

Full Length Research Paper

Staphylococcus aureus protein A gene typing by PCR-RFLP

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Staphylococcus aureus protein A coding gene (*spa*) is a proper gene for typing of *S. aureus* and used to trace in the epidemiological studies. The aim of this study was to explore the typing of *S. aureus* by polymerase chain reaction restriction fragment-length polymorphism (PCR-RFLP) *spa* gene. Hae II restriction enzyme (Fermentase Co, Germany) method was used to digest PCR product of *spa* gene of 178 *S. aureus* which was isolated from carrier and healthy subjects within Gorgan located in the northern Iran. 3 to 10 samples from each *spa* types were sequenced (ABI Big Dye Biosystems, CA, USA). The statistical comparison of findings was done by χ^2 , the $p < 0.05$ was considered as significant. The *S. aureus* results were divided in 3 groups and 8 types by PCR-RFLP *spa* gene. The group 1 (3 bands), group 2 (2 band) and group 3 (4 band) with following ratios 69.7%, 26.4% and 3.9%, were detected respectively. Groups 1, 2 and 3 consist of 2, 3, 3 types, respectively. 20.8% of isolated *S. aureus* did not have either of A or B IgG binding region. The group 3 only was seen on the healthy carrier. The frequency of aforementioned groups among the patients and healthy carrier showed to have a significant difference ($p < 0.001$). In conclusion, Hae II enzyme can be applied perfectly for typing of *S. aureus*. Due to deletion of either A or B IgG binding regions of *spa* in some isolated *S. aureus*, it is suggested to use *spa* PCR-RFLP HaeII enzyme for *S. aureus* typing simultaneously with *spa* Xr typing.

Key words: *Staphylococcus aureus*, protein A, *spa*, typing, polymerase chain reaction restriction fragment-length polymorphism (PCR-RFLP).

INTRODUCTION

Staphylococcus aureus is one of the most important recognized bacterial pathogens in diseases such as bacteremia, endocarditis, toxic shock syndrome and skin infections as sporadic and epidemic (Saderi et al., 2009). Among the most important characterization of this bacteria is the high capability of pathogenesis, toxins, different enzyme production and its ability in acquiring rapid resistance toward antibiotics. Due to the high incidence of the diseases occurred by this bacteria, the application of different typing methods particularly molecular techniques have been developed for

epidemiological tracing. Protein A is one of the main cell wall protein, unique in this bacteria and can also binds to Fc IgG (except IgG3). In the structural of this protein, 4 to 5 IgG binding site (A, B, C, D, E) are present, and also it can be considered as the pathogen (Hallin et al., 2009; Movitz, 1974). In recent years, *spa* (*S. aureus* protein A gene) typing has been used frequently as a typing method (Baum et al., 2009; Sorum et al., 2013). *spa* consist of 6 regions, A to E (their products behave as immunoglobulin binding site) and X which is a length variable region with a 24 bp which have been repeated 2

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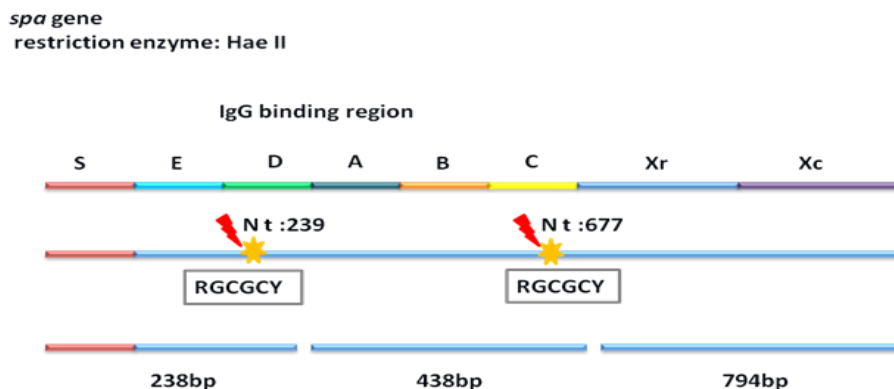


Figure 1. Restriction site of HaeII on the *spa* gene in *S. aureus* strain 8325-4, expected according OLIGO software.

to 16times. The later observation in X- region of protein A is being used for the typing of *S. aureus*, due to its reproducibility and type ability by high differentiation power (Mehndiratta et al., 2009) (Arvind et al., 1989).

