	No founder
t9127	1	MSSA	Neg	07–23–12–34–34–12–36–23–02–12–23	84
t9128	1	MSSA	Neg	26–23–34–23–31–05–17–17–25–16–28	Singletons
t9129	1	MSSA	Neg	7–16–21–17–34–34–34–33–34	267
t9830	1	MSSA	Neg	04–17–34–17–32–23–24–24	Singletons
t9831	2	MRSA	Neg	07–23–12–12–20–17–12–12–12	No founder
t9832	2	MRSA	Neg	125–13–23–31–29–25–17–25–16–28	Singletons

*mec*A, methicillin resistance encoding gene; PVL, Panton–Valentine leukocidin gene; *spa*-CC, *spa* clonal complex; MSSA, methicillin-susceptible *Staphylococcus aureus*; MRSA, methicillin-resistant *Staphylococcus aureus*.

Ireland (7%), Romania (12%), Portugal (4%), and other areas in Europe.[37,38] Finally, 12 isolates were assigned to nine new *spa* types assigned by Ridom StaphType software (http://spaserver.ridom.de/) (Table 2).

Thirty-six isolates consisting of 11 MRSA and 25 MSSA were MLST-typed. The isolates were chosen to represent all the 15 *spa*-CC groups, five of the singletons, and one of the excluded isolates (Table 3). There were seven major MRSA clones defined as isolates with the same sequence type (ST) and SCC*mec* type. These clones were associated with complexes CC5, CC22, CC30, CC72, CC80, and CC239. On the other hand, 20 MSSA clones were identified and these were associated with complexes CC1, CC6, CC7, CC8, CC15, CC25, CC30, CC45, CC88, CC97, CC121, CC361, CC398, CC641, CC770, and CC789. Twenty-five allelic profiles were generated with the most prevalent STs being ST80 (4/36; 11%) and ST30 (3/36; 8%), which is in agreement with previous results from Lebanon, Jordan, Kuwait, and other neighboring countries.[10,33,39–41]

Table 3
Overview of representative MRSA and MSSA *spa* types and their corresponding MLST clones

Clone[a]	MLST-CC	*spa* type (number of isolates)	*spa*-CC	PVL
ST5-MRSA-IV	5	t002 (2)	306/002	Neg
ST5-MRSA-IV	5	t214 (2)	306/002	Neg
ST6-MSSA	6	t304 (4)	304/121	Neg
ST361-MSSA	361	t315 (2)	306/002	Neg
ST199-MSSA	15	t084 (4)	084	Neg
ST199-MSSA	15	t9210 (1)	084	Neg
ST15-MSSA	15	t774 (1)	084	Neg
ST8-MSSA	8	t008 (2)	304/121	Pos
ST30-MSSA	30	t012 (1)	021	Neg
ST30-MSSA	30	t021 (7)	021	Pos
ST30-MRSA-IV	30	t019 (2)	021	Pos
ST34-MSSA	30	t2096 (1)	No founder	Neg
ST34-MSSA	30	t166 (2)	No founder	Neg
ST80-MRSA-IV	80	t021 (1)	021	Neg
ST80-MRSA-IV	80	t044 (5)	044	Pos
ST80-MRSA-IV	80	t131 (3)	044	Pos
ST80-MRSA-IV	80	t6476 (1)	sg No. 9	Pos
ST720-MSSA	121	t659 (1)	No founder	Neg
ST1-MSSA	1	t127 (5)	sg No. 3	Neg
ST22-MRSA-IV	22	t9832 (2)	sg No. 15	Neg
ST508-MSSA	45	t861 (1)	sg No. 7	Neg
ST46-MSSA	45	t132 (2)	Excluded	Neg
ST88-MSSA	88	t186 (2)	186/786/690	Neg
ST72-MRSA-IV	72	t3468 (1)	No founder	Neg
ST72-MRSA-V	72	t9831 (2)	No founder	Neg
ST97-MSSA	97	t9129 (1)	267	Neg
ST97-MSSA	97	t044 (1)	044	Neg
ST291-MSSA	398	t937 (1)	No founder	Neg
ST291-MSSA	398	t1149 (5)	No founder	Neg
ST7-MSSA	7	t091 (5)	No founder	Neg
ST789-MSSA	789	t9806 (1)	No founder	Neg
ST770-MSSA	770	t377 (2)	sg No. 5	Neg
ST641-MSSA	641	t005 (2)	032	Neg
ST25-MSSA	25	t078 (1)	No founder	Neg
ST239-MRSA-III	239	t037 (1)	021	Neg
ST1995-MSSA	25	t349 (1)	No founder	Neg

