Study the Association of Accessory Gene Regulator Types and Methicillin Resistance/Sensitivity of Staphylococcus aureus Isolated in Gorgan, Iran

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Background: In this study, we investigated the prevalence of Staphylococcus aureus agr groups to detect the predominant type according to the source of isolation and assessed the possible relationship between agr groups, types of infection and susceptible or resistance to methicillin.

Materials and Methods: DNA of 194 S. aureus isolates were extracted by lysozyme-phenol chloroform method that included 85 clinical samples, 58 samples were isolated from nose of health care workers and 51 were obtained from food products in Gorgan, North of Iran. PCR-based assays were used for the identification of agr specificity group and mecA gene.

Results: The majority of isolates belonged to agr group I (43.3%), followed by agr group III (28.87%), agr group II (22.68%), agr group IV (5.15%) and 40.7% of strains were MRSA. In our study, the majority of S. aureus isolates recovered from health care workers and food products were agr group I and isolates recovered from patients were agr group III, these differences were statistically significant (P-value <0.05). There was no statistical difference between the agr groups, infection type and susceptibility or resistance to methicillin. However, agr group III was the predominant group in MRSA strains.

Conclusion: The agr group I was predominant among isolates of health care workers and food products specimens in Gorgan, North of Iran, while agr group III was predominant in MRSA strains and the isolates from patients. Investigation of the possible role of agr group III in S. aureus infections in the further studies is recommended.

Keywords: S. aureus, agr genes, PCR

1. Background

Staphylococcus aureus is a human commensal and cause of a different infections including hospital-acquired infections, subcutaneous abscesses, furuncles, sepsis, scalded skin syndrome, pyogenic arthritis, necrotizing pneumonia, and toxic shock syndrome (TSS) (1).

Methicillin resistant Staphylococcus aureus (MRSA) is a major human pathogen with many clinical appearance and their frequency are too vary between countries. (1, 2).

The accessory gene regulator (agr) locus controls and regulation of the production of virulence factors. This two-component system is composed of, the agr-locus and a secreted auto-inducing-peptide (AIP). The agr locus have two various transcriptional units, RNAII and RNAIII, driven by the P2 and P3 promoters (2, 3).

The P2 promoter encodes four proteins (AgrA, AgrB, AgrC, and AgrD) that generate the agr sensing mechanisms and P3 promoter encoding the effector molecule (RNAIII). RNAIII which contains many genes that encoding toxin and other secreted virulence factors(2, 4, 5).

Based on agr operon including agrA, B, C and D, Staphylococcus aureus agr groups can be divided into four agr groups. (6).

The relation between agr types and Staphylococcal disease had been proven in several study. Jarray and colleague (7), described that Staphylococcus aureus TSST-1-producing isolates belonged to agr group III. Boubaker and colleague (8), showed that strains cause of noninvasive infections and invasive infections especially bacteremia belonged to agr group III and agr group I, respectively. Chini et al (9) described that TSS toxin 1-producing isolates belonged to agr group I and III. Strommenger and colleague (10) found that agr group I were common in MRSA strains.

2. Objectives

In this study, we first investigation of the prevalence of agr groups and detect the predominant type and for the second stage, the possible relationship between agr groups, infection type and sensitive or resistant to methicillin were determined.

3. Materials and Methods

3.1. Bacterial isolates

One hundred and ninety four isolates of S. aureus were collected from patients (85 samples), health care workers (58 samples), and food products (51 samples) from Gorgan, Iran between 2009 and 2012. The isolates were identified by phenotypic methods such as Gram Staining, Catalase, Coagulase and Dnase test (11).

3.2. Genomic DNA Extraction

DNA extraction were done based on the method that was mentioned earlier. Briefly, 1ml of each S. aureus fresh culture were lysed with lysozyme-phenol chloroform method and treated with N-lauroyl sarcosine sodium salt 2% (300µL), proteinase k 100 µg (50µL), and RNase A (5µL). (11).

3.3. The agr and mecA typing

Identification of the agr groups and mecA genes were carried out by PCR with specific primers which are shown in Table 1 (12).

