Original Article

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

Elahe Soltani Fard1, Mohammad Roayaei Ardakani1,2*, Hossein Motamedi1,2*

1Department of Biology, Faculty of Science, Shahid Chamran University of Ahvaz, Ahvaz, Iran; 2Biotechnology and Biological Science Research Center, Shahid Chamran University of Ahvaz, Ahvaz, Iran

Received Dec. 04, 2018; Accepted Jun. 30, 2019

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 mecA gene. The diversity of MRSA isolates was investigated by amplification of the coa, spa, aroA, and gap genes followed by RFLP analysis using the AluI, HindIII, TaqI and RsaI 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 AluI 13 types, and spa with HindIII 13 types were identified. Also, the RFLP analysis of gap gene with AluI revealed 3 types, and of aroA gene with TaqI and RsaI, 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 Infect 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, aroA, 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 aroA 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].

*Correspondence:* Hossein Motamedi
Department of Biology, Faculty of Science, Shahid Chamran University of Ahvaz, Ahvaz, Iran, 4313544337.

Email: motamedih@scu.ac.ir
Tel/Fax: +98 (61) 33331045

http://jommid.pasteur.ac.ir
Glyceraldehyde-3-phosphate dehydrogenase is a product of gap gene that is a part of the glycolytic operon, which encodes transfer binding protein (Tptn). 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, spa, aroA, 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.

Table 1. The primers used for amplification of mecA, coa, spa, aroA, and gap genes in this study

| Target gene | Sequence (5' to 3') | Expected Fragment size bp | Reference |
|-------------|---------------------|---------------------------|-----------|
| MECA | F GTAGAATAGCTGACGTTCCGATAA | 310 bp | 19 |
| | R CCAATCCCAAGTGGTTCCGCTCA | | |
| COA | F ATAGAAGAGCTGACGTTACAGG | variable | 20 |
| | R GCTTCGGATTTGCTGATG | | |
| SPA | F ATCTGTTGCGTAACACCTG | variable | 21 |
| | R CGTGCACCTAAACGCTAAATG | | |
| AROA | F AAGGCGAAAATAGAATGCAGGCC | 1153 bp | 15 |
| | R CACAAGCAACTGCAAGCAT | | |
| GAP | F ATGTTTTGGTGAATTTGTCGTTTA | 933 bp | 22 |
| | R GACATTCCGTTATCATACCAAGCTG | | |

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 mecA 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 pm), 1U Taq DNA polymerase, 2mM MgCl₂, 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].

Amplification of coa, spa, aroA, and gap genes. The coa, spa, aroA, and gap genes were targeted 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 aroA 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 (DNAzist Asia, Mashhad, Iran).

RFLP analysis of coa, spa, aroA and gap genes. The PCR products of coa and gap genes were digested with AluI in reactions containing 10 μl PCR product, 1 μl AluI, 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 aroA gene product was digested with TaqI and RsaI enzymes with the same protocol except for the incubation procedure, which was 2 h at 65°C with TaqI, and 2 h at 37°C with RsaI. 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 mecA 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, aroA 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
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 AluI 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 AluI 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 aroA gene has various target sites for TaqI and RsaI restriction endonuclease. According to the genotyping methods defined by other studies [15, 23, 1], we detected 3 types in RFLP patterns following digestion of aroA gene with TaqI (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 TaqI. The frequency of types in clinical samples is reflected in Table 9.

Following digestion of the 1153 bp PCR product with RsaI, 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 AluI 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 aroA, 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 aroA subtype A2 (RsaI) 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%) | Spa1a | 1100             | 6      |
|            | Spa1b  | 1200             | 3      |
|            | Spa1c  | 1300             | 2      |
|            | Spa1d  | 1500             | 3      |
|            | Spa2a  | 300, 1200        | 3      |
| Spa2 (2 bands) (20%) | Spa2b | 350, 1300        | 3      |
|            | Spa2c  | 350, 1100        | 1      |
|            | Spa2d  | 150, 1200        | 1      |
|            | Spa2e  | 400, 1500        | 2      |
|            | 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      |
| Spa3 (3 bands) (34%) | Spa4a | 150, 300, 400, 1200 | 3 |
|            | Spa4b  | 150, 300, 600, 1100 | 1 |
| Spa4 (4 bands) (8%) | Spa5a | -                | -      |
| Spa5 (no band) (10%) | Spa5b | -                | -      |
Table 4. Typing of MRSA isolates based on the RFLP pattern of *coa* gene digested with *Aul*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      | -     | -      | 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     | -         | -       | -      | -     | -      | 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)   |
Table 8. Typing of MRSA isolates based on the RFLP pattern of aroA gene digested with TaqI

| 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 aroA gene digested with Rsal