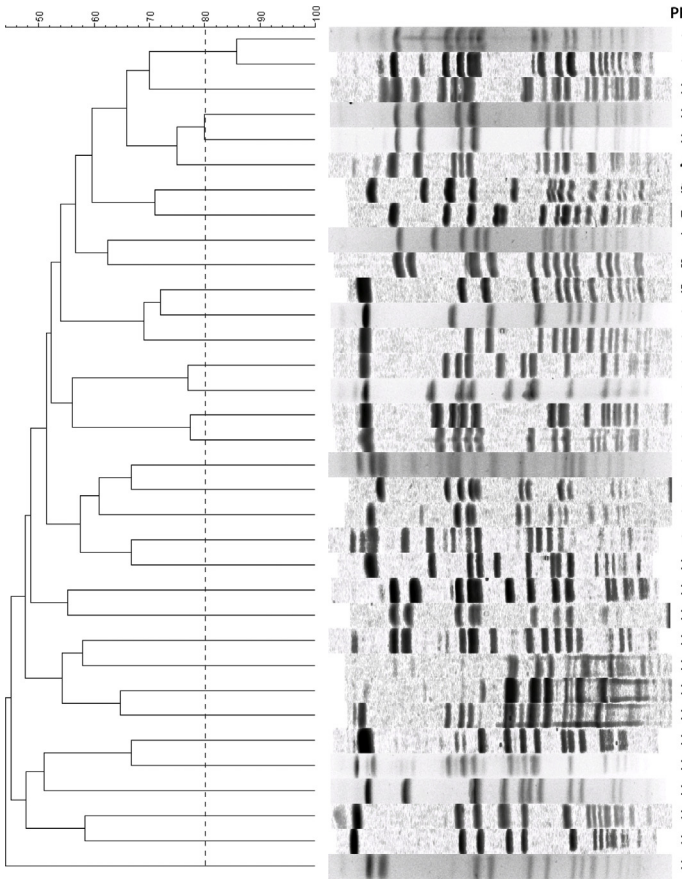
MRSA, methicillin-resistant *Staphylococcus aureus*; MSSA, methicillin-susceptible *Staphylococcus aureus*; MLST, multilocus sequence typing; *spa*-CC, *spa* clonal complex; MLST-CC, multilocus sequence type clonal complex; PVL, Panton–Valentine leukocidin gene; sg, singleton.

[a] Thirty-six strains were typed by MLST representing all 15 *spa*-CC groups, five of the singletons, and one of the excluded isolates.

Clustering SCC*mec* with MLST identified seven MRSA and 20 MSSA clones, with PVL-positive ST80-MRSA-IV being the dominant clone (9/36; 7%), followed by PVL-positive ST30-MSSA (7/36; 5%) (Table 3). The predominance of the ST80 clone has previously been reported in the region,[33,39–42] while ST30-MSSA, known as the Southwest Pacific clone, has been shown to prevail in other areas, in particular Kuwait, Abu Dhabi, the UK, Germany, and Australia,[2] and to probably have been introduced through air travel. One strain among the ST80-IV was PVL-negative harboring *spa* type t021; this is not common within CC80, as it is usually associated with strains belonging to CC30 (http://spaserver2.ridom.de/spa-t021.shtml).[43]

All isolates typed using PFGE were typeable by *Sma*I macro-restriction and produced different pulsotypes according to previously defined standards.[44] Thirty-two groups were defined by employing an 80% similarity cutoff value, with only two groups containing more than one isolate. Clusters were designated with numbers from 1 to 32 (Figure 2). Typing results combining MLST and PFGE showed that several isolates belonging to the same MLST CC were assigned to different PFGE clusters; CC30 and CC80 strains were present in more than one PFGE cluster. On the other hand, strains belonging to the same PFGE cluster generally belonged to the same MLST CC (e.g., CC15). Similarly, isolates harboring the same *spa* types were found to belong to distinct PFGE clusters: t021 belonging to PFGE types 9 and 28, and t044 belonging to PFGE types 12 and 19. Of note, PFGE grouped isolates belonging to the same *spa*-CCs as genetically close (*spa*-CC 084 were assigned to PFGE types 3 and 4). This could indicate higher diversity being detected with the use of PFGE compared to that defined by *spa* typing and/or MLST, making PFGE the 'gold standard' typing technique.[45,46] Although, PFGE is highly reproducible and remains an important tool for micro- and macro-epidemiological surveys, the technique suffers from several disadvantages. These include: the high cost during outbreaks, technical challenges, it is time-consuming and labor intensiveness, and it requires complex inter-laboratory exchange of results; the hampered typeability of isolates with methylated *Sma*I sites is also a drawback.[47,48]

Seventy-four (56%) out of the 132 isolates were sensitive to all tested antibiotics. Among the remaining 58 (44%), resistance was detected to one or more of the antibiotics used. According to the definitions proposed by Magiorakos et al.,[49] 22% of the isolates were multidrug-resistant (MDR) bacteria by being MRSA and 5%



