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Molecular typing of MRSA isolates by *spa* and PFGE

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ABSTRACT

Background: Methicillin resistant *S. aureus* (MRSA) strains circulating among populations and crossing borders constitute a major problem for health control and require a fast and simple genotypic approach.
Methods: In the current study we compared staphylococcal protein A (*spa*) and pulsed field gel electrophoresis (PFGE) typing methods to genotype 106 MRSA clinical isolates.
Results: The genetic spectrum of the isolates was very diverse as revealed by the two typing approaches. In total, we identified 35 *spa* types in the study. The most frequently detected *spa* type in the study was t044 (30.18%), followed by t127 and t304 (5.6% each), t363 (4.6%), and t1200 and t002 (3.8% each). The rest of isolates were detected in low frequency and many were singletons. PFGE genotyping identified 34 pulsogroups. Most of the isolates were clustered in pulsogroup J. There was no clustering of the *spa* types into the pulsogroups.
Conclusions: MRSA isolates are very diverse in the region. In light of the observed MRSA diversity the *spa* typing could constitute a preferable approach for MRSA typing.

1. Introduction

Methicillin resistant *S. aureus* (MRSA) gains tremendous attention in medical practice and microbial research because of the ascending challenge of therapy and control. MRSA causes annual deaths higher than any other infectious agent in USA (Klevens et al., 2007). In 2011 the Center for Disease Control and Prevention (CDC) estimated that more than 80,000 invasive infections and more than 11,000 deaths were caused by MRSA (Dantes et al., 2013). Furthermore, patients infected with MRSA required longer period of hospitalization and higher health care cost compared to patients infected with other staphylococcus species (Cosgrove et al., 2005). Meta-analysis study comparing MRSA infection to methicillin sensitive *S. aureus* (MSSA) bacteremic infection revealed that MRSA caused markedly higher mortality, which was attributed to prescribing ineffective early therapy (Cosgrove et al., 2005).

Considering the spread of MRSA strains across the borders, stringent strategies are required to control their dissemination and trace their nosocomial reservoirs including active surveillance and general infection control measures such as screening, decolonization and contact isolation. However, to ensure the effectiveness of these strategies, knowledge of the common MRSA genotypes, especially of the ones circulating in the population, is required. Several molecular typing methods employing DNA banding patterns are available such as pulsed-field gel electrophoresis (PFGE) or utilizing DNA sequencing such as the staphylococcal protein A (*spa*) typing and multilocus sequence typing (MLST).

Staphylococcal cassette chromosome which harbor mec (SCC*mec*) typing method is a polymerase chain reaction (PCR)-based and detect the type of SCC*mec* cassette but not its structure and is therefore less discriminative (Boye et al., 2007; Zhang et al., 2005). PFGE is a very discriminative approach for *S. aureus* typing because it is based on fragmentation of the bacterial chromosome

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using Serratia marcescens endonuclease I (*Sma*I) enzyme and separation of the digested DNA fragments on agarose gel using alternating pulses. However, it is laborious, time consuming and lacks the reproducibility among different laboratories in addition to its cost ineffectiveness. Furthermore, efforts to harmonize standard protocols were only successful on the national levels in some countries but not internationally (Murchan et al., 2003; Tenover et al., 1994; van Belkum et al., 1998). Sequence-based typing methods are preferable to DNA-size typing methods. MLST typing was established to analyze the allelic sequence of short DNA fragments (450–500 bp) of seven *S. aureus* housekeeping genes (Enright et al., 2000). This method is an excellent tool for screening and very useful in studying the molecular evolution of *S. aureus*. However, it is expensive, laborious, and time consuming and therefore not suitable for diagnostic purposes as for each *S. aureus* isolate seven PCR reactions and 14 sequencing reactions should be performed apart from the data analysis.

Spa typing method targets region X of the *spa* gene, which contains a tandem repeats (about 24 bp in length) (Frenay et al., 1996). Different *spa* types vary by the type of repeats and their copy numbers. *Spa* typing became very popular since it is based on sequencing of a single locus, less expensive and less time consuming than other methods. Moreover, it has more discriminative power compared to MLST (Malachowa et al., 2005). The availability of the *spa* types on the central *spa* server (http://spaserver.ridom.de) and its unified nomenclature provided another important advantage. The use of these typing tools contributes to understanding clonal diversity and transmission of MRSA in the hospital and community settings.