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

Table 11. Frequency of aroA 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 AluI

| 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)
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 *AluI* 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).
Fig. 5. PCR-RFLP pattern of *aroA* 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 *aroA* 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).
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_, _aroA_, and _gap_. In our study, out of the 100 _S. aureus_ isolates, 50 (50%) turned to be MRSA as indicated by amplification of the _mecA_ 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 _AluI_ 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 _HaeIII_ (an enzyme with a similar restriction site to _AluI_) 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 _AluI_ 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 _AluI_ and _HaeIII_, 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 _HaeII_ 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 _HaeII_ enzyme [32]. Other restriction enzymes have also been used for _spa_ typing; e.g., using _RsaI_ [39], _HaeII_ [6] and _Bsp143I_ [3], 9, 5, and 7 types were identified, respectively.

The _aroA_ gene is present in many MRSA isolates with a genetic variation suitable for typing. In our study, RFLP typing of _aroA_ with _TaqI_ revealed 3 types including A, B, and H, with the majority of isolates (75.55%) from tracheal (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 _RsaI_ produced 2 subtypes which majority (73.52%) of them were from the subtype A2. Based on RFLP analysis by _TaqI_, 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 _aroA_ 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 _aroA_ 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 _AluI_ 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.
ACKNOWLEDGMENT

The authors wish to thank the vice chancellor of research of the Shahid Chamran University of Ahvaz for providing the research grant (Grant No. 31400/02/3/95).