In *spa* typing, the main concern is related to variable X region and on this base the various online sites such as NCBI and *spa* Ridom type for gene typing of this protein are available. Based on this fact, the alterations on the number of antibody complementary binding region are undetectable and practically are missed during this typing procedure. Therefore, this study was designed with the aim of *S. aureus* typing based on Polymerase Chain Reaction Restriction Fragment-Length Polymorphism (PCR-RFLP) *spa* encoding gene.

MATERIAL AND METHODS

Staphylococcus aureus isolation

This study was carried out on 178 isolated *S. aureus* which was collected from 77(43.3%) healthy carriers and 101(56.7%) from patients referred either to teaching hospital or private medical diagnostic laboratory in our region. As in our earlier study in this area of research, diagnostic biochemical tests initially were used to identify *S. aureus* which was followed by Glutamate synthesizes gene detection by PCR technique to confirm the primary diagnosis. The specific primer of *mecA* was applied to assess the methicillin-resistant *S. aureus* (MRSA) strains, and it was found that, 49(27.5%) and 129(82.5%) of *S. aureus* isolates were MRSA and MSSA (methicillin-sensitive *S. aureus*), respectively (Shakeri et al., 2010).

DNA extraction and PCR *spa*

DNA extraction and *spa* gene amplification was carried out according to the previous study (Shakeri et al., 2010) which can be explained briefly as follow: the DNA extraction was done using the lysostaphin, phenol-chloroform and specific primers mentioned as follows was used for PCR with product length varied 1150 to 1500 bp (Mehndiratta et al., 2009).

ATCTGGTGGCGTAACACCTG-3' - 5 *spa*1

CGCTGCACCTAACGCTAATG-3" -2 5 *spa*

PCR RFLP

The HaeII enzyme (Fastdigest HaeII- Fermentas) was used to digest the final PCR product of *spa* gene. The OLIGO software 5th version was also applied to detect the restriction sites on the gene. The restriction sites of this enzyme is outside of X region and recognized the RGCGCY sequence in genes. On condition of no gene mutation, we expect to observe 3 bands in electrophoresis, according to standard *S. aureus* strain 8325-4 (Figure 1). The final volume of enzymatic mixture for PCR-RFLP was 30 µl; consist of 17 µl distilled water, 2 µl reaction buffer, 1 µl of Hae II enzyme and 10 µl of PCR product of *spa*. The sample was incubated in 37 for 15 min than the resulting products were diagnosed by gel agarose 1.5% electrophoresis.

Sequencing

3 to 10 samples from each *spa* types were sequenced (ABI Big Dye Biosystems, CA, USA) to determine the differences existed among various types. The findings were analyzed using Gene Runner software and were compared by *S. aureus* 8325-4.

Statistical analysis

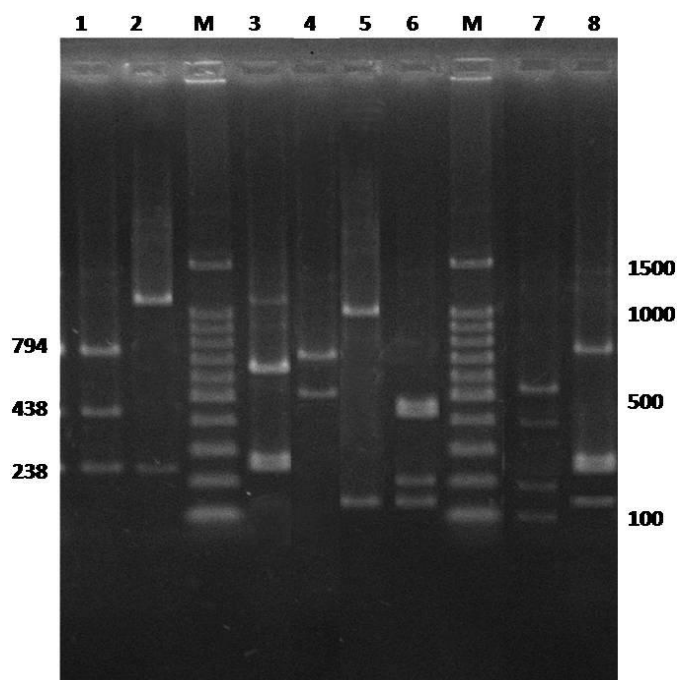
The statistical comparison of results was done by χ^2 and the $p < 0.05$ was considered as significant.

