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Molecular characterization of *Staphylococcus aureus* in Lebanon

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SUMMARY

Staphylococcus aureus is an important human pathogen and is a growing public health concern. In this study, 130 *S. aureus*, 93 methicillin-resistant *S. aureus* (MRSA) and 37 methicillin-sensitive *S. aureus* (MSSA), clinical isolates recovered from Lebanon were typed by protein A gene (*spa*) sequencing and multi-locus sequence typing (MLST). Forty-eight different *spa* types were identified and clustered into 30 different groups. MLST revealed 10 sequence types (STs) among the isolates. There were eight major MRSA clones defined as isolates with the same ST and the same SCC*mec* type. The majority of the PVL-positive isolates (53%) were ST80-MRSA-IVc. Systematic surveillance of both hospital and community isolates in Lebanon together with measures designed to limit the spread are required.

Key words: Bacterial infections, bacterial typing, methicillin-resistant *S. aureus* (MRSA), *Staphylococcus aureus*, staphyococcal infections.

INTRODUCTION

Staphylococcus aureus is recognized as one of the most prevalent pathogens isolated from hospitalized patients and is of increasing importance in the community setting [1]. It is associated with a wide spectrum of infections ranging from mild superficial skin lesions to life-threatening systemic infections [2, 3]. The ongoing evolution of antimicrobial resistance particularly methicillin-resistant strains of *S. aureus* (MRSA) has complicated the treatment of infections with such isolates. Hospital-acquired MRSA (HA-MRSA) strains have been and remain endemic in many countries worldwide, but in recent years community-acquired (CA-MRSA) strains have emerged to cause serious invasive and life-threatening infections in young, healthy patients without significant healthcare contacts [1].

Resistance to methicillin is encoded by the staphylococcal cassette chromosome *mec* (SCC*mec*) element, composed of the *mec* gene complex, and the *ccr* (cassette chromosome recombinase) gene complex, encoding for the recombinase gene [2, 4, 5]. SCC*mec* elements have been classified into eight major types (I–VIII) some of which are differentiated further into subtypes [6]. SCC*mec* types I, II, and III, and types IV and V have been associated with HA- and CA-MRSA, respectively [4, 5, 7]. CA-MRSA lineages have also been identified in healthcare-associated infections, sometimes causing invasive bloodstream infections [8, 9].

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The production of Panton–Valentine leukocidin (PVL) is highly associated with the most common genetic lineages of CA-MRSA particularly those prevalent in North America [10, 11] but some lineages identified in the Far East lack the toxin [12, 13]. Although PVL has been shown to be a significant virulence factor in necrotizing pneumonia [14, 15], its role in promoting the virulence of CA-MRSA in skin and soft tissue infections has been questioned [16].

The most widely used methods for genotyping *S. aureus* are multi-locus sequence typing (MLST) and pulsed-field gel electrophoresis (PFGE) [17]. MLST groups strains into sequence types (STs), and these can be sorted by their relatedness into clonal complexes (CCs) [10, 18]. Enright *et al*. identified 11 major MRSA clones within five groups of related genotypes in a large international strain collection of *S. aureus* and suggested that major MRSA clones evolved from successful epidemic methicillin-sensitive strains [19]. DNA macrorestriction analysis by PFGE on the other hand, is excellent for the determination of strain relatedness in outbreak situations but does not inform the evolutionary relatedness of the overall populations of strains [18]. It also lacks the portability of MLST owing to difficulties in reproducibility between laboratories [10]. Genetic analysis of strain types of *S. aureus* can also be performed by *spa*-sequence typing. A region of the *spa* gene consists of a variable number of direct repeats which exhibit extensive strain-dependent polymorphisms [20]. Its advantage over the preceding methods is that it provides data informative of the population structure and evolution of strains as well as identifying strain relationships for studies of hospital outbreaks. However, the discriminating power of the method is sometimes limited by the occurrence of the same or related *spa* types in different clonal lineages [10].