CONFLICT OF INTEREST

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

REFERENCES

1. Saei HD, Ahmadi M, Mardani K, Batavani R. Molecular typing of Staphylococcus aureus isolated from bovine mastitis based on polymorphism of the coagulase gene in the north west of Iran. Vet Microbiol. 2009; 137 (1): 202-6.
2. Gould IM, David MZ, Esposito S, Garau J, Lina G, Mazzei T, et al. New insights into meticillin-resistant Staphylococcus aureus (MRSA) pathogenesis, treatment and resistance. Int J Antimicrob Agents. 2012; 39 (2): 96-104.
3. Shakeri F, Ghaemi EA, Kochaksaarei MB. Staphylococcus aureus typing by digestion of protein A coding gene using Bsp143I. Jundishapour J Microbiol. 2014; 7 (6).
4. Hookey J, Edwards V, Cookson B, Richardson J. PCR-RFLP analysis of the coagulase gene of Staphylococcus aureus: application to the differentiation of epidemic and sporadic methicillin-resistant strains. J Hosp Infect. 1999; 42 (3): 205-12.
5. Tenover FC, Arbeit R, Archer G, Biddle J, Byrne S, Goering R, et al. Comparison of traditional and molecular methods of typing isolates of Staphylococcus aureus. J Clin Microbiol. 1994; 32 (2): 407-15.
6. Mostafa SI. Molecular typing of meticillin resistant Staphylococcus aureus by spa gene polymorphism. Afr J Microbiol Res (AJMA). 2013; 7 (9): 755-9.
7. Tambic A, Power E, Tambic T, Snur J, French G. Epidemiological analysis of meticillin-resistant Staphylococcus aureus in a Zagreb trauma hospital using a randomly amplified polymorphic DNA-typing method. Euro J Clin Microbiol Infect Dis. 1999; 18 (5): 335-40.
8. Talebi-Satlow R, Ahmadi M, Dastmalchi Saei H. Restriction fragment length polymorphism genotyping of human Staphylococcus aureus isolates from two hospitals in Urmia region of Iran using the coa gene. Jundishapour J Microbiol. 2012; 5 (2): 416-420.
9. Zadoks R, Van Leeuwen W, Barkema H, Sampimon O, Verbrugh H, Schukken YH, et al. Application of Pulsed-Field Gel Electrophoresis and Binary Typing as Tools in Veterinary Clinical Microbiology and Molecular Epidemiologic Analysis of Bovine and Human Staphylococcus aureus Isolates. J Clin Microbiol. 2000; 38 (5): 1931-9.
10. Willems RJ, Hanage WP, Bessen DE, Feil EJ. Population biology of Gram-positive pathogens: high-risk clones for dissemination of antibiotic resistance. FEMS Microbiol Rev. 2011; 35 (5): 872-900.
11. Afrough P, Pourmand MR, Zeinalinia N, Yousefi M, Abdossamadi Z, Bagherzadeh et al. Molecular typing of clinical and nasal carriage isolates of Staphylococcus aureus by spa gene patterns. J Mazandaran Univ Med Sci. 2012; 22 (94): 28-34.
12. Mehdinattra P, Bhalla P, Ahmed A, Sharma Y. Molecular typing of meticillin-resistant Staphylococcus aureus strains by PCR-RFLP of spa gene: a reference laboratory perspective. Indian J Med Microbiol (IJMM). 2009; 27 (2): 116.
13. Agius P, Kreiswirth B, Naidich S, Bennett KP. Typing Staphylococcus aureus using the spa gene and novel distance measures. IEEE/ACM Trans Comput Biol Bioinform. 2007; 4 (4): 693-704.
14. Baum C, Haslinger-Löffler B, Westh H, Boye K, Peters G, Neumann C, et al. Non-spa-typeable clinical Staphylococcus aureus strains are naturally occurring protein A mutants. J Clin Microbiol. 2009; 47 (11): 3624-9.
15. Marcos JY, Soriano AC, Salazar MS, Moral CH, Ramos SS, Smeltzer MS, et al. Rapid Identification and Typing of Staphylococcus aureus by PCR-Restriction Fragment Length Polymorphism Analysis of the arA Gene. J Clin Microbiol. 1999; 37 (3): 570-4.
16. Yugueros J, Temprano A, Berza B, Sánchez Ma, Hernanz C, Luengo JM, et al. Glyceraldehyde-3-Phosphate Dehydrogenase-Encoding Gene as a Useful Taxonomic Tool for Staphylococcus spp. J Clin Microbiol. 2000; 38 (12): 4351-5.
17. Brown DF, Edwards DI, Hawkey PM, Morrison D, Ridgway GL, Towner KJ, et al. Chemotherapy JWPotBSFA. Guidelines for the laboratory diagnosis and susceptibility testing of meticillin-resistant Staphylococcus aureus (MRSA). J Antimicrob Chemother. 2005; 56 (6): 1000-18.
18. Ausubel FM, Brent R, Kingston RE, Moore DD, Seidman J, Smith JA, et al. Short protocols in molecular biology. 1992.
19. McClure J, Conly JM, Lau G. Novel multiplex PCR assay for detection of the staphylococcal virulence marker Panton-Valentine leukocidin genes and simultaneous discrimination of meticillin-susceptible from resistant staphylococci. J Clin Microbiol. 2006; 44: 1141-4.
20. Hookey JV, Richardson JF, Cookson BD. Molecular typing of Staphylococcus aureus based on PCR restriction fragment length polymorphism and DNA sequence analysis of the coagulase gene. J Clin Microbiol. 1998; 36 (4): 1083-9.
21. Wichelhaus TA, Hunfeld K-P, Böddinghaus B, Kraiczky P, Schafer V, Brade V. Rapid molecular typing of meticillin-resistant Staphylococcus aureus by PCR-RFLP. Control Hosp Epidemiol. 2001; 22 (05): 294-8.
22. Yugueros J, Temprano A, Sánchez Ma, Luengo JM, et al. Glyceraldehyde-3-Phosphate Dehydrogenase-Encoding Gene as a Useful Taxonomic Tool for Staphylococcus spp. J Clin Microbiol. 2000; 38 (12): 4351-5.
23. El-Huneidi W, Bdlour S, Mahasneh A. Detection of enterotoxin genes seg, seh, sei, and sej and of a novel arA genotype in Jordanian clinical isolates of Staphylococcus aureus. Diagn Microbiol Infect Dis. 2006; 56 (2): 127-32.
24. Maleki SaHa Z. Comparison of the sensitivity of the results issued by two different anti-germ disc and E test for oxacillin and vancomycin antibiotics. Islamic Azad Univ J Med Sci. 2007; 16 (4): 211-15.
25. Afragh P, Pourmand MR, Sarajian AA, Saki M, Saremy S. Molecular investigation of *Staphylococcus aureus*, *coa* and *spa* genes in Ahvaz hospitals, staff nose compared with patients clinical samples. Jundishapur J Microbiol. 2013; 6 (4).

