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Molecular Investigation of *Staphylococcus aureus*, *coa* and *spa* Genes in Ahvaz Hospitals, Staff Nose Compared With Patients Clinical Samples

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A B S T R A C T

Background: *Staphylococcus aureus* is one of the important human pathogens which are mainly isolated from wound, skin and contaminated respiratory excretions. Because many of hospital staff and patients carry this pathogen in their nose or skin, close contacts and touching have special role in spreading the infection in hospitals. Also, antibiotic resistant *S. aureus*, especially Methicillin Resistant *S. aureus* (MRSA) have been seen among subjects. Thus, there should be an investigation for Bacteria colonization in nose of hospital staff and patients. Furthermore, investigation of antibiotic resistance pattern and examination of genotyping properties of resistant strains have a high efficacy in control and recognition of infection origin.
Objective: The current study aimed to determine the characteristics of *S. aureus* isolated from patients and staff in hospitals and compare them based on *coa* and *spa* typing methods.
Materials and Methods: In the current study, 157 clinical specimens were collected from patients who were treated at the Ahvaz medical university hospitals including 79 specimens (50.3%) from Sina hospital, 34 specimens (21.7%) from Imam Khomeini hospital, and 44 specimens (28%) from Golestan hospital and 157 nose swab specimens from the staff of these hospitals were collected during 2010. *coa, spa* genes of isolated Bacteria were amplified using PCR.
Result: PCR results showed seven different patterns for staff and five different patterns for patients based on *spa* gene, and for *coa* gene five and six different patterns respectively. In addition, the prevalence of MRSA was 52.5 in staff and 83.7 in patients' specimens. Comparison of genetic diversity of *spa*, and *coa* genes in Ahvaz university hospitals doesn't show significant difference (Chi-square and fisher's exact test).
Concloutions: The outcome of this study show that spa and coa typing are suitable methods for MRSA isolates typing because it is easy to use and interpret them, and that these methods can be useful in infection source detection and its control especially in epidemic situations

Keywords: Staphylococcus aureus; ProA, Coagulase

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Implication for health policy/practice/research/medical education:
Results of the current study showed that the repeat region of *coa* and *spa* genes can be useful for typing.

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1. Background

Staphylococcus aureus is the most important pathogen of human resources mainly wound, respiratory tract and skin infections. Infection spreads in hospitals because of close contact and the point that hospitals staff and patients carry antibiotic resistant *S.aureus* specially (MRSA) in their nose or on their skin (1, 2). High risk of staphylococcus infection in infants, surgery, chemotherapy and ICU wards makes enough evidence to examine the patients and staff for bacteria colonization in their noses. As a result, determination of antibiotic resistance pattern and examination of genotyping properties of isolated Bacteria has a special role in recognition of infection origin and its control.

Although Pulse Field Gel Electrophoresis (PFGE) is a standard method for Bacteria gene typing, using PCR for multiplication of *coa, spa* genes is a better technique forthe current experiments, because of its lower cost, no need for experts, more rapid competency (rapidity) and high throughput ability (3-5). Protein A is coding by *spa* gene and has a polymorphic x region with short sequence. This protein is one of the *S.aureus* surface proteins that belong to a group of adhesins called Microbial Surface Components Recognizing Adhesive Matrix Molecules (MSCRAMMS). This protein reacts to Antibodies and has an important role in immunologic and laboratory diagnosis (6-8).

The coagulase enzyme is also a virulence factor in addition to its important role in *S.aureus* diagnosis. The *coa* gene that codes coagulase enzyme has diversity in studied polymorphism of strains (9, 10). Since about 20 percent of healthy persons were permanent vectors and 60 percent of them were recurring vectors (11), and multi-drug resistant staff for different species, therefore the risk of these species transferring from hospital staff to patients or vice versa (12) is a serious problem for hospitals. The main goal of this study was to type the collected clinical isolates of Ahvaz medical university hospitals (Golestan, Sina and Imam Khomeini) and to compare them with typing of vector staff isolates.

2. Objectives

The current study aimed to determine the characteristics of *S.aureus* isolated from patients and staff of hospitals and to compare them based on *coa* and *spa* typing methods.

