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Original Article

Molecular Diversity of Methicillin-resistant *Staphylococcus aureus* Isolates Originated from Patients in Ahvaz Hospitals, Iran

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Introduction: *Staphylococcus aureus* is among the primary cause of hospitals and community-acquired infections. The emergence of methicillin-resistant *S. aureus* (MRSA) strains has resulted in the treatment failure of the infections caused by these bacteria. Hence, regional data on antibiotic resistance of *S. aureus* strains is necessary to adopt appropriate treatment regimens. This study aims to identify MRSA isolates' diversities and frequencies by molecular analysis of four genes. **Methods:** In a cross-sectional study, 100 *S. aureus* isolates from patients hospitalized in two hospitals of Ahvaz, Iran were collected and identified. The MRSA isolates were identified by phenotypic method and amplification of the *mec*A gene. The diversity of MRSA isolates was investigated by amplification of the *coa*, *spa*, *aro*A, and *gap* genes followed by RFLP analysis using the *Alu*I, *Hind*III, *Taq*I and *Rsa*I restriction enzymes. **Results:** In this study, we identified 50 MRSA isolates. Based on the analysis of *coa* gene, 8 types, *spa* gene 5 types and 17 subtypes, *coa* gene with *Alu*I 13 types, and *spa* with *Hind*III 13 types were identified. Also, the RFLP analysis of *gap* gene with *Alu*I revealed 3 types, and of *aro*A gene with *Taq*I and *Rsa*I, 3 types and 2 subtypes, respectively. **Conclusion:** Our PCR-RFLP analysis revealed that diversities are present among MRSA isolates originated from clinical samples and showed that this method is simple, reproducible, and cost-effective. *J Med Microbiol Infec Dis, 2019, 7 (1-2): 19-28.*

Keywords: Methicillin-resistant, *Staphylococcus aureus*, Restriction Fragment Polymorphism, Iran.

INTRODUCTION

Infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA) strains are among the significant causes of mortality worldwide [1]. The hospital or community-acquired MRSA is the primary cause of skin and bloodstream infections and ventilator-associated pneumonia (VAP) [2]. Knowledge of the origin of MRSA strains can be useful for control and spread of these bacteria [3]. This issue can be more critical when the emergence of multidrug-resistant strains lead to treatment failure [4]. Hence, planning surveillance and monitoring program is necessary for control of these pathogens [5]. Currently, both phenotypic and genotypic approaches are available for the diagnosis of MRSA strains. However, in many countries genotyping methods are increasingly used for identification of MRSA isolates, their origins and distribution pattern in communities and hospitals [6]. The genotypic methods are not affected by laboratory conditions, are reproducible, rapid and culture-independent, and suitable for fastidious bacteria. Also, in comparison with phenotypic methods, genotypic methods are of higher sensitivity for detection of the bacteria and differentiating the strains within a species [7, 8]. PCR-RFLP has shown to be a useful assay for the identification of bacterial strains [9]. This method can analyze large numbers of specimens in a short period and has a broad application for epidemiologic studies. The *S. aureus* infections might originate from hospitals or communities, and their origin can influence their antibiotic susceptibility pattern and consequently, their response to treatment. Genotypic identification of *S. aureus* isolates can provide insights about their origin and their relation with other strains [10]. Through molecular typing, it would be possible to reduce the infections caused by these pathogens and prevent outbreaks [11]. Various molecular markers are available for assessment of *S. aureus* diversity, among which are *spa*, *coa*, *aro*A, and *gap* genes. The *spa* gene codes protein A, a superficial and virulence protein in *S. aureus*. The X region in the C-terminal of this gene contains 24 repeated base pair with high polymorphism among strains, which can differentiate between epidemic and endemic strains [12-14]. The *coa* gene encodes coagulase, a virulent factor of *S. aureus*; it has a high heterogeneity at 3' end, which makes it as another candidate for MRSA typing [4, 8]. The *aro*A gene is another genetic marker for genotyping of *S. aureus* isolates. This gene codes 5-enolpyrovyl shikimate-3-phosphate synthase, a key enzyme in the biosynthesis of aromatics amino acids and folate [15].

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Glyceraldehyde-3-phosphate dehydrogenase is a product of *gap* gene that is a part of the glycolytic operon, which encodes transferrin binding protein (Tpn). The polymorphism in this gene was used in PCR-RFLP to investigate the diversity of Staphylococcal species [16].

The present study aims to find the genotypic diversity among MRSA isolates from the patients in Ahvaz hospitals, by PCR-RFLP analysis of *coa*, s*pa*, *aro*A, and *gap* genes. The obtained results can be used to find the origin of isolates and to control outbreaks by these bacteria.

MATERIAL AND METHODS

Clinical samples. In this cross-sectional study, during six months, from July to October 2016, we collected specimens from blood, wounds, tracheal secretions, urine, and sputum of patients hospitalized in Razi and Golestan hospitals of Ahvaz, Iran.

