**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 X², 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 Haell 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 Agene) 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
to 16 times. 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).

ATCTGGTGCGTAACACCTG-3' - 5'

CGCTGCACCTAAGCTATG-3' -25 spa

**PCR RFLP**

The Haell enzyme (Fastdigest Haell- 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 RGGCGY 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 Haell 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 X² 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 Haell, 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 *HaeII* enzyme.

<table>
<thead>
<tr>
<th>Group</th>
<th>Band type</th>
<th>Length of bands</th>
<th>Number (%)</th>
<th>Total length (bp)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-</td>
<td>238</td>
<td>438</td>
<td>794</td>
<td>1470</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>238</td>
<td>288</td>
<td>750</td>
<td>1276</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>N=124(69.7%)</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td>238</td>
<td>-</td>
<td>1035</td>
<td>1273</td>
</tr>
<tr>
<td>4</td>
<td>-</td>
<td>-</td>
<td>475</td>
<td>676</td>
<td>1152</td>
</tr>
<tr>
<td>5</td>
<td>-</td>
<td>-</td>
<td>147</td>
<td>1085</td>
<td>1232</td>
</tr>
<tr>
<td>6</td>
<td>150</td>
<td>238</td>
<td>438</td>
<td>644</td>
<td>1470</td>
</tr>
<tr>
<td>7</td>
<td>98</td>
<td>238</td>
<td>438</td>
<td>644</td>
<td>1418</td>
</tr>
<tr>
<td>8</td>
<td>150</td>
<td>238</td>
<td>288</td>
<td>815</td>
<td>1491</td>
</tr>
</tbody>
</table>

**Figure 2.** The types of *S. aureus* following the digestion of *spa* gene by *HaeII* 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 (*HaeII*) 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 HaeII enzyme, according to the isolation sources and among MRSA and MSSA.

<table>
<thead>
<tr>
<th>Group samples</th>
<th>1 (79.2%)</th>
<th>2 (20.8%)</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carrier</td>
<td>44(57.1%)</td>
<td>26(33.8%)</td>
<td>7(9.1%)</td>
</tr>
<tr>
<td>Patient</td>
<td>80(79.2%)</td>
<td>21(20.8%)</td>
<td>0(0%)</td>
</tr>
<tr>
<td>MRSA</td>
<td>40(81.6%)</td>
<td>9(18.4%)</td>
<td>0(0%)</td>
</tr>
<tr>
<td>MSSA</td>
<td>84(65.1%)</td>
<td>38(29.5%)</td>
<td>7(5.4%)</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Group isolation sites</th>
<th>1 (84.4%)</th>
<th>2 (15.6%)</th>
<th>3 (0%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine</td>
<td>27(84.4%)</td>
<td>5 (15.6%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Wound</td>
<td>18(81.8%)</td>
<td>4 (18.2%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Blood</td>
<td>16 (66.7%)</td>
<td>8 (33.3%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Other</td>
<td>19(82.6%)</td>
<td>4 (17.4%)</td>
<td>0 (0%)</td>
</tr>
</tbody>
</table>

*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 HaeII 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 Xr-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 HaeII 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 HaeII 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.

REFERENCES