The aim of our study is to genotype MRSA isolates using *spa* typing to determine the circulating MRSA strains, which will enable a better design of surveillance protocols and control strategies. The study also aims at comparing the *spa* typing approach to that based on PFGE.

2. Methods:

2.1. Bacterial isolates:

MRSA isolates (106 isolates) were collected from King Fahd Hospital of the University in Alkhubar (KFHU), Saudi Arabia. All bacterial isolates were of clinical origin. Fifty one isolates were obtained from infection sites such as wound, abscess, respiratory infections, blood stream and eye infection. Fifty five isolates were obtained from carrier colonization sites such as nose, groin, or throat. Further details about the samples can be found elsewhere (Alkharsah et al., 2018). All bacterial isolates were maintained as glycerol stock and stored at −80 °C. The isolates were designated numbers M1-M114. All isolates were identified as previously described (CLSI, 2016).

We obtained the Ethical approval for the study from the Institutional Review Board at Imam Abdulrahman Bin Faisal University (IRB-2017-13-142).

2.2. Spa typing:

Extraction of DNA was performed using Qiagen DNA extraction kit following the standard protocol with addition of lysozymes (Qiagen, Germany). DNA concentration and quality assessment was done by NanoDrop (life technologies, USA). The spa1095F (5′-AAAGACGATCCTTCGGTGAGC-3′) and spa1517R (5′-GCTTTTGC AATGTCATTTACTG-3′) primers were used to amplify the target fragment of the *spa* gene by PCR according to the protocol previously described (Harmsen et al., 2003). The amplified DNA product was separated on 1% agarose by gel electrophoresis and the amplicons were purified by gel extraction kit from Qiagen (Qiagen, Germany). The purified DNA product was sequenced in both forward and reverse directions using the same PCR primers in combination with the big dye termination mix kit and the ABI genetic analyzer 3500 (Applied Biosystems, USA). All DNA sequences were trimmed to the required length and the full fragment sequence was obtained by aligning the forward and reverse sequences for each isolate using the DNAGear software (F et al., 2012). *Spa* types were allocated to each isolate according to the repeats listed in the *spa* typing website (http://www.spaserver.ridom.de/) using the DNAGear software (F et al., 2012). Fig. 1 illustrates the flow of *spa* typing method (Fig. 1).

2.3. Pulse field gel electrophoresis (PFGE):

All the 106 isolates were subjected to PFGE analysis. PFGE was performed as per the protocol defined by the Center for Disease Control and prevention (CDC) with few modifications (https://www.cdc.gov/pulsenet/pathogens/pfge.html). Briefly MRSA colonies from overnight cultures grown on trypticase soy agar were inoculated in 5 ml of Brain heart infusion broth and further incubated at 37 °C for 18 hrs. Cell suspension turbidity was adjusted to 0.9–1.0. Cell pellet of 200 μl of adjusted cell suspension was added to 200 μl of Tris-EDTA (TE) buffer and 4 μl of Lysostaphin in 300 μl of 1.8% Seakem agarose prepared in TE buffer. This mixture was quickly dispensed into plug mold. After 10–15 mins plugs were removed from mold and placed into a tube with 3 ml of Lysis buffer (1M tris, 0.5 M EDTA, 0.5% Brij58, 0.2% Sodium deoxycholate, 0.5% sodium laureyl sarcosine) at 37 °C overnight. Later Lysis buffer was decanted and replaced by 3 ml of ESP lysis buffer (10 mM tris, 1 mM EDTA, 1% SDS, 1 mg/ml proteinase K) at 50 °C for 4 hrs. After

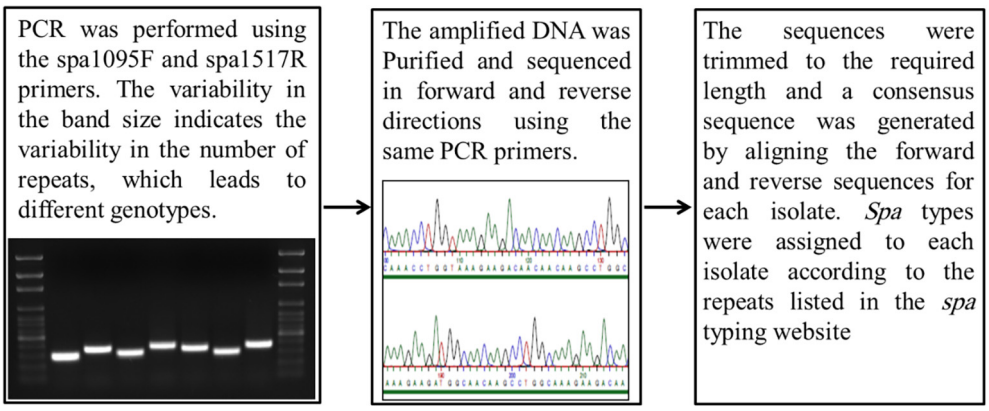

Fig. 1. Illustration of the procedure and flow of *spa* typing method.