Identification of *S. aureus* and MRSA isolates. The *S. aureus* isolates were obtained and identified using the standard biochemical tests as described before [17]. The DNA from the isolates was extracted by the boiling method [18], and MRSA isolates were screened by the amplification of a 310 bp fragment of *mec*A gene using the primers (Table 1) designed in previous studies [19]. The 25 µl PCR reactions contained 1µl of each of forward and reverse primers (10 ρm), 1U *Taq* DNA polymerase, 2mM $MgCl_2$, 1 µl of template DNA, 200 µM dNTPs, and 2.5 µl PCR buffer (CinnaGen, Tehran, Iran). The amplification program included an initial denaturation of 94°C for 10 min, followed by 10 cycles at 94°C for 45 s, 55°C for 45 s, 72°C for 75 s, and 25 cycles at 94°C for 45 s , 50°C for 45 s, 72°C for 75 s and a final extension at 72°C for 10 min [19].

Table 1. The primers used for amplification of *mec*A, *coa*, *spa*, *aro*A, and *gap* genes in this study

Target gene		Sequence (5' to 3')	Expected Fragment size bp	Reference
MECA	F	GTAGAATGACTGAACGTCCGATAA	310 bp	19
	R	CCAATTCCACATTGTTTCGGTCTAA		
COA	F	ATAGAGATGCTGGTACAGG	variable	20
	R	GCTTCCGATTGTTCGATG		
SPA	F	ATCTGGTGGCGTAACACCTG	variable	21
	R	CGCTGCACCTAACGCTAATG		
AROA	F	AAGGGCGAAATAGAAGTGCCGGGC	1153 bp	15
	R	CACAAGCAACTGCAAGCAT		
GAP	F	ATGGTTTTGGTAGAATTGGTCGTTTA	933 bp	22
	R	GACATTTCGTTATCATACCAAGCTG		

Amplification of *coa, spa, aro*A, and *gap* genes. The *coa*, *spa*, *aro*A, and *gap* genes were targetted by the primers designed by others (Table 1). Amplification of *coa* gene was programmed for an initial denaturation at 94°C for 3 min, 30 cycles of denaturation at 94°C, 1 min, annealing at 55°C for 1 min, and extension at 72°C for 1 min, and a final extension at 72°C for 2 min [20]. The program for amplification of *spa* gene was included an initial denaturation of 94°C, for 4 min followed by 35 cycles including 94°C for 1 min, 56°C for 1 min and 72°C for 3 min, and a final extension at 72°C for 5 min [21].

Amplification of *aro*A gene was began with an initial denaturation of 94°C for 2 min, and 35 cycles of 94°C for 1min, 61°C for 1 min, and 72°C for 90 s, and ended with a final extension at 72°C for 10 min [15].

Amplification of the *gap* gene was included an initial denaturation at 94°C for 2 min, 40 cycles of 94°C for 20 s, 55°C for 30 s, and 72°C for 40 s, and a final extension at 72°C for 10 min [22]. All PCR products were resolved on 1% agarose gel containing DNA safe stain, visualized under UV, and photographed.

The primers used in this study were synthesized by a commercial company (DENAzist Asia, Mashhad, Iran).

RFLP analysis of *coa, spa, aro*A and *gap* genes. The PCR products of *coa* and *gap* genes were digested with *Alu*I in reactions containing 10 µl PCR product, 1 µl *Alu*I, 3 µl 10X rapid digest buffer and 16 µl DNase free water. The reactions were incubated at 37°C for 1 h and 2 h with *coa* and *gap* genes, respectively.

The *spa* gene was digested with *Hind* III using the above protocol and an incubation time of 2 h. The 1153 bp *aro*A gene product was digested with *Taq*I and *Rsa*I enzymes with the same protocol except for the incubation procedure, which was 2 h at 65°C with *Taq*I, and 2 h at 37°C with *Rsa*I. All digestions were electrophoresed in 2% agarose gel containing DNA safe stain , and photographed under UV. The isolates were grouped based on the number and size of generated bands.

All the restriction enzyme were purchased from a commercial company (CinnaGen, Alborz Iran).

RESULTS

MRSA isolates. One hundred *S. aureus* was isolated from clinical samples from which 50 (50%) were positive for *mec*A gene indicating MRSA isolates. The distribution of MRSA in clinical specimens was as follows: wound (n=25), infectious secretion (n=2), tracheal specimens (n=15), sputum (n=4), urine (n=2) and blood (n=2).

***coa, spa, aro*A and *gap* PCR.** The amplification of *coa* gene yielded bands of various size ranging from 450 bp to 900 bp with the majority of the isolates (50%) showing a 650 bp band. Among 50 isolates, 48 exhibited a single band, and two had 2 bands of different sizes (Fig. 1). The MRSA

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isolates were grouped into 8 different types based on the size of their amplicons (Table 2).

Amplification of the *spa* gene resulted in the production of various bands ranging from 100 bp to 1500 bp exhibiting five different types (Fig. 2, Table 3). Within types, based on the PCR product size, 17 subtypes were also defined, and Spa 3 with 34% frequency was the predominant type.