PFGE-Type	Sample	*mec*A	PVL	SCC*mec*	*spa* Type	*spa*-CC	ST Type	MLST CC
1	HST 075	MSSA	Neg.		t304	304/121	6	6
1	HST 089	MSSA	Neg.		t091	No founder	7	7
2	HST 067	MSSA	Neg.		t127	Singletons	1	1
3	HST 081	MSSA	Neg.		t774	84	15	15
3	HST 120	MSSA	Neg.		t084	84	199	15
4	HST 021	MSSA	Neg.		t9210	84	199	15
5	HST 077	MRSA	Neg.	III	t037	21	239	239
6	HST 110	MSSA	Neg.		t186	186/786/690	88	88
7	HST 069	MSSA	Neg.		t315	306/002	361	361
8	HST 098	MSSA	Neg.		t1149	No founder	291	398
9	HST 096	MSSA	Pos.		t021	21	30	30
10	HST 112	MSSA	Neg.		t012	21	30	30
11	HST 095	MSSA	Neg.		t166	No founder	34	30
12	HST 091	MRSA	Pos.	IV	t044	44	80	80
13	HST 119	MRSA	Pos.	IV	t131	44	80	80
14	HST 084	MRSA	Neg.	V	t9831	No founder	72	72
15	HST 121	MSSA	Pos.		t008	304/121	8	8
16	HST 043	MSSA	Neg.		t2096	No founder	34	30
17	HST 116	MSSA	Neg.		t044	44	97	97
18	HST 073	MSSA	Neg.		t078	No founder	25	25
19	HST 023	MSSA	Neg.		t9129	267	97	97
20	HST 111	MSSA	Neg.		t377	Singletons	770	770
21	HST 041	MRSA	Neg.	IVa	t002	306/002	5	5
22	HST 132	MSSA	Neg.		t005	32	641	641
23	HST 024	MSSA	Neg.		t937	No founder	291	398
24	HST 038	MRSA	Neg.	IVa	t214	306/002	5	5
25	HST 040	MRSA	Neg.	IV	t3468	No founder	72	72
26	HST 078	MRSA	Pos.	IV	t6476	Singletons	80	80
27	HST 060	MRSA	Neg.	IVa	t9832	Singletons	22	22
28	HST 130	MRSA	Neg.	IV	t021	21	80	80
29	HST 115	MSSA	Neg.		t659	No founder	720	121
30	HST 080	MSSA	Neg.		t132	Excluded	46	45
31	HST 088	MSSA	Neg.		t861	Singletons	508	45
32	HST 009	MRSA	Pos.	IVc	t019	21	30	30

Figure 2. Dendrogram of PFGE clusters and genotypic relationships of *Staphylococcus aureus* isolates. *Sma*I macrorestriction patterns were analyzed using the Dice coefficient and visualized by unweighted pair-group method, using average linkages with 1% tolerance and 1% optimization settings. The similarity cutoff of 80% is indicated by the vertical line. PFGE groups determined by cluster analysis are numbered from 1 to 32. The *spa* types, *spa*-CC, ST types, and MLST-CC are also included.

were MDR for being non-susceptible to one or more agents in three or more antimicrobial categories. Only two isolates (1.5%) were extensively drug-resistant (XDR) with the possibility of being pandrug-resistant (PDR) for being non-susceptible to one or more agents in all but two or fewer antimicrobial categories. Moreover, the major resistance profile detected within the MSSA included resistance to clindamycin and erythromycin (6/93; 6%), with all strains showing an inducible clindamycin resistance. However, all isolates were sensitive to teicoplanin, vancomycin, and rifampin. Table 4 summarizes the percentages of the 16 generated antibiotic resistance profiles in this study, as well as their distribution between MRSA and MSSA.

In conclusion, genotype analyses showed a high diversity and a varied range of *spa* types in both MSSA and MRSA isolates. The high diversity along with the lack of any equivalence in the genetic backgrounds of the major MSSA and MRSA clones could be indicative of MRSA clones being imported instead of arising from successful MSSA clones and warrants further study.

Table 4
Distribution of antimicrobial resistance profiles observed among MRSA and MSSA

Resistance profile (%)	Number among MSSA	Number among MRSA
CIP (1.5)	2	-
CIP, DA (0.8)	1	-
CIP, ERY (0.8)	1	-
CIP, TET (0.8)	1	-
DA, ERY (4.5)	6	-
DA, ERY, TET (0.8)	1	-
ERY (0.8)	1	-
SXT, TET (0.8)	1	-
TET (3.8)	5	-
AUG, CIP, DA, ERY, KF, OXA (2.3)	-	3
AUG, CIP, DA, ERY, KF, OXA, SXT, TET (0.8)	-	1
AUG, CIP, ERY, KF, OXA (0.8)	-	1
AUG, DA, ERY, KF, OXA, TET (1.5)	-	2
AUG, ERY, KF, OXA (0.8)	-	1
AUG, KF, OXA (16.6)	-	22
AUG, KF, OXA, TET (6.8)	-	9

MRSA, methicillin-resistant *Staphylococcus aureus*; MSSA, methicillin-susceptible *Staphylococcus aureus*; AUG, augmentin; CIP, ciprofloxacin; DA, clindamycin; ERY, erythromycin; KF, cephalothin; OXA, oxacillin; SXT, trimethoprim–sulfamethoxazole; TET, tetracycline.

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Conflict of interest: The authors declare that they have no conflicts of interest.

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.2013.10.007.

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