decanting ESP buffer, plugs were washed several times with TE buffer then stored at 4 °C. A slice of 2 × 10 mm was aseptically cut from a prepared plug and equilibrated for 30 mins in 200 μl of water buffer (buffer stock, bovine serum albumin [BSA], grade 1 water). Later water buffer was decanted and replaced by 200 μl of enzyme mixture (buffer stock, BSA, SmaI 4 μl/slice, grade 1 water) for minimum 4 hrs at 30 °C. Digested plugs were loaded into the wells of a 1% agarose gel and run in 0.5 TBE using a CHEF-DR III system (Bio-Rad Laboratories, Inc, CA, USA) according to the following parameters: 6 V, temperature 14 °C, initial switch time5 s, final switch time 40 s, included angle 120, with a run time of 21 hrs. Two controls were included in each run. The bacteriophage lambda ladder was used a PFGE marker and the NCTC 8325 strain was used a reference control. Gel was stained using ethidium bromide solution (final concentration 1 mg/ml) for 30–40 min on a rocking shaker in a covered container. Destaining was done thrice with distilled water on shaker and then the gel was visualized and captured.

Gel pictures were processed and analyzed using the software BioNumerics v6.5. Dice coefficient was employed to compare the obtained PFGE fingerprints. GelCompare and Bionumerics software v6.5 were employed to perform analysis of cluster employing the Unweighted Pair Group Method with Arithmetic Means (UPGMA). Further parameters including the band tolerance as well as optimization settings were set to 1% and 0.5%, respectively. We used the suggested recommendation by Tenover *et al.* to cluster the PFGE groups (Tenover et al., 1995). The similarity coefficient was set to 80% as recommended by Struelens *et al.* (Struelens et al., 1992).

2.4. Data analysis

SPSS version 23 software was used in the statistical analysis.

3. Results

In total, thirty-five *spa* types were identified in our cohort. The most common *spa* type in our study was t044 constituting 30.18% of the isolates (Table 1). *Spa* types t127 and t304 were the second most common types (5.6% each), followed by t363 (4.6%), and t1200 and t002 (3.8% each) (Table 1). The rest of the isolates were clustered either in less frequent *spa* types or as singletons (Table 1). Seven isolates had no matching *spa* type in the database. Three of the seven isolates had new repeats and four isolates had known repeats but the arrangement of the repeats didn't match with previously identified *spa* types (Table 1).

SmaI digestion and PFGE identified the presence of 34 pulsogroups designated as A to T (Fig. 2). PFGE group J was the largest and comprised 24 of the studied isolates. Other pulsogroups (A, B, E, I, P, and S) comprised 4–7 isolates each (Fig. 2).

There was no significant clonal association between *spa* types and PFGE groups. There was clustering of some spa types within some of the pulsogroups. However, it was statistically non-significant, most probably due to the small numbers of isolates of such spa types. Additionally, the seven isolates that potentially represent new *spa* types clustered within the identified pulsogroups.

No association was found between neither of the typing methods and the clinical data nor demographic data such as gender, age

Table 1
Frequency of the spa types among the PFGE pulsogroup in the study.