3. Materials and Methods

3.1. Sample Collection and Bacterial Species Identification

In the current study 157 clinical specimens were selected from patients who were treated at the Ahvaz medical university hospitals including 79 specimens (50.3%) from sina hospital, 34 specimens (21.7%) from Imam Khomeini hospital, and 44 specimens (28%) from Golestan hospital. Furthermore 157 nose swab specimens from the mentioned hospitals staff were collected during 2010. The *coa,spa* genes showed relevance to *coa*gulase and protein A, respectively.

Using microbiological standard methods including, catalase, *coa*gulase and manitol fermentation on manitol salt agar, the isolated *S.aureus* was confirmed. Then the sensitivity of isolates was examined by disk diffusion method based on CLSI guide direction, against antibiotics including methicillin (1μg), vancomycin (30μg), penicillin (10μg), and mupirocin (5μg). All antibiotic disks were prepared form MAST Company (England).

3.2. Polymerase Chain Reaction for Detection of coa and spa Genes

The *S.aureus* genome obtained from 24 hours cultures, extracted with DNA extraction kit (Bioneer Korea) based on kit manual and for amplification of *coa ,spa* genes the below primers were used,

coa 1: (-CCAGACCAAGATTCAATAAQ-)
*coa*2: (- AAAGAAAACCACTCACATCGT -)
*spa*1: (-GATCTGTAACTTTAGGTACATTAC-)
*spa*2: (-ATAGTTCGCCACGACGTC-)

PCR was performed in 50 μl reactions containing:

5 μl MgCl2, 2.5 μl Buffer 10x, 1.5 μl dNTP, 0.3 μl Taq DNA polymerase, 1.5 μl primer F (20 pm), 1.5 μl primer R (20pm), and 37.2 ml D.W. Reaction was performed in the thermo cycler (Eppendorf-Germany) by denaturation at 94 for three minutes, followed by 30 cycles of 94˚Cfor 45 seconds, 55˚C for 30 seconds and 72˚Cfor 90 seconds. Final extension was five minutes at 72˚Cin the end of cycles. The products of the PCR were analyzed by electrophoresis in a 1% agarose gel and the results were analyzed by SPSS ver.14 software. In all of the analyses *P* value =0.05 was considered.

4. Results

4.1. The Results of Patients

Among 157 patients under study, 52 patients (33.1%) women and 105 patients (66.9%) were men. 79 patients (50.3%) were from Golestan hospital, 37 patients (21.75%) from Imam Khomeini hospital and 44 patients (28%) from Sina hospital. Most of the collected specimens were from wound (46, 29.3%), blood (36, 22.9%), urine (17, 10.8%), trachea (15, 4.6%) body fluids (12, 7.6%), abscess (8, 5.1%), respiratory apparatus (7, 4.5%) and other specimens (16,

10.2%). Among these specimens, 49 (31.2%) were positive for *S. aureus* including 27 specimens (18.3%) from Golestan hospital, seven specimens (4%) from Imam Khomeini hospital and 15 specimens (9.8%) from Sina hospital. 41 isolates (83.7%) were methicillin resistant (MRSA) and all isolates were vancomycin sensitive. The most sensitivity after vancomycin was seen against mupirocin. The sensitivity of isolated *S. aureus* against Antibiotics are mentioned in *Table 1*.

Six different patterns based on *coa* gene were obtained from *S. aureus* isolates: C 1 (850bp) with 21.7% had the highest frequency, and C 7 (900bp) with 0.6% had the lowest frequency. Other patterns included C 2 (800bp) with 2.5%, C 3 (650 bp) with 3.2%, C 5 (750bp) with 1.9% and C 6 (1000bp) with 1.3%, from 49 clinical isolates, 27 (55.1%) from Golestan hospital, 15 (30.6%) from Sina and 7 (14.3%) from Imam Khomeini hospital. All Imam Khomeini hospital isolates had C 1 pattern. The C 6 adC 7 patterns were not observed in Sina hospital isolates (*Table 3* and *Figure 2*).

Among patients` isolates five different patterns based on *spa* gene were obtained. S 1 (1400bp) with 19.1% had the highest frequency, and other patterns including S 5 (1500hp) with 7%, S 3 (1200bp) with 2.5% , S 4 (1100bp) with 1.3% and S 6 (900bb) with 1.3% followed. 27 specimens (55.1%) were from Golestan hospital, 15 specimens (30.6%) from Sina and seven specimens (14.3%) from Imam Khomeini hospital. In Imam Khomeini hospital only S 1 pattern was detected and in Sina hospital S 4 pattern was not detected (*Table 2* and *Figure 2*).