RFLP analysis. The RFLP pattern of the *coa* gene following digestion with *Alu*I produced 13 types (Table 4), type 3 showed the highest frequency (44%). The isolates originated from wounds distributed among 10 different types reflecting a high genetic diversity among them. Table 5 represents the frequency of PCR-RFLP types based on the clinical sample. Digestion of *coa* gene with *Alu*I produced 1-3 fragments of 81 bp-700 bp length (Fig. 3).

Out of 50 MRSA isolates, 45 showed amplification of *spa* gene. Digestion of amplifications with *Hind* III revealed 13 different types (Table 6, Fig 4). Table 7 shows the frequency of *spa* types within clinical specimens.

The *aro*A gene has various target sites for *Taq*I and *Rsa*I restriction endonuclease. According to the genotyping methods defined by other studies [15, 23, 1], we detected 3 types in RFLP patterns following digestion of *aro*A gene with *Taq*I (Fig. 5), with the type A exhibiting the highest frequency (75.55%). None of the isolates belonged to the types C, D, and N. (Table 8). The product of five isolates was not digested with *Taq*I. The frequency of types in clinical samples is reflected in Table 9.

Following digestion of the 1153 bp PCR product with *Rsa*I, genotype A revealed 2 subtypes, A1 (26.47%) and A2 (73.52%) (Fig. 6 and Table 10). Table 11 shows the frequency of subtypes in clinical samples.

Digestion of *gap* gene with *Alu*I enzyme resulted in 3 types (Fig. 7) with the majority of type I (94%) (Table 12). The frequency of *gap* types in clinical samples is reflected in Table 13.

Comparison of RFLP patterns of the isolates from two hospitals revealed more diversity in Golestan hospital. Also, RFLP patterns of *aro*A, *coa*, and *spa* showed 8 isolates of a similar fingerprints.

Based on the origin of the specimens, the frequency of *coa* type 2, *spa* type 4, and *aro*A subtype A2 (*Rsa*I) was similar in both hospitals.

The comparison of PCR-RFLP patterns of *spa* and *coa* genes showed that 8 isolates belonged to *coa* type 3 and *spa* type 1. About 60% of *coa* types were from Golestan hospital, and 40% from Razi hospital. Also, 70% and 30% of *spa* types were from Golestan and Razi hospitals, respectively.

The most common types were *coa* type 3 (16 isolates) and *spa* type 1 (10 isolates) that were originated from Golestan hospital. Furthermore, the frequency of *coa* type 2 and *spa* type 4 was the same, and both types were uniformly distributed in both hospitals.

Table 2. The size and number of amplicons produced by PCR amplification of *coa* gene in MRSA isolates

Type	Product (bp)	Frequency (%)
1	450	3 (6)
2	600	1 (2)
3	650	25 (50)
4	700	9 (18)
5	750	9 (18)
6	800	1 (2)
7	(450 and 600)	1 (2)
8	(500 and 900)	1 (2)

Table 3. The size and number of amplicons produced by PCR amplification of *spa* gene in MRSA isolates

Type	Subtype	PCR product (bp)	Number
Spa1 (1 band) (28%) 14	Spa1a	1100	6
	Spa1b	1200	3
	Spa1c	1300	2
	Spa1d	1500	3
Spa2 (2 bands) (20%) 2	Spa2a	300, 1200	3
	Spa2b	350, 1300	3
	Spa2c	350, 1100	1
	Spa2d	150, 1200	1
	Spa2e	400, 1500	2
Spa3 (3 bands) (34%) 17	Spa3a	100, 200, 1200	3
	Spa3b	200, 300, 1200	7
	Spa3c	300, 400, 1100	1
	Spa3d	350, 900, 1100	1
	Spa3e	150, 250, 1100	4
	Spa3f	100, 350, 1200	1
Spa4 (4 bands) (8%) 4	Spa4a	150, 300, 400, 1200	3
	Spa4b	150, 300, 800, 1100	1
Spa5 (no band) (10%) 5	-	-	-

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Table 4. Typing of MRSA isolates based on the RFLP pattern of *coa* gene digested with *Alu*I

Type	PCR product (bp)	RFLP pattern (bp)	Frequency (%)
1	700	243-286	4 (8)
2	750	81-243-405	9 (18)
3	650	243-405	22 (44)
4	700	324-405	3 (6)
5	800	81-243-324	1 (2)
6	500, 900	243-324-486	1 (2)
7	450, 600	162-243-324	1 (2)
8	650	243-324	1 (2)
9	650	405	2 (4)
10	700	700	2 (4)
11	450	162-243	2 (4)
12	600	81-162-243	1 (2)
13	450	243	1 (2)

Table 5. Frequency of *coa* gene types in MRSA clinical specimens

Type	Wound	Secretion	Trachea	Sputum	Urine	Blood	Total (%)
1	1	1	2	-	-	-	4 (8)
2	5	1	1	1	-	1	9 (18)
3	9	-	11	2	-	-	22 (44)
4	2	-	-	-	-	1	3 (6)
5	1	-	-	-	-	-	1 (2)
6	1	-	-	-	1	-	1 (2)
7	-	-	-	-	-	-	1 (2)
8	1	-	-	-	-	-	1 (2)
9	1	-	1	-	-	-	2 (4)
10	2	-	-	-	-	-	2 (4)
11	1	-	-	-	1	-	2 (4)
12	1	-	-	-	-	-	1 (2)
13	-	-	-	1	-	-	1 (2)