There have been several studies on the molecular characterization of *S. aureus* isolates in the Middle East, two of which revealed the prevalence of ST239 MRSA isolates in Tehran and Turkey [21, 22]. The absence of such reports from Lebanon provided the rationale of the present study of the molecular characterization of *S. aureus* isolated from Lebanon.

METHODS

Bacterial isolates

A total of 130 *S. aureus* clinical isolates recovered from different patients between January 2006 and May 2007 at the Clinical Microbiology Section of the American University of Beirut in Lebanon was used in this study. The isolates were randomly chosen targeting primarily those recovered from wound and respiratory specimens. DNA was extracted using QIAamp DNA Mini extraction kit (Qiagen, USA) and the efficiency of the DNA extraction method was tested using 16S rDNA PCR [23].

Multiplex PCR for SCC*mec* subtyping

The SCC*mec* Multiplex-PCR (M-PCR) assay for types and subtypes I, II, III, IVa, IVb, IVc, IVd and V and PCRs for *mecA* and *ccr* genes were as previously described [24]. The following control strains for SCC*mec* types and subtypes were kindly provided by Dr T. Ito; NCTC 10442 (type I), N315 (type II), 85/2082 (type III), JCSC 4744 (type IVa), JCSC 2172 (type IVb), JCSC 4788 (type IVc) and WIS (type V). All PCR assays were performed using 1·0 U platinum *Taq* DNA polymerase (Invitrogen Inc., USA). The SCC*mec* subtype IVc failed to amplify even with the control strain and so primers for this subtype were replaced by the set recommended by Kondo *et al*. [25]. All PCR assays run incorporated a negative control (without template DNA), and a PCR control with *Escherichia coli* DNA. PCR detection of the PVL gene was performed as previously described [26] with *S. aureus* ATCC 49775 as a positive control.

spa typing

The polymorphic X region of the protein A gene (*spa*) was amplified using the primers spa-1113f and spa-1514r [27]. All sequencing reactions were carried out using the ABI Prism BigDye Terminator version 3.1 cycle sequencing ready reaction kit (Applied Biosystems, USA). *spa* types were assigned using the Ridom StaphType software version 1.5.13 (Ridom GmbH, Germany) as described by Harmsen *et al*. [27] and further grouped into *spa* clonal complexes (*spa* CCs) using the BURP algorithm with the calculated cost between members of a group being $\leqslant 4$ and excluding *spa* types fewer than five repeats [28].

MLST

MLST was performed on 15 isolates of the most common *spa* types by sequencing the *arc*, *aroE*, *glp*, *gmk*, *pta*, *tpi* and *yqiL* genes as described by Enright *et al*. [19] using the BigDye Terminator version 3.1 cycle

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Table 1. *Demographics and molecular characterization of MRSA and MSSA isolates from Lebanon*

Aspect	Number (%)* found in isolates	
	MRSA ($n=93$)	MSSA ($n=37$)
Specimen source		
Wound/cyst/abscess	54 (58)	12 (32)
Respiratory	10 (11)	3 (8)
Blood	3 (3)	2 (5)
Catheter	2 (2)	1 (3)
Urine	1 (1)	1 (3)
Other†	23 (25)	18 (49)
Sex		
Female	36 (39)	13 (35)
Male	57 (61)	24 (65)
Age (years)		
Median	47	50
Range	<1 year to 89 years	<1 year to 85 years
Specimen origin		
Outpatient	66 (51)	
In-patient	64 (49)	
PVL		
Positive	59 (62)	7 (20)

PVL, Panton–Valentine leukocidin.
* Decimals are rounded.
† Other: sputum, DTA, brain, neck mucus, urine, gall bladder, joint fluid, semen, tissue.

sequencing ready reaction kit (Applied Biosystems). MLST types were assigned by submitting the sequences to the *S. aureus* database on the website (http://www.mlst.net/).