4.2. The Results of Hospitals Staff

Among hospitals staff, 157 specimens from different hospitals, Golestan (79 persons , 50.3%), Imam Khomeini hospital (34 persons , 21.7%) and Sina (44 persons ,28%) were collected. Most of the specimens were obtained from nurses (82 specimen,52.2%) , doctors(10 specimens), servants(39 specimens) and nurse's aides(26 specimens). After special tests 40 *S. aureus* (25.4%) were isolated. The isolates were , 16 (10.2%) from Golestan hospital, 12 (7.6%) from Imam Khomeini hospital and 12 (7.6%) from Sina hospital. 21 isolates (52.5%) were methicillin resistant (MRSA).

All isolates were vancomycin sensitive and the most considered resistance related to penicillin with 85% (*Table 1*). Five different patterns of *coa* were obtained from staff positive specimens using PCR method. C 4 (700bp) with 8.9% had the highest frequency and other patterns including C 2 (800bp) with 5.7%, C 3 (650bp) with 1.9%, C 10 (400bp) ,C 12 (600bp) with 7% followed. From 16 *coa* specimens from Golestan hospital, all patterns were detected except C 10 , whereas in 12 specimens from Sina hospital all patterns except C 3 were obtained (*Table 3* and *Figure 1*).

Seven different patterns from *spa* gene were obtained among which S 3 (1200bp) with 8.9% was more frequent than other patterns including S 4 (1000bp) with 5.7%, S 6 (900bp) with 1.9%, S 7 (800bp) with 1.9%, S 8 (700bp) with 1.3%, S 9 (650bp) with 1.9% and S 11 (850bp) with 3.8% .Only S 3, S 4 and S 6 were seen in Golestan hospital. All patterns except S 9 were seen in Imam Khomeini hospital (*Table 2* and *Figure 1*).

Based on the obtained results no significant different among *coa,* and *spa* genes patterns with infection source and hospital wards were detected (P = 0.90- fisher's exact test). Furthermore based on chi^2 exam, distribution frequency of obtained patterns in different hospitals weren't similar. df: 5 , chi^2: 212.480 , P $\leq$ 0.0001.

5. Discussion

The *S.aureus* infections occur recurrently in hospitalized patients and in spite of Antibiotic therapy, cause severe complications (13). Considering the increasing prevalence of methicillin resistant *S. aureus*, inhibition of these infections and determination of spreading center in hospitals are definitely important subjects, the carriers of methicilln resistant strains have the original role in bacteria transmission (14). The current study showed methicillin resistance in 83.6% of clinical isolates and 52.5% of hospitals Staff `s.

Various studies have shown different results of bacterial resistance and carrierswhich may be related to various bacterial detecting methods. Aligholi *et al.* showed 70% methicillin resistant among 338 clinical isolates (15). Maleki *et al.* showed 42% MRSA among 100 clinical isolates (16). Mohraz *et al.* (2003) reported 46.5%, (17). Rahimi *et al.* in a study on 321 clinical isolates in Ahvaz reported the MRSA 73%, (18). Bagherzade *et al.* (2007) reported this frequency 62.1%, (19).

Table 1. Frequency Distribution of Hospitals Staff `s and Patients` Isolated *S. aureus* Based on Antibiotic Resistance in Antibiogram

Antibiotic	Patients specimens			Staffs specimens		
	Resistant, No. (%)	Sensitive, No. (%)	Intermediate, No. (%)	Resistant, No. (%)	Sensitive, No. (%)	Intermediate, No. (%)
Penicillin(P)	44(89.7)	5(10.3)	-	34(85)	6(15)	
Vancomycin(V)	-	49(100)	-	-	40(100)	
Mupirocin(M)	8(16.3)	41(83.7)	-	5(12.5)	35(87.5)	
Oxacillin(OX)	1(83.7)	8(16.3)	-	21(52.5)	19(47.5)	

Table 2. Frequency Distribution of Ahvaz Medical University Hospitals Staff `s and Patients` Isolated *S. aureus spa* gene