Table 6. Typing of MRSA isolates based on the RFLP pattern of *spa* gene digested with *Hind* III

Type	RFLP pattern (bp)	Frequency (%)
1	350, 700	12 (30)
2	200, 300, 700	10 (25)
3	400, 1000	3 (7.5)
4	300	4 (10)
5	450, 650, 900	2 (5)
6	200, 350, 850	2 (5)
7	250. 400, 600, 700	1 (2.5)
8	500, 700, 900	1 (2.5)
9	400, 450, 500, 700	1 (2.5)
10	200, 900	1 (2.5)
11	200, 350, 400, 800	1 (2.5)
12	350, 900	1 (2.5)
13	750, 900	1 (2.5)

Table 7. Frequency of *spa* types among MRSA specimens

Type	**Wound**	**Secretion**	**Trachea**	**Sputum**	**Urine**	**Blood**	**Total (%)**
1	2	1	6	2	1	-	12 (30)
2	2	-	5	2	1	-	10 (25)
3	3	-	-	-	-	-	3 (7.5)
4	2	1	1	-	-	-	4 (10)
5	1	-	-	-	-	1	2 (5)
6	1	-	1	-	-	-	2 (5)
7	1	-	-	-	-	-	1 (2.5)
8	1	-	-	-	-	-	1 (2.5)
9	-	-	1	-	-	-	1 (2.5)
10	1	-	-	-	-	-	1 (2.5)
11	1	-	-	-	-	-	1 (2.5)
12	1	-	-	-	-	-	1 (2.5)
13	1	-	-	-	-	-	1 (2.5)

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Table 8. Typing of MRSA isolates based on the RFLP pattern of *aro*A gene digested with *Taq*I

Type	RFLP pattern (bp)	Frequency (%)
A	536, 254, 244, 87, 32	34 (75.55)
B	536, 341, 244, 32	10 (22.22)
C	533, 499, 87, 32	-
D	341, 300, 244, 220, 50	-
N	297, 259, 254, 244, 87	-
H	568, 254, 244, 87	1 (2.22)

Table 9. Frequency of *aroA* gene types among MRSA clinical specimens

Type	Wound	Secretion	Trachea	Sputum	Urine	Blood	Total (%)
A	16	-	13	3	2	-	34 (75.55)
B	7	-	2	-	-	1	10 (22.22)
H	1	-	-	-	-	-	1 (2.22)

Table 10. Typing of MRSA isolates based on the RFLP pattern of *aro*A gene digested with *Rsa*I

Type	Subtype	RFLP pattern (bp)	Percentage
A	A1	837, 197, 119	26.47 %
	A2	693, 218, 123, 119	73.52%

Table 11. Frequency of *aro*A gene subtypes in MRSA clinical samples

Subtype	Wound	Secretion	Trachea	Sputum	Urine	Blood	Total (%)
A1	3	-	4	2	-	-	9 (26.47)
A2	13	-	9	1	2	-	25 (73.52)

Table 12. Typing of MRSA isolates based on the RFLP pattern of *gap* gene digested with *Alu*I

Type	RFLP pattern (bp)	Frequency (%)
1	50, 150, 300	47 (94)
2	100, 250, 350	1 (2)
3	150, 250, 300	2 (4)

Table 13. Distribution of *gap* PCR-RFLP types among MRSA clinical isolates

Type	Wound	Secretion	Trachea	Sputum	Urine	Blood	Total (%)
1	23	2	14	4	2	2	47 (94)
2	1	-	-	-	-	-	1 (2)
3	1	-	1	-	-	-	2 (4)