RESULTS

Table 1 shows that approximately equal numbers of *S. aureus* isolates were recovered from outpatients and in-patients. The majority (93/130) were MRSA. More than half of the MRSA and a third of the methicillin-sensitive *S. aureus* (MSSA) isolates were from cutaneous sites (wounds, cysts, abscesses). The prevalence of the PVL gene in MRSA isolates was 62% compared to 20% in MSSA isolates (Table 1). Most (87%) of MRSA isolates were of SCC*mec* type IVc followed by type III (10%), with types II, IVb and V occurring in 1% each. The PVL gene was detected only in isolates harbouring SCC*mec* type IVc, and in the single isolate with type IVb.

spa typing

All isolates were assigned to 48 different *spa* types, varying in length between 2 (t524) and 16 (t032) repeats. The most common *spa* types identified were t044 (37·7%) and t037 (4·6%), and occurred only in MRSA isolates. All *spa* t044 isolates harboured SCC*mec* type IVc and the PVL gene, except for isolate S44, which was of type III and PVL negative. It is noteworthy that a novel repeat was identified in this study which resulted in a new type designated as *spa* t4099; this isolate was PVL positive with SCC*mec* subtype IVc.

Using the BURP algorithm, *spa* types were clustered into 23 different groups, with 11 groups comprising more than one *spa* type and 12 'singletons'. Since clustering parameters excluded *spa* types shorter than five repeats, one type (t524) was excluded. Six of the 11 *spa* CCs had designated group founders (*spa* CC021, *spa* CC008, *spa* CC660, *spa* CC044, *spa* CC279, *spa* CC005). The group founder, if present in a group, is defined as the *spa* type with the highest founder score and is assigned to the *spa* type with the most closely related *spa* types and strains. Twenty-eight different *spa* types were identified in the MSSA isolates compared to 27 in the MRSA isolates. Both groups of isolates were distributed into all identified clusters, except for *spa* CC44, which comprised only MRSA strains. CC 21 (*spa* CC21) and CC44 together accounted for 60% of the isolates; 16% of MSSA isolates fell in CC5 and CC8.

MLST

MLST revealed 11 STs in the 15 isolates tested. There were eight major MRSA clones defined as isolates with the same ST and the same SCC*mec* type. These clones were associated with complexes CC80, CC30, CC8, CC22, CC5, CC97 and CC6 (Table 2). ST80, PVL-positive, SCC*mec* subtype IVc, was the predominant genotype in the 15 tested population. PVL gene positive MRSA fell in clones CC80, CC30 and CC5 and PVL-negative isolates in other clonal complexes. None of the PVL-positive MRSA had the same clonal class as the PVL-negative MRSA. However, MSSA strains were distributed within CC5, CC30, CC80, CC1, CC5 and CC121.

DISCUSSION

The present study is, to our knowledge, the first comprehensive comparison of the genetic background

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Table 2. *An overview of the major MRSA and MSSA* spa *types and their corresponding MLST clones recovered from Lebanon*

Clone	*spa* type	*spa*-CC	PVL	MLST ST	MLST CC
ST80-MRSA-IVc	t044 (37·7%) t131 (1·5%)	*spa*-CC044	+	ST80	CC80
ST80-MSSA	t937 (0·8%)	*spa*-CCd	−		
ST239-MRSA-III	t037 (4·6%) t030 (3·1%)	*spa*-CC021	−	ST239	CC8
ST30-MRSA-IVc	t318 (0·8%)	*spa*-CC021	+	ST30	CC30
ST30-MSSA	t012 (2·3%)		−		
ST5-MSSA	t002 (3·8%)	*spa*-CCb	−	ST5	CC5
ST97-MRSA-V	t267 (3·1%)	sg no. 6	−	ST97	CC97
ST8-MRSA-IVc	t008 (3·1%)	*spa*-CC008	−	ST8	CC8
ST121-MSSA	t159 (2·3%)	sg no. 3	+	ST121	CC121
ST6-MRSA-IVc	t304 (2·3%)	*spa*-CC008	−	ST6	CC6
ST22-MRSA-IVc	t032 (2·3%)	*spa*-CC005	−	ST22	CC22
ST1-MSSA	t127 (1·5%)	sg no. 2	−	ST1	CC1
ST5-MRSA-IVc	t311 (0·8%)	*spa*-CCb	+	ST5	CC5