	Staffs			Patients		
Hospital	**Present of isolated Staff aureus**	***spa* variability Based on Hospital Wards**	**Percent of MRSA in Every Hospital**	**Percent of isolated *S.aureus***	***spa* variability Based on Hospital Wards**	**Percent of MRSA in every hospital**
Golestan (79)	16(20.3)	S3 :N(2)-D(1)-icu(1)-inter M(1)-inter F(1)-N.S.F(1)-S.F(2)-S.M(2)-OPD(1) S4 :N(2) N.S.M(1) S6 :S.F(1)	10(25)	27(34.1)	S1 : N(1)-inter M(2) -S F(1)-ICU(2)-CCU(5)-KTP(2)-S M(1) S5: inter F(1)-D(2)-icu(2)-OPD(1)-S M(1)-O(1) S3 :S F(1)-icu(1) S4 :O(1)-icu(1) S6 :D(1)	24(30.3)
Sina (44)	12(27.2)	S3:N.S(1) S4:icu(1)-N.S(2) S7:icu(1) S9:D(1)-icu(1)-U(1) S10:D(1)-N.S(2)-U(1)	6(15)	15(34)	S1: D(2)-icu(1)-N.S M(2)- N.S F(1)-U(2)-infect(1)- S5: S M(1)-U(1)-infect(1) S3: S M(1)-N.S M(1) S6 :icu(1)	12(27.2)
Imam Khomeini (34)	12(35.2)	S3:ccu(1) S4:ccu(1)-KTP(1) Infect(1) S6:S F(1)-KTP(2) S7:icu(1)-S M(1) S8:icu(1) S10:KTP(1)-infect(1)	5(12.5)	7(20.6)	S1:N(2)-D(2)-N.S M(1) S F(1)-inter F(1)	5(14.7)
Total (157)	40(25.4)		21(13.4)	49(31.2)		41(26.1)

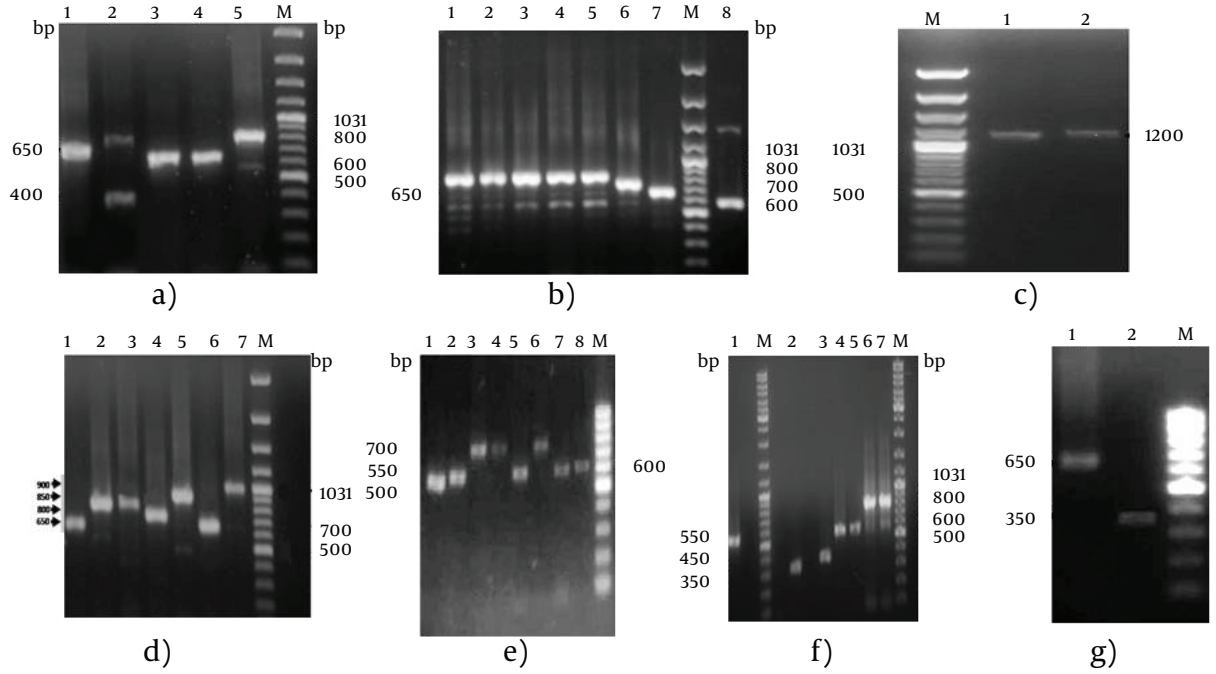
Abbreviations: N, Neurology; N.S, Neurosurgery; U, Urology; O, Orthopedic; D, Dialysis; inter, Internal; S, Surgery; KTP, Transplant: M, male; F, female; infect, infectious; c, *coa* gene; s, *spa* gene)