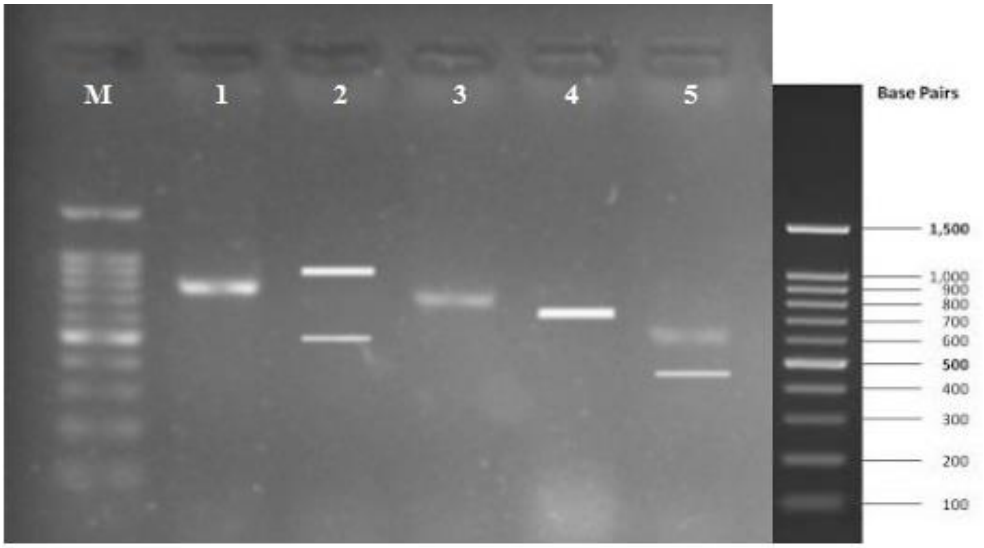


Fig. 1. PCR amplification of *coa* gene of MRSA isolates. M, 100 bp molecular marker; lanes 1, 3, type 6 (800 bp); lane 2, type 8 (500 and 900 bp); lane 4, type 5 (750 bp); lane 5, type 7 (450 and 600 bp)

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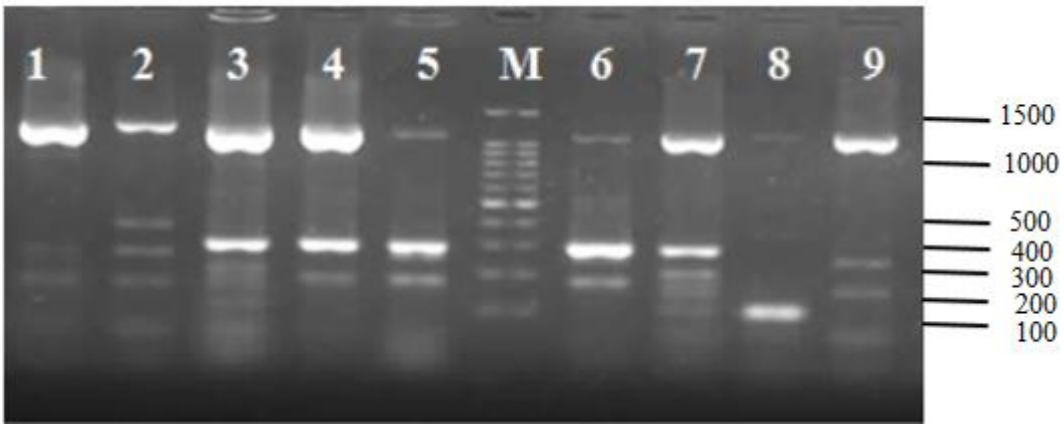


Fig. 2. PCR amplification of *spa* gene in MRSA isolates. M, 100 bp molecular marker, lane 1, type 3 (Spa3e); lane 2, type 4 (Spa4a); lane 3, type 2 (Spa2a); lanes 4, 5, 6, 7, and 9, type 3 (Spa3b); lane 8, type 2 (Spa2d)

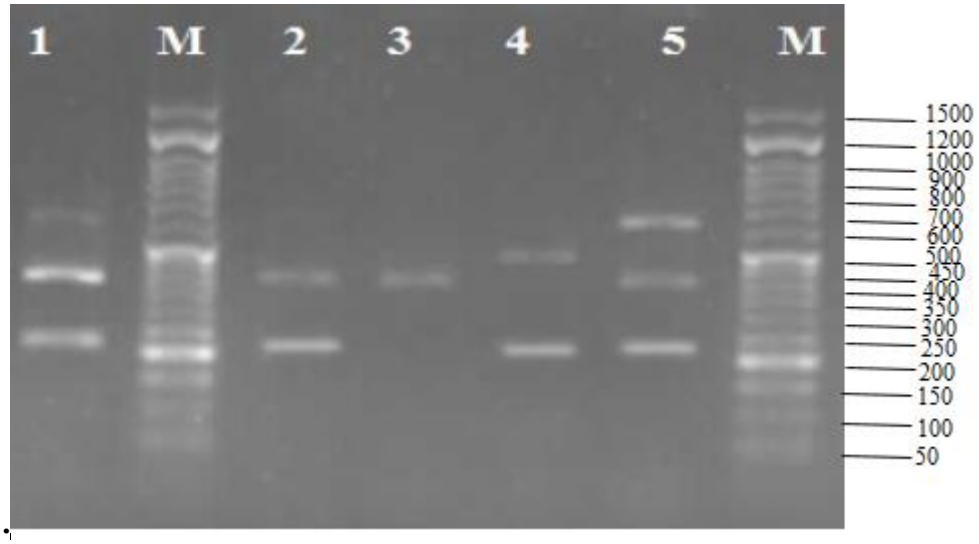


Fig. 3. RFLP pattern of *coa* gene digested with *Alu*I in MRSA isolates. M, 50 bp molecular weight, lane 1, 2, and 5, type 3 (243 bp and 405 bp); lane 3, type 9 (403 bp); lane 4, type 1 (243 bp and 486 bp). There is an extra band in the lanes 1 and 5, due to incomplete digestion of the 650 bp PCR product.