26. Zeinali E, Moniri R, Safari M, Mousavi SGA. Molecular characterization and SCCmeC typing in meticillin-resistant *Staphylococcus aureus* isolated from clinical samples. J Kaums (FEYZ). 2011; 14 (4): 439-46.

27. Khosravi AD, Hoveizavi H, Farshadzadeh Z. The prevalence of genes encoding leukocidins in *Staphylococcus aureus* strains resistant and sensitive to methicillin isolated from burn patients in Taleghani Hospital, Ahvaz, Iran. Burns. 2012; 38 (2): 247-51.

28. Japoni A, Jamalidoust M, Farshad S, Ziyaeyan M, Alborzi A, Japoni S, et al. Characterization of SCCmeC types and antibacterial susceptibility patterns of methicillin-resistant *Staphylococcus aureus* in Southern Iran. Japanese J Infect Dis (JJID). 2011; 64 (1): 28-33.

29. Lee BY, Singh A, David MZ, Bartsch SM, Slayton RB, Huang SS, et al. The economic burden of community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA). Clin Microbial Infect. 2013; 19 (6): 528-36.

30. Ishino K, Tsuchizaki N, Ishikawa J, Hotta K. Usefulness of PCR-restriction fragment length polymorphism typing of the coagulase gene to discriminate arbekacin-resistant methicillin-resistant *Staphylococcus aureus* strains. J Clin Microbiol. 2007; 45 (2): 607-9.

31. Janwithayanuchit I, Ngam-Ululert S, Paungmoung P, Rangsipanurat W. Epidemiologic study of methicillin-resistant *Staphylococcus aureus* by coagulase gene polymorphism. Science Asia. 2006; 32: 127-32.

32. Omar NY, Ali HAS, Harfoush RAH, El Khayat EH. Molecular typing of methicillin resistant *staphylococcus aureus* clinical isolates on the basis of protein A and coagulase gene polymorphisms. Int J Microbiol. 2014; 2014.

33. da Silva ER, da Silva N. Coagulase gene typing of *Staphylococcus aureus* isolated from cows with mastitis in southeastern Brazil. Can J Vet Res. 2005; 69 (4): 260.

34. Himabindu M, Muthamilsettyan DS, Bishi DK, Verma RS. Molecular analysis of coagulase gene polymorphism in clinical isolates of methicillin resistant *Staphylococcus aureus* by restriction fragment length polymorphism based genotyping. Am J Infect Dis. 2009; 5: 170-6.

35. Schmitz FJ MS, HV. Tichy, et al. Typing of meticillin-resistant *Staphylococcus aureus* isolates from Dusseldorf by six genotypic methods. J Med Microbiol. 1998; 47 (4): 341-51.

36. Montesinos I, Salido E, Delgado T, Cuervo M, Sierra A. Epidemiologic genotyping of methicillin-resistant *Staphylococcus aureus* by pulsed-field gel electrophoresis at a university hospital and comparison with antibiotyping and protein A and coagulase gene polymorphisms. J Clin Microbiol. 2002; 40 (6): 2119-25.

37. Adesida SA, Likhoshvay Y, Eisner W, Coker AO, Abioye OA, Ogunsole FT. Repeats in the 3’ region of the protein A gene is unique in a strain of *Staphylococcus aureus* recovered from wound infections in Lagos, Nigeria. Afr J Biotechnol. 2006; 5 (20): 1858.

38. Soltan-Dallal M, Salehipour Z, Mehrabadi JF. Molecular epidemiology of *Staphylococcus aureus* in food samples based on the protein A gene polymorphic region DNA sequence. Can J Microbiol. 2010; 56 (1): 18-21.

39. Mahmoudi H, Arabestani MR, Mousavi SF, Ghafel S, Alikhani MY. Study of polymorphism *spa* gene (encoding protein A) of *Staphylococcus aureus* in clinical isolates and nasal carriers. Tehran Univ Med J. 2015; 73 (1): 24-30.

40. Talebi-Satou R, Ahmadi M, Ghavam F, Saei HD. Use of 5-Enolpyruvylshikimate-3-Phosphate synthase encoding gene for typing of *Staphylococcus aureus* isolated from skin and urinary tract infections of human. Iran J Basic Med Sci (ijbms). 2011; 15(4): 975-982.