Table 3. Frequency Distribution of Ahvaz Medical University HospitalsStaff `s and Patients` Isolated *S. aureus*, *coa* gene

	Staffs			patients		
Hospital	**Present of Isolated *S . aureus***	***coa* variability Based on Hospital Wards**	**Percent of MRSA in Every Hospital**	**Present of Isolated *S.aureus***	***coa* variability Based on Hospital Wards**	**Percent of MRSA in Every Hospital**
Golestan(79)	16(20.3)	C3: S F(1) C4: N(1)-inter F(1)-S F(1)- S M(1) –D(1) -N.S(2)-OPD(1) C2: N(2) C12: N(1)-inter M(1)-icu(1)-N.S(1)-SM(1)	10(25)	27(34.1)	C1: N(1)-S F(1)-S M(2)-D(2)- ccu(5)-KTP(2)-inter M(1)-inter F(1)-icu(1) C2: inter M(1)-icu(1) C3:S M(1)-U(1)-icu(2) C6: D(2) C7:O(1)- C5: ICU(2)	24(30.3)
Sina (44)	12(27.2)	C2: D(1)-icu(1)-S M(1)-U(1) C4: N.S M(1) icu(2)-U(1) D(1) C10: N.S M(1) C12: N.S.M(2)	6(15)	15(34)	C1: S M(1)-D(2)- U(2)- icu(1)-N.S F(1)-infect(2)-N.S M(2) C2: S M(1)-U(1) C3: N.S M(1) C5: ICU(1)	12(27.2)
Imam Khomeini (34)	12(35.2)	C2: S M(1)- ccu(1) KTP(1) C10:infect(1) SM(1) C3: KTP(1)-infect(1) C4: KTP(1) C12: SM(1)-CCU(1)-ICU(1)-KTP(1)	5(12.5)	7(20.6)	C1: N(2)-inter F(1)-S F(1)-D(2)-N.S M(1)	5(14.7)
Total (157)	40(25.4)		21(13.4)	49(31.2)		41(26.1)

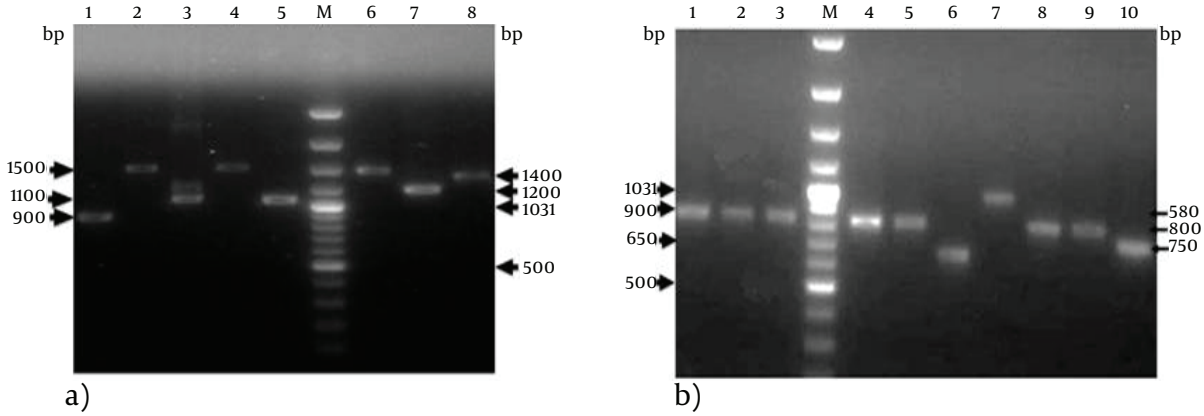
Abbreviations: N, Neurology; N.S, Neurosurgery; U, Urology; O, Orthopedic; D, Dialysis; inter, Internal; S, Surgery; KTP, Transplant: M, male; F, female; infect, infectious; c, *coa* gene; s, *spa* gene