Isolate	PFGE group	spa type	Isolate	PFGE group	spa type	Isolate	PFGE group	spa type
M96	A	t127	M24	I	t044	M59	L	t002
M57		t044	M73		t934	M37	M	t13180
M90		t127	M70		t002	M38		t044
M92		t127	M71		t127	M79	MA	t002
M58	AA	t044	M72		t044	M81	MB	t3364
M101	B	t304	M74		t044	M86	N	t019
M108		t304	M76		t002	M87		t267
M95		t304	M14	IA	t044	M82	NA	t1339
M105		t304	M61	J	t13180	M85	O	t044
M18		NR1	M69		t044	M88		t044
M91		t2297	M65		t223	M89		t044
M12	C	t8400	M68		t044	M83		t044
M32		t363	M13		NT1	M1	OA	t852
M34		t363	M52		t223	M7		t362
M102		t037	M94		t044	M2	P	t037
M25	D	t1200	M45		t044	M3		t127
M28		NR2	M46		t693	M4		t044
M10	DA	t304	M43		t311	M6		NR3
M40	E	t044	M104		t1247	M5	PA	t363
M41		t657	M31		t044	M8		t1200
M42		t044	M100		t044	M75	PB	t657
M44		t044	M97		t044	M77		t1200
M39		t044	M99		t044	M54	Q	t311
M35	EA	t657	M47		NT2	M55		t037
M50	F	t021	M49		t044	M11	R	t223
M51		t8154	M17		t044	M19		t008
M9	FA	t304	M53		t309	M56	S	t932
M112	FB	t362	M66		t442	M64		t044
M63	G	t13180	M93		t044	M22		NT3
M67		t8154	M103		t044	M23		NT4
M115		t690	M29		t044	M111	T	t4573
M20		t309	M30		t363	M113		t127
M21		t690	M106	K	t044			
M16		t690	M110		t044			
M114	GA	t1339	M119		t004			
M117	H	t224	M60	KA	t1200			
M62		t224	M15	L	t363			

NR: New repeat lead to new spa type. NT: new type due to new repeat arrangement.

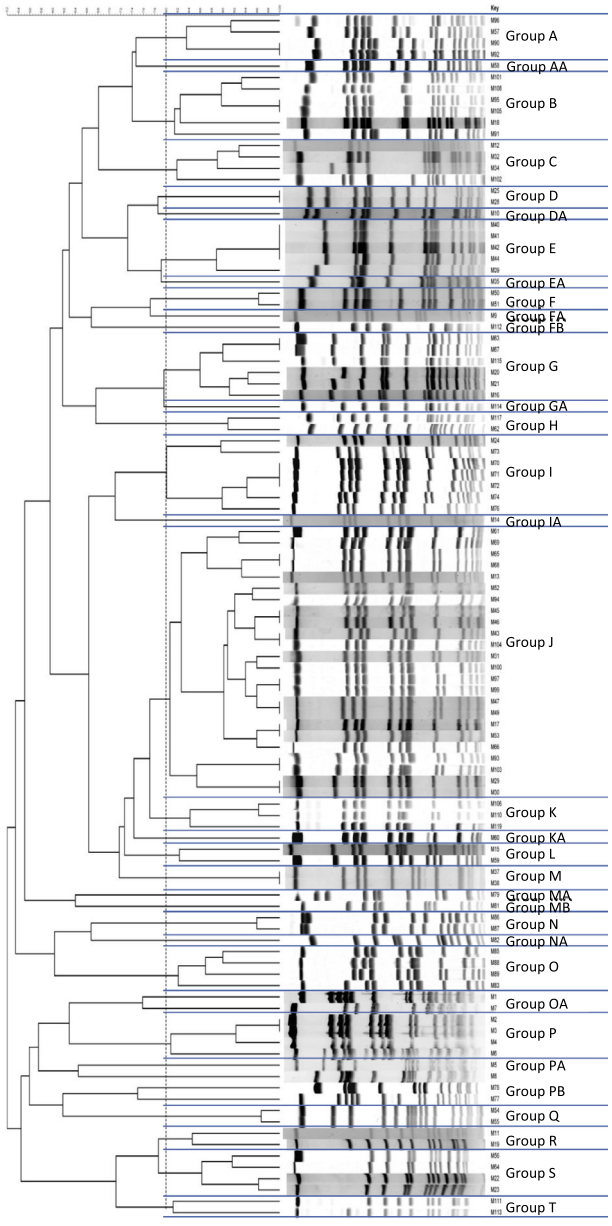

Fig. 2. Pulsed field gel electrophoresis groups based on 80% Dice coefficient.

group, source of isolates, or antibiotic susceptibility pattern. However, some *spa* types (t002 and t363) were confined to isolates recovered from a set of infection sites (wound, abscess, and endotracheal aspirate), while others were more frequently associated with isolates recovered from colonization sites (t304 and t690). There was no relation between spa types and severity of infection.