The PCR assay was performed in 25µL of reaction mixture containing: 1.5U of Taq DNA polymerase (Fermentas), 200µM dNTPs (Fermentas), 5mM MgCl2(200mM), 2.5µL of 10X PCR buffer, 5µL of the nucleic acid solutions and a 1µM
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concentration of each primers. The PCR conditions were an initial denaturation step at 94°C for 6 min followed by 32 cycles of denaturation at 95°C for 45s, annealing at 56°C for 1min, and elongation at 72°C for 70s and final extension step at 72°C for 8 min (12). PCR product was electrophoresed in a 1.5% agarose gel and stained with ethidium bromide. Statistical analyses were calculated by using SPSS software (version 16), X² Statistical test and P-value <0.05 was considered significant.

4. Results
One hundred and ninety four S. aureus isolates investigated in this study were collected from patients, health care workers and food products (such as meat, dairy and cookies). The prevalence of agr groups were agr group I (43.3%), agr group III (28.87%), agr group II (22.68%) and agr group IV (5.15%), respectively (Figure1). In all, 79 (40.7%) of isolates were methicillin resistant and 115 (59.3%) of them were sensitive. The agr group I was the common agr groups in S. aureus that were recovered from health care workers and food products, but agr group III was predominant in patients samples with statistically significant (P-value <0.05). Interestingly, agr group IV shown the least prevalence in all these sources (Table 2).

Table 1. The specific primers used in this study.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward Primers</th>
<th>Reverse Primers</th>
<th>Product size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>agr I</td>
<td>5-ATG CAC ATG GTG CAC ATG C-3</td>
<td>5-TAT TAC TAA TTG AAA AGT GGC CAT AGC-3</td>
<td>441</td>
</tr>
<tr>
<td>agr II</td>
<td>5-GTC ACA AGT ACT ATA AGC TGC CAT GAT-3</td>
<td>5-GTA AT TAT  TAC  TAA TTG AAA AGT GGC CAT AGC-3</td>
<td>575</td>
</tr>
<tr>
<td>agr III</td>
<td>5-GTA ATG TAA TAG CTT GTA TAA TAC CCA G-3</td>
<td>5-CGA TAA TGC CGT AAT ACC CG-3</td>
<td>323</td>
</tr>
<tr>
<td>agr IV</td>
<td>5-GTTCTCAGTACCCGATTTGTC-3</td>
<td>5-GTTCTCAGTACCCGATTTGTC-3</td>
<td>533</td>
</tr>
</tbody>
</table>

Table 2. Distribution of different S. aureus agr types based on source of bacteria isolation.

<table>
<thead>
<tr>
<th>Place of isolation</th>
<th>agr I</th>
<th>agr II</th>
<th>agr III</th>
<th>agr IV</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>patient</td>
<td>28(34.1%)</td>
<td>15(17.6%)</td>
<td>35(41.2%)</td>
<td>67(14%)</td>
<td>85(100%)</td>
</tr>
<tr>
<td>health worker</td>
<td>28(48.3%)</td>
<td>13(22.4%)</td>
<td>16(27.6%)</td>
<td>1(1.7%)</td>
<td>58(29.9%)</td>
</tr>
<tr>
<td>food product</td>
<td>27(22.9%)</td>
<td>16(31.4%)</td>
<td>5(9.8%)</td>
<td>3(5.9%)</td>
<td>51(26.3%)</td>
</tr>
<tr>
<td>Total</td>
<td>84(43.3%)</td>
<td>44(22.7%)</td>
<td>56(28.9%)</td>
<td>10(5.1%)</td>
<td>194</td>
</tr>
</tbody>
</table>

Table 3. Distribution of different S. aureus agr gene types isolated from patients.