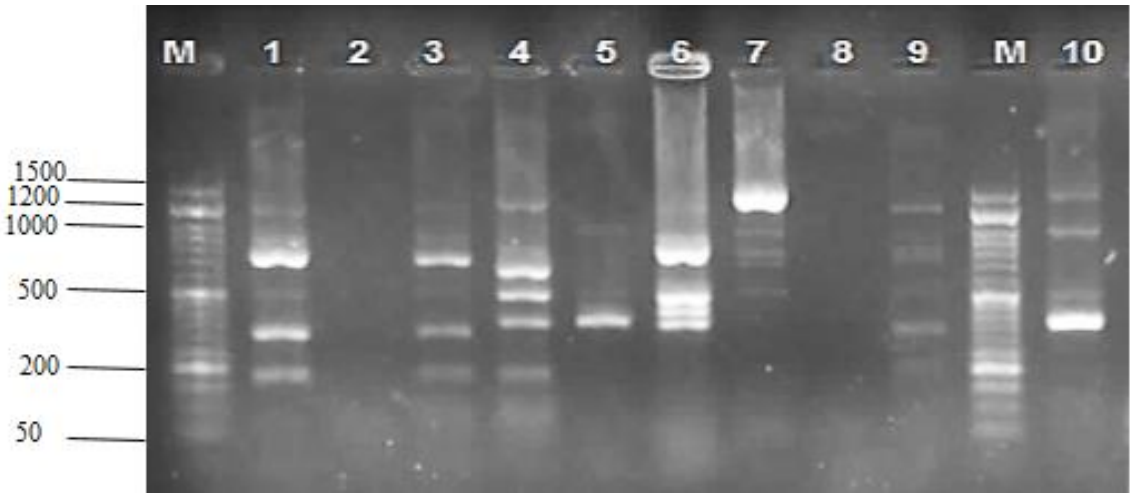


Fig. 4. PCR-RFLP of pattern of *spa* gene among MRSA isolates following digestion with *Hind* III. M, 50 bp molecular weight; lanes 1, 3, and 9, type 2 (200 bp, 300 bp, and 700 bp); lane 4, type 13 (750 bp, 900 bp); lanes 5 and 10, type 3 (400 bp and 1000 bp); lane 6, type 9 (400 bp, 450 bp, 500 bp, 700 bp); lane 7, type 8 (500 bp, 700 bp, and 900 bp); lanes 2 and 8, negative (in some isolates, digestion of amplicons yielded very small DNA fragments that were not resolved in gel electrophoresis).

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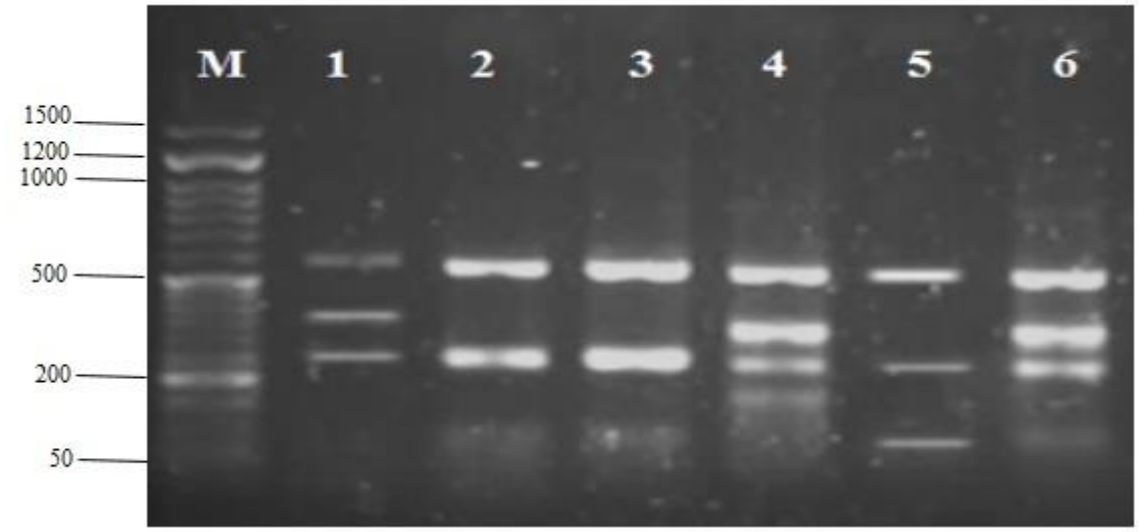


Fig. 5. PCR-RFLP pattern of *aro*A gene among MRSA isolates following digestion with *Taq*I. M, 50bp molecular weight; lanes 1, 4, and 6, type B (536 bp, 341 bp, 244 bp, 32 bp); lanes 2, 3, and 5, type A (536 bp, 254 bp, 244 bp, 87 bp, 32 bp).