RESULTS

The length of *spa* band in 178 isolated bacteria was varied from 1150 to 1500 bp (Shakeri et al., 2010) and after digestion by HaeII, three patterns with 2, 3 and 4 fragments were observed. These patterns were classified in 8 types according to the size and number of aforementioned fragments. Group 1 with 3 fragments (two restriction site), group 2 with 2 fragments (one restriction site), and group 3 with 4 fragments (three restriction sites) including 69.7, 26.4 and 3.9% of studied *S. aureus* respectively (Table 1 and Figure 2). Following

Table 1. The prevalence of *S. aureus* groups and types after digestion of *spa* PCR product by HaeIII enzyme.

Group	Band type		Length of bands			Number (%)	Total length (bp)	Total
	1	2	3	4	5			
1	1	-	238	438	794	95(53.4%)	1470	N=124(69.7%)
	3	-	238	288	750	29(16.3%)	1276	
2	2	-	238	-	1035	39(21.9%)	1273	N=47(26.4%)
	4	-	-	475	676	5(2.8%)	1152	
	5	-	-	147	1085	3(1.7%)	1232	
3	6	150	238	438	644	3(1.7%)	1470	N= 7(3.9%)
	7	98	238	438	644	2(1.1%)	1418	
	8	150	238	288	815	2(1.1%)	1491	

**Figure 2.** The types of *S. aureus* following the digestion of *spa* gene by HaeIII enzyme in PCR RFLP method. M (DNA marker 100 bp, Fermentase company, Germany), number designated the various types.

sequencing *spa* gene, it was found that the A IgG binding site in types 3, 4 and B IgG binding site in type 5 were deleted, but the 5 IgG binding sites in other types were conserved (Figure 3). The most common type belong to type 1 (group 1) which contain all of 5 IgG binding sites, 2 restriction sites (HaeIII) and is similar to standard 8325-4 strain. All of the seven isolated bacteria in third group (type6-8) obtained from carriers and were MSSA; none of them were isolated from patients and MRSA. The prevalence of three groups among the patients and healthy carrier show significant difference ($P < 0.001$), but their prevalence among isolated MRSA and MSSA did

not show significant differences ($P = 0.058$) (Table 2). The mean age of cases in groups 1, 2, 3 were 33.6, 30.4 and 36.5, respectively, this findings statistically was not significant.

It should be mentioned that bacteria belong to groups 1 and 2 were found among all tested clinical samples (Table 3).

DISCUSSION

The typing of pathogenic bacteria is an important

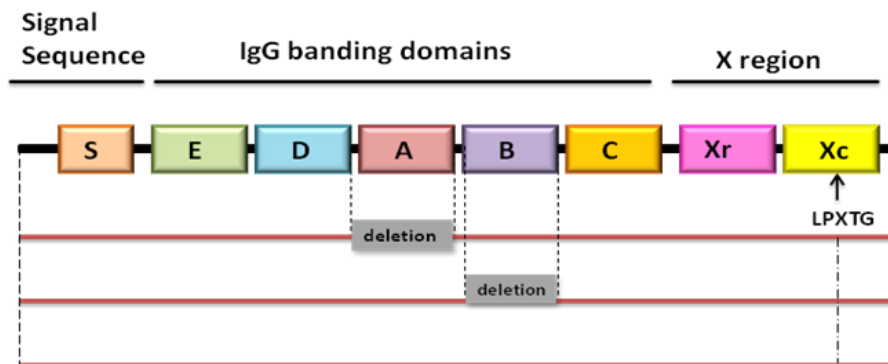


Figure 3. Deletion IgG binding sites in different *spa* gene types. Types 3, 4 loss the A and type 5 loss the B IgG binding sites.

Table 2. The frequency of *S. aureus* *spa* groups after digestion of *spa* PCR product by HaeIII enzyme, according to the isolation sources and among MRSA and MSSA.