ST, MLST sequence type; CC, clonal complex; sg, singleton.

of isolates of MRSA and MSSA in Lebanon. It revealed that the majority of MRSA harboured the PVL gene and the most prevalent clone was ST80-MRSA-IVc, *spa* type 44. Three out of the five major PVL-positive CA-MRSA clones (ST80, ST30, ST8) disseminating worldwide were detected in this collection, and the identification of ST5-MRSA-IVc with the PVL gene was of particular concern since this lineage has been associated with a high capacity to spread and thus become epidemic [10]. The distribution of isolates with the predominant SCC*mec* subtype IVc (86%) according to specimen source revealed that almost 75% were from soft tissue and 20% from respiratory sources, which is in accord with several other studies [29] as was the distribution of SCC*mec* elements and the frequency of PVL gene positivity in different age groups, although some reports have highlighted PVL-positive MRSA mainly in younger people [30–32].

A variety of genotyping techniques is available to determine staphylococcal clonal relatedness. Strommenger *et al*. [33] have previously shown a wide similarity of clustering results obtained by *spa* typing/BURP analysis with those obtained by well-established methods (*Sma*I macrorestriction analysis and MLST/eBURST) while Enright *et al*. used ST and SCC*mec* type to define MRSA clones [19]. However, a potential problem with *spa* typing is that it involves sequencing of only one small region of the chromosome, which is subject to recombination between unrelated clones. This could result in isolates exhibiting the same *spa* type when they are unrelated by other methods [34]; *spa* t037 isolates have arisen from a single recombination event that involved the exchange of a 200-kb DNA fragment including the *spa* gene between MLST30 and MLST239 [35, 36].

MSSA strains in this study were distributed within CC5, CC30, CC80, CC1, CC5 and CC121; the majority of which represent international MSSA pandemic clones [33]. The increased diversity within the MSSA isolates was in accord with the findings of Strommenger *et al*. [28].

Of the eight major MRSA clones (ST80, ST30, ST8, ST239, ST22, ST5, ST97, ST6) identified here, the ST8 clone is known to have disseminated in Europe and USA, ST30 in Australia, Europe and South America, ST80 in Asia, Europe and the Middle-East, ST5 in Africa, Europe and South America and ST22 in parts of Europe. Other MRSA clones detected in this study included: ST30-MRSA-IVc observed in Australia, Europe and South America, ST1-MRSA-IVc clone observed in Asia, Europe and USA and ST239-MRSA-III the Brazilian/Hungarian clone [35].

ST80-MRSA-IVc, the major MRSA clone in Lebanon, was frequently associated with skin sources (wounds, abscesses) which is in accord with Larsen and colleagues who found that the 'European CA-MRSA' clone CC80-MRSA-IV primarily caused CA infections, predominantly skin and soft tissue, in young people outside hospitals [37], with some of the isolates being recovered from hospitalized patients. However, European CA-MRSA seemed less adapted

to persist in hospital environments, and patients with a recent history of travel or family relation to the Mediterranean or Middle East were highly over-represented in their study.

In conclusion, we present here the first comprehensive study on the genetic population structure of *S. aureus* clinical isolates from Lebanon. The widespread distribution of the PVL gene positive ST80-MRSA-IV clone in different regions of the world is probably related to international travel. If the prevalence of this clone reflects increased community acquisition of *S. aureus* infections in Lebanon, focused studies are urgently needed to define the disease burden and to establish their association with clinical presentation and patient outcomes. The data presented here represent a starting point defining the major genetic populations of both MRSA and MSSA in Lebanon and provide a basis for clinical epidemiological studies to determine their prevalence in disease and carriage and inform the development of measures to control the spread of these potentially serious infections.

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DECLARATION OF INTEREST

None.

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