<table>
<thead>
<tr>
<th>Specimens</th>
<th>agr I N (%)</th>
<th>agr II N (%)</th>
<th>agr III N (%)</th>
<th>agr IV N (%)</th>
<th>Total N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>urine</td>
<td>6(28.6%)</td>
<td>6(28.6%)</td>
<td>11(39.3%)</td>
<td>3(10.3%)</td>
<td>28(100%)</td>
</tr>
<tr>
<td>wound</td>
<td>9(45.0%)</td>
<td>1(4.5%)</td>
<td>10(45.5%)</td>
<td>2(9.1%)</td>
<td>22(50%)</td>
</tr>
<tr>
<td>blood</td>
<td>6(37.5%)</td>
<td>4(25.0%)</td>
<td>5(31.2%)</td>
<td>1(6.2%)</td>
<td>12(25.5%)</td>
</tr>
<tr>
<td>others</td>
<td>6(31.6%)</td>
<td>4(21.1%)</td>
<td>9(47.4%)</td>
<td>0</td>
<td>19(43.2%)</td>
</tr>
<tr>
<td>Total</td>
<td>26(34.1%)</td>
<td>15(17.6%)</td>
<td>35(41.2%)</td>
<td>6(7.1%)</td>
<td>85(100%)</td>
</tr>
</tbody>
</table>

Table 4. The distribution of different S. aureus agr types based on resistance/sensitive to methicillin.

<table>
<thead>
<tr>
<th>agr group</th>
<th>MRSA</th>
<th>MSSA</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>agr I</td>
<td>18</td>
<td>50</td>
<td>68</td>
</tr>
<tr>
<td>agr II</td>
<td>12</td>
<td>2(70%)</td>
<td>40</td>
</tr>
<tr>
<td>agr III</td>
<td>40(61.5%)</td>
<td>25</td>
<td>65</td>
</tr>
<tr>
<td>agr IV</td>
<td>9</td>
<td>12</td>
<td>21</td>
</tr>
<tr>
<td>Total</td>
<td>79</td>
<td>115</td>
<td>194</td>
</tr>
</tbody>
</table>

Although agr group III was more common in the patients samples, but, in blood samples the agr group I and III were more frequency than other groups. agr group IV prevalence was similar in wound, urine and blood samples (Table 3). Finally, no significant differences observed between the agr group and the samples source (P-value >0.05).

The agr group III with 61.5% was the main agr groups in MRSA strains and agr group II with 70% was the predominant in MSSA strains, however, there were not significant differences between the agr group and susceptibility to mecthillin (Table 4).
Boubaker and colleagues (2006) in Tunis (8) showed that out of a total of 57 S. aureus strains that were isolated from patients, agr group III were predominant type in MRSA strains and Strommenger and colleagues (2004) in Germany (10) found that all of the MRSA strains that were isolated from Central Europe belonged to agr group I.

Our study could not show a distinction between certain types of diseases and agr type. However, studies on strains that were isolated from patients with certain disease can clarify the role of agr types in pathogenesis.

Boubaker and colleagues (2006) in Tunis (8), showed that agr group III strains were associated with non-invasive infections and agr group I strains were related to invasive infections, especially bacteremia which confirm our findings showing that the frequency of agr group I in bacteria isolated from blood cultures was higher than the other groups.

One of the purposes of bacterial typing is for understanding the epidemiology of infectious diseases, such as agr typing and other methods like spa typing, MLST, coa typing and PFGE as these methods can be useful tools to achieve this purpose.

These findings suggest that the prevalence of predominant agr specificity groups differs according to epidemiological and regional factors and is useful for finding the relationship with clinical signs. In Golestan province, North of I.R. Iran, the agr group III was predominant in MRSA and the clinical isolates.

Our findings indicate that higher virulence and resistance among agr group III in comparison to other groups may be accidental. Thus we suggest larger scale studies on S. aureus strains from various infections.

6. Conclusion
The results of this study illustrate that agr group I was predominant among health care workers and food product specimens in Gorgan, North of Iran; however, agr group III was predominant among MRSA and clinical strains. Investigation of the possible role of agr group III in S. aureus infection in the next studies is recommended.

Conflict of Interests
The authors declare they have no conflict of interests.

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Authors’ Contributions
Meysm Hasannejad Bibalan and Fatemeh Shakeri performed the experiments and wrote the manuscript; Naeme Javid analyzed data and Ezzat Allah Ghaemi designed the experiments and analyzed data.

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