Figure 1. Study of Pattern Diversity of *coa, spa*, in Ahvaz Hospitals Staff Specimens



A) Column one, *coa*3 (650bp) gene type, column two, *coa*10 (400bp) gene type, column three, four, *coa* 12 (600bp) gene type, column 5 *coa*2 (800bp) gene type B) Column one to, five, *coa2* (800bp) gene type, column six, *coa4* (700bp) gene type, column seven, *coa* 3 (650bp) gene type, column eight, *coa*12 (600bp) gene type C) Column one, two, *spa*3 (1200bp) gene type D) Column one, *spa*9 (650bp) gene type, column two, three, *spa*11 (8500bp)gene type, column four, *spa*7 (800bp) gene type, column five, *spa*6(900bp)gene type, column six, *spa*8 (700bp) genotype ,column seven, *spa*4 (1000bp) gene type.

Figure 2. Study of Pattern Diversity of *coa*, *spa*, in Ahvaz Hospitals Patients Specimens



M: molecular DNA marker (100 plus -fermentase company) . A) Column one, *spa*6 gene type (900 bp), column two, five, *spa*5 gene type(1500 bp), Column three, five, *spa*4 gene type (1100), column six, *spa*5 gene type (1500 bp), column seven, *spa*3 gene type (1200 bp), column eight, *spa*1 gene type (1400 bp) B) Column one, *coa*7 (900bp) gene type, column two, three, *coa*1 (850bp) gene type, column six, *coa*3 (650bp) gene type, column six *spa*5 gene type (1500 bp), column seven, *coa*6 (1000bp) gene type, column 10, *coa*5(750bp) gene type, column eight nine, *coa*2 gene type (800 bp).

The current study is more similar to Rahimi`s study based on MRSA frequency of staff specimens (18). The current study could detect 49 isolates of *S. aureus* (32%) in clinical specimens and based on *spa, coa* genes 40 isolates (25.4%) in hospital staff, further more it could determine the different patterns in these isolates. Present study shows nine different patterns based on *coa* gene, and in this respect, is similar to Ishino *et al.* (2007) study which determined eight different patterns of *coa*gene in Japan (20).

Bagherzadeh *et al.* also showed 10 different patterns of *coa* gene in 103 clinical isolates in Tehran that was similar to the current study result (20). Shittu *et al,* also determined 4 different patterns of *coa* gene in *staphylococcus aureus*isolates of South Africa using *coa* typing method in their study to C4, C3, C2 and C2 of the current study (21).

Janwithayanuchi *et al.* determined 4 different patterns of *coa* gene in 129 MRSA isolates from 17 hospitals in Thailand. The most frequent pattern in their study was the 111 pattern (37520) that was similar to C4 pattern in size (22). Mitain *et al*, also detect 6 different patterns for *coa* gene in 35 MRSA isolates in Japan based on PCR-RFLP method (23). Li Q *et al*, study also showed S11 type of C1 and C2 region of *coa* gene in hospital isolates (24). In the current study, 10 different patterns of *spa* gene were determined by PCR, which were similar to those of Harmsen *et al*. study in Germany, they could determine 10 different patterns of *spa* gene in 191 MRSA isolates of a university hospital by *spa* typing (25).

Modley *et al*. could determine five types in 320 clinical isolates in south Africa in 2010 by *spa* typing method (26). Montensinos *et al*. also could detect 4 different patterns of *spa* gene in MRSA isolates using *spa* and *coa*typing methods (27). And the Mitanni study in Japan in 2005 showed different patterns of *spa* gene using PCR-RFLP method on *coa, spa* genes (23).

The comparison of genetic diversity of *coa* and *spa* genes from patients and staff of different parts of Ahvaz medical university hospitals didn't show significant difference based on chi-square and fisher's exact tests. Comparison of determined patterns of patients and staff of Golestan, Sina and Imam Khomeini hospitals, showed genetic diversity in the most specimens. However in some cases similar bands were seen that may have occurred as the results of bacterial transmission among patients and hospital staff.

The outcome of this study and the other similar researches, show that *spa* and *coa* typing are suitable methods for MRSA isolates typing because it is easy to use and interpret them, and that these methods can be useful in infection source detection and its control especially in epidemic situations.

Authors' Contribution

None declared.

Financial Disclosure

None declared.

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