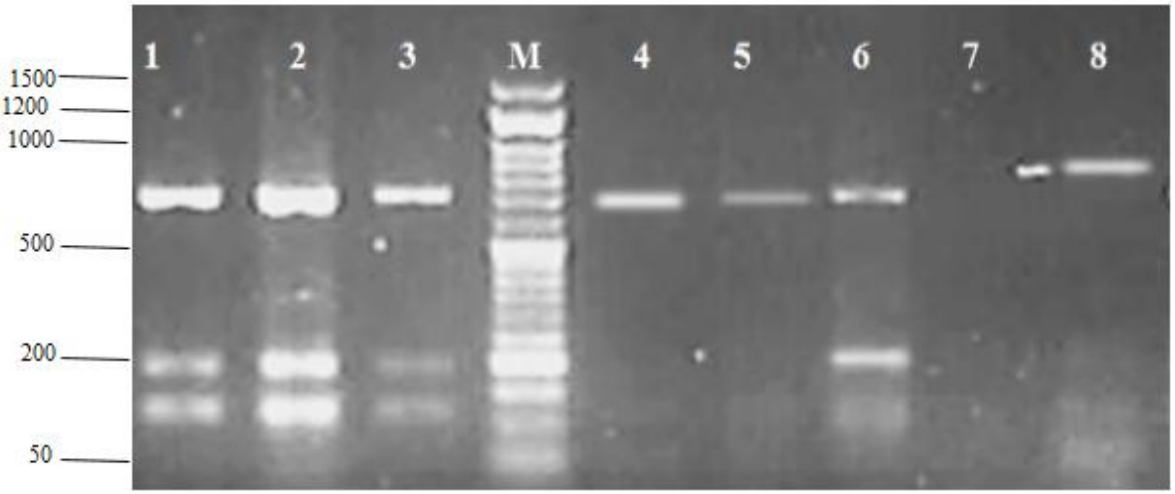


Fig. 6. The *aro*A gene PCR product (genotype A) digested with *Rsa*I. M: 50 bp molecular weight; lane 7: negative; lane 2, 3, 4, 5 6: subtype A2; lane 8: subtype A1

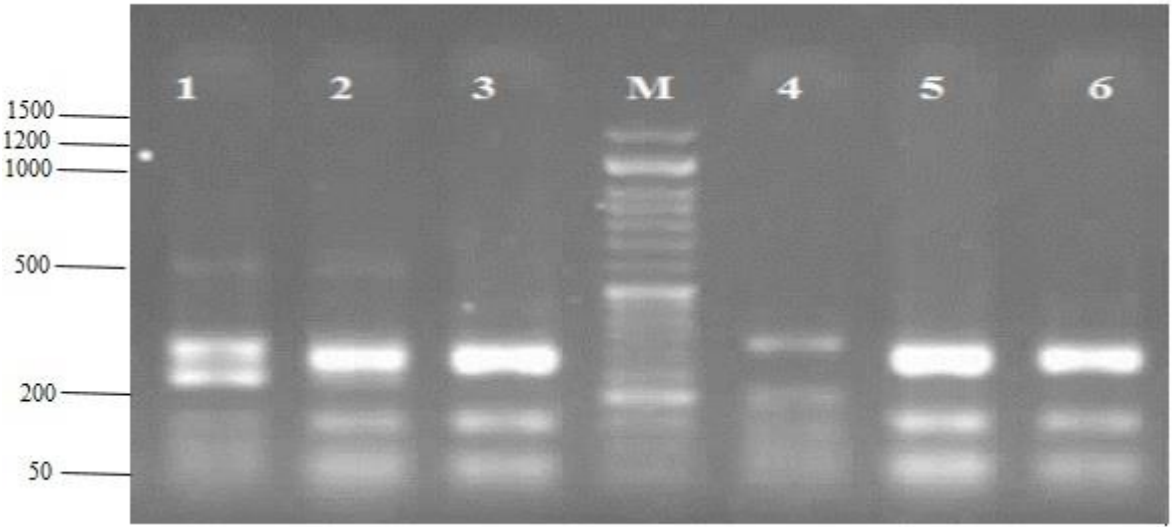


Fig. 7. PCR-RFLP pattern of *gap* gene digested with *Alu*I enzyme. M, 50 bp molecular weight; lane 1, type 3 (150 bp, 250 bp, 300 bp), lanes 2, 3, 5, and 6: type1 (50 bp, 150 bp, 300 bp); lane 4, type 2 (100 bp, 250 bp, 350 bp).

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DISCUSSION

During the last decades, the emergence of antibiotic-resistant bacteria has become a significant threat to public health. Among these, are MRSA isolates, a major cause of life-threatening infections in hospitals and health care settings [8]. Regarding the impact and increase of MRSA bacteria, knowledge of their origin can be useful for the control of infections caused by these bacteria. Molecular typing methods have been proved as useful tools for finding the possible relationship between MRSA isolates and the source of clinical samples. In the present study, the diversity of MRSA was investigated based on four genes: *coa, spa, aro*A, and *gap*. In our study, out of the 100 *S. aureus* isolates, 50 (50%) turned to be MRSA as indicated by amplification of the *mec*A gene. Previous surveys in Ahvaz hospitals reported the prevalence rates of 42% and 83.7% for MRSA [24, 25]. Additional studies in Iran from 2000 to 2012 showed prevalence rates of 19.5% to 90% [26-28]. This rate in Japan, Singapore, and Taiwan was 60% and in Italy and Portugal 50% [29]. The difference between the results of various studies, to some extent, might be attributed to the sensitivity of diagnostic methods.