4. Discussion

Effective strategies are required to control the dissemination of MRSA worldwide such as active surveillance and institutional preventive measures, which requires insight into the molecular epidemiology of the circulating genotypes. MRSA prevalence in Saudi Arabia ranges between 12 and 49% depending on the region (Austin et al., 2003; Baddour et al., 2006; Bukharie and Abdelhadi, 2001; El Amin and Faidah, 2012). The estimated carriage rate of MRSA among health care workers is too high reaching 67% (Iyer et al., 2014) leading to an increase in the risk of hospital-acquired infections. Two studies were recently published using the *spa* typing approach but had either a small number of isolates or studied a selected group of patients (Abou Shady et al., 2015; Alreshidi et al., 2013). Based on that, we have initiated this study, which revealed a clear genotypic diversity within the MRSA population.

More than thirty types were identified using *spa* or PFGE typing approaches among 106 isolates. This genotypic diversity of MRSA strains could be attributed to the diversity in the population living in the area; people from different nationalities work and live in the region and hence importing the diversity observed. Additionally, it could be attributed to low rate of nosocomial cross-transmission due to stringent MRSA screening program adopted for more than a decade, and the selective pressure effect of antimicrobials over various circulating strains (Argudin et al., 2009).

Spa typing method relies on the PCR amplification followed by sequencing of the highly variable X fragment of the staphylococcal protein A, which makes it simple and fast compared to the MLST approach. All of the isolates, except seven, had *spa* types matching a known type on the database. Our isolates with new spa type were obtained from skin and nasal samples.

Spa type t044 (30.18%), which was the most common in this study, was previously reported as being the second most common after type t037 in Riyadh (Alreshidi et al., 2013). This indicated the high prevalence of type t044 in Saudi Arabia with some regional variation. However, in another study from Buraydah city in Al-Qassim district near to Riyadh none of the two former common *spa* types were detected, which could be attributed to the low sample size (15 MRSA isolate) (Abou Shady et al., 2015).

Type t044 represents the so called ST80 MRSA, which is very common in the Middle East. It was detected in other neighboring countries at variable frequencies such as Jordan (Al-Bakri et al., 2013; Bazzoun et al., 2014); however, it was only reported in adults and healthcare workers but not in children (Aqel et al., 2015). This was in contrast to our results, as type t044 was detected in all age groups in our study. It was also reported from Lebanon (Harastani et al., 2014), United Arab Emirate (Sonnevend et al., 2012), Kuwait (Udo and Al-Sweih, 2017), Iran (Goudarzi et al., 2016), and in one but not all studies from Tunisia (Ben Nejma et al., 2013; Ben Said et al., 2016; Kechrid et al., 2011), and widely disseminated in Europe, Australia (Larsson et al., 2014), and USA (Fluit et al., 2015). No correlation between type t044 and the source of the isolate was reported. However, some *spa* types were confined to isolates obtained from a specific site of infection; for example t002 and t363 were isolated from wound, abscess, and endotracheal aspirate. However, the small number of isolates (5 each) precludes firm conclusion. There was no relation between spa types and severity of infection.

Three new *spa* types were reported, and four isolates had known repeats but the repeat sequence did not match any previously identified *spa* types on database. The X region of the *spa* protein is very polymorphic, with 761 repeats being so far registered on the Ridom *spa* server. The random arrangement of these repeats generated 7643 *spa* types. New *spa* types are generated by the rearrangement of the repeats in a recombination event or by generation of new repeats due to DNA polymerase error, deletion, or duplication events.

Despite its limitations, *spa* typing has shown, in this work, its utility to study local MRSA epidemiology and to help in clustering isolates. It also showed a better discriminative power. The lack of association between the *spa* type and PFGE pattern could be attributed to the large genetic diversity of the studied isolates.

5. Conclusion

The data presented showed high diversity of MRSA in our population providing a baseline for further molecular characterization in the Region. *Spa*-typing can be considered a practical approach to investigate and manage MRSA-linked outbreaks because of its simplicity, relative low cost, high throughput and discriminative power besides the standardized nomenclature and portability into an international database. Infrequently when misclassification or non-typeability is a concern, a sophisticated typing tool, such as PFGE in this study, is an informative supplementary addition.

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Conflict of interest

The authors have no conflict of interest to declare.

Author contribution

KRA: overall design and plan of the work and raising fund.
SR: PFGE experimental work.
AA: spa data analysis.
AD: spa experimental design.
AH: PFGE experimental design.
ST: Analysis of PFGE data.
All authors read and edited the manuscript.

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