Group samples	1	2	3	Total (%)
Carrier	44(57.1%)	26(33.8%)	7(9.1%)	100
Patient	80(79.2%)	21(20.8%)	0(0%)	100
MRSA	40(81.6%)	9(18.4%)	0(0%)	100
MSSA	84(65.1%)	38(29.5%)	7(5.4%)	100

Table 3. The frequency of *S. aureus* groups according to the clinical sample sites using PCR-RFLP with HaeIII enzyme.

Group isolation sites	1	2	3
Urine	27(84.4%)	5(15.6%)	0(0%)
Wound	18(81.8%)	4(18.2%)	0(0%)
Blood	16(66.7%)	8(33.3%)	0(0%)
Other *	19(82.6%)	4(17.4%)	0(0%)

**S. aureus* isolated from other samples such as: Synovial fluid, sputum, throat culture and CSF.

procedure in the epidemiological studies and the control of nosocomial infections. *Spa* typing and PCR-RFLP in addition to PFGE are among the efficient methods which are applied to differentiated *S. aureus*, particularly MRSA strains (Go'mez et al., 2006; Lim et al., 2012). In this study, isolated *S. aureus* were classified by PCR-RFLP method with HaeIII enzyme, divided in 3 groups and 8 types. The *spa* gene length in the isolated cases were varied from 1150 to 1500 bp, which were slightly longer than data reported from studied in India (1150 to 1420 bp). The maximum repetitive X-region in our samples was 16 times, but the same item in India was 13 (Mehndiratta et al., 2009). In this present study we were able to determine 8 different types from *spa* gene, contrary to the findings in India which was 5, this difference was due to the fact that, we examined both MRSA and MSSA strains but study carried out in India only was on MRSA strains. We found types 6, 7 and 8

only among MSSA strains. It should be mentioned that types 1 and 3 in our study and India were similar. First group which is the most common group, divided in 2 types on the bases of Xr length and the number of IgG binding sites. We found that the length of *spa* in *S. aureus* isolated in the north of Iran was varied from 1150 to 1500 bp, this variation is due to two phenomenon; change in the number of Xr region and deletion of A or B region in *spa* gene. We found that at least in 3 out of 8 types (20.8%), this deletion occurred; this finding can be considered one of the reasons behind variation among *spa* gene length.

The majority of recent researches in typing of MRSA strains are based on Xr region but Baum et al. (2009) demonstrated this technique not to have enough efficacy, and the amplification of PCR were lost because in some cases the deletion of IgG binding site particularly in C region occurred (Baum et al., 2009) due to the requirement

ment of this IgG binding site for Xr amplification. In our study, we do not observe the deletion of C region, it means all *S. aureus* isolated in our region can be typed by Xr *spa* typing method, therefore, it is suggested to apply PCR-RFLP *spa* total gene, by HaeIII enzyme, simultaneously with random repeated Xr region to have a proper knowledge *S. aureus* typing. The third group which is a nascent group in *S. aureus* consists of 3 HaeIII restriction sites and 4 bands in electrophoresis. In this group, all of IgG binding site were available, although, this group had very low prevalence but have high variation and is classified in 3 types, probably produced from different colons of *S. aureus*.

Our findings indicated that all of the isolated *S. aureus* belong to third group were collected from healthy carriers, also it should be mentioned that all of them were MSSA. These findings can be considered as their novelty of these groups in this region. On the other hand, our findings showed that the variability of different types in MSSA is more than MRSA, in such way that in MRSA, only 5 types and in MSSA all of 8 types were recognized. Some other studies showed that the types distribution and variability among MSSA are more than MRSA, for example Fenner et al. (2008) which classified MSSA and MRSA by *spa* typing found that 65 types were present among 101 MSSA isolate, but 42 types among 200 MRSA isolates, which is an indicative of high variation of *spa* types among MSSA (Fenner et al., 2008).

Strommenger in a study on 1459 *S. aureus* isolates demonstrated that the types variations of MSSA (128 types for 283 isolate) was more than MRSA types variations (121 types in 1176) (Strommenger et al., 2008); the later workers believed that the restriction in MRSA variability can be an indicative that the majority of these types are produced from a specific sort of bacteria.

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