In our study, amplification of *coa* gene revealed 8 amplicon types among 50 MRSA isolates with the majority (50%) belonging to type 3 (650 bp). In Japan, a similar study detected the same types following amplification of *coa* gene [30]. In Urmia, Iran, four *coa* types were identified, of which the type yielding a 700 bp fragment showed the highest frequency [8]. In Thailand, 4 *coa* types were identified among MRSA isolates with the highest frequency for type 3 [31].

Digestion of *coa* gene with *Alu*I produced 13 patterns comprising 1-3 bands of 81-700 bp length. The 243 bp fragment was present in 11 types and digestion of *coa* type 3 produced 3 RFLP patterns. Digestion of *coa* gene with *Hae*III (an enzyme with a similar restriction site to *Alu*I) in MRSA isolates from Egypt revealed 5 types and 11 subtypes with the 243 bp fragment present in all types [32]. In another study, in Brazil 49 different RFLP patterns were reported following digestion of *coa* gene with *Alu*I in 64 *S. aureus* isolates from the milk of cows with mastitis. [33]. Also in India, 85 MRSA clinical isolates were collected from various clinical sources and subjected to molecular typing. As a result 31 and 21 types were reported following digestion of *coa* gene with *Alu*I and *Hae*III, respectively [34]. Diversity in RFLP patterns of *coa* gene of different regions can be attributed to changes in the coagulase gene, which creates different sites for restriction endonucleases enzymes. Amplification of various bands of *coa* gene with the same primers in different studies is a reflection of the polymorphism in the coagulase gene. Deletions and insertions at 3' end of this gene seem to cause polymorphism in the size of this gene and consequently in its antigenic properties.

The polymorphism at the X region of the *spa* gene is due to a variable number of 24 bp repeated sequences. Amplification of this region produces bands of different lengths. In this study, *spa* typing revealed 5 types and 17 subtypes. The *spa* types seem to vary according to geographical areas. This gene has shown various types and subtypes in Egypt [32], Germany [35], Canary Islands [36], and Iran [3]. In this study, the *spa* gene was not present in 10% of MRSA isolates. Similar studies in Iran and other countries have also shown that some of MRSA isolates were negative for the *spa* gene as the gene was not amplified by PCR [3, 32, 37].

The PCR-RFLP of the *spa* gene with *Hind* III revealed 13 types, and tracheal isolates included the highest frequencies of types 1 and 2 (Table 6). The wound isolates distributed among 12 types indicating their high genetic diversities. In our study, the *spa* gene of five isolates was not digested with *Hind* III. However, in a similar study in Tehran, among 93 *S. aureus* isolates from food samples, the *spa* gene showed polymorphism once digested with *Hae*II and *Hind* III enzymes [38]. In another study in Egypt, 5 types and 12 subtypes of MRSA from various clinical specimens were reported after the digestion of PCR product with *Hae*II enzyme [32]. Other restriction enzymes have also been used for *spa* typing; *e.g.*, using *Rsa*I [39], *Hae*II [6] and *Bsp*143I [3], 9, 5, and 7 types were identified, respectively.

The *aro*A gene is present in many MRSA isolates with a genetic variation suitable for typing. In our study, RFLP typing of *aro*A with *Taq*I revealed 3 types including A, B and H, with the majority of isolates (75.55%) from trachea (n=13), sputum (n=3), urine (n=2) and wounds (n=16) displaying type A. The wound isolates were of A and B types. In the present study, C, D, and N genotypes were not detected among the isolates (Table 8).

Digestion of genotype A product with *Rsa*I produced 2 subtypes which majority (73.52%) of them were from the subtype A2. Based on RFLP analysis by *Taq*I, in West Azerbaijan province, Iran, the genetic diversity of 26 *S. aureus* isolates recovered from human skin and urinary tract infections were investigated. As a result, 4 *aro*A types including N (80.77%), B (7.69%), H (7.69%) and A (3.85%) were reported [40]. A similar study in Iran, reported 4 types, A (41.38%), B (50%), N (1.7%), and H (6.9%) [1]. In Spain 38.9% of the *S. aureus* isolates belonged to the type A (60% A1, and 40% A2), followed by types B (13.5%), C (3.3%), and D (44.1%) [15]. Also In Jordan, 4 *aro*A types were reported [23]. Variation of *aroA* types in different countries indicates the uniform distribution of types in different geographical areas, which may be related to different environmental conditions and the genetic diversity of the isolates. RFLP analysis of *gap* gene with *Alu*I revealed 3 types in our MRSA isolates with the type 1 comprising 94% of the isolates. Except one tracheal and 2 wound isolates, all the MRSA isolates in the present study had similar RFLP pattern.

The results of this study showed high genetic variations among MRSA isolates originated from Ahvaz hospitals. This variation might be linked to properties such as antibiotic resistance, gene acquisition, and virulence factor of MRSA and affect the outcome of infection and treatment. Continuous surveillance and monitoring of the common types in geographical regions can provide useful information about the status of these bacteria and the emergence of new isolates.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest associated with this manuscript.

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