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 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* were divided into four agr groups. (6)

The relation between agr types and Staphylococcal disease had been proven in several study. Jarraud 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 bacteraemia 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-lauryl sarcosine sodium salt 2% (300µL), protease 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
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</th>
<th>Reverse</th>
<th>Product size (bp)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>agr I</td>
<td>5-ATG CAC ATG GTG CAC ATG C-3</td>
<td>-</td>
<td>441</td>
<td>12</td>
</tr>
<tr>
<td>agr II</td>
<td>5-GTC ACA AGT ACT ATA AGC TGC GAT-3</td>
<td>-</td>
<td>575</td>
<td>12</td>
</tr>
<tr>
<td>agr III</td>
<td>-</td>
<td>5-TAT TAC TAA TTG AAA AGT GGC CAT AGC-3</td>
<td>323</td>
<td>12</td>
</tr>
<tr>
<td>agr IV</td>
<td>5-GTA ATG TAA TAG CTT GTA TAA TAC CCA G-3</td>
<td>-</td>
<td>659</td>
<td>12</td>
</tr>
<tr>
<td>mec A</td>
<td>5-AAA ATCG ATGT GAA GGTT GGC-3</td>
<td>533</td>
<td>12</td>
<td></td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Place of isolation</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>patient</td>
<td>29(14.1%)</td>
<td>44(22.7%)</td>
<td>56(28.9%)</td>
<td>18(9.5%)</td>
<td>104(52.9%)</td>
</tr>
<tr>
<td>health worker</td>
<td>28(48.3%)</td>
<td>10(17.6%)</td>
<td>16(27.6%)</td>
<td>5(8.8%)</td>
<td>69(44.2%)</td>
</tr>
<tr>
<td>food product</td>
<td>27(22.9%)</td>
<td>16(31.4%)</td>
<td>50(9.8%)</td>
<td>10(5.1%)</td>
<td>93(25.6%)</td>
</tr>
<tr>
<td>Total</td>
<td>84(43.3%)</td>
<td>15(26.7%)</td>
<td>85(43.8%)</td>
<td>28(14.3%)</td>
<td>194(100%)</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>8(25.9%)</td>
<td>6(21.3%)</td>
<td>11(39.3%)</td>
<td>1(3.4%)</td>
<td>22(33.3%)</td>
</tr>
<tr>
<td>wound</td>
<td>9(28.1%)</td>
<td>1(4.5%)</td>
<td>10(45.5%)</td>
<td>3(10.7%)</td>
<td>25(41.2%)</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(18.8%)</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(22.4%)</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 mexitellicin.

<table>
<thead>
<tr>
<th>agr group</th>
<th>MRSA N (%)</th>
<th>MSSA N (%)</th>
<th>Total N (%)</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>26(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 and resistance to mexitellicin (Table 4).
5. Discussion

S. aureus is the major cause of both community and hospital acquired infections. It is a member of the human microbial flora, responsible for infections ranging from subacute infectious abscesses or furuncles to scalded skin syndrome, sepsis, necrotizing pneumonia, and toxic shock syndrome (TSS). Many of the cell surface proteins, secreted exotoxins, enzymes and virulence factors of S. aureus are regulated by agr locus (1).

In our study, S. aureus has been classified based on agr locus in four agr groups. First time Dufour and colleagues (13) used this method for the classification of Staphylococcus aureus and showed that these bacteria can be divided into four groups I, II, III, IV. Although agr specific group IV was absent in many previously reported studies (12, 14, 15). We detected agr group IV in blood, wound and urine samples.

In our region similar to previously reported, agr group I was the most prevalent agr type. For example, Shopen and colleagues (12) found that agr specific group I (42%) was prevalent among children and their guardians, while in the van Leeuwen and colleagues (14) collection of 192 S. aureus strains, 71% of strains belonged to agr group I and in the Najer Peay and colleagues (6) collection of 212 S. aureus strains, 55.1% of strains belonged to agr group I. In a more recent study, Indrawattana and colleagues in 2013 in Thailand found that the agr specific group I (58.7%) was prevalent among all the groups investigated (16).

Staphylococcal food poisoning is a gastrointestinal illness. It is caused by eating foods contaminated with toxins produced by S. aureus. The true incidence of Staphylococcal food poisoning is unknown for a number of reasons, including poor response from victims during interviews with health officials and misdiagnosis of the illness, which may be symptomatically similar to other types of food poisoning.

The predominant agr type isolated from food products in our study was agr I and agr group II with an incidence of 31.4%, subsequent to agr group I; however, in a study conducted by Montaz and colleagues (2010), agr group II was most prevalent among S. aureus isolated from milk in Iran (17).

S. aureus which belongs to agr group III was predominant in patients, however in the carriers and food products, it was less frequent. Boubaker and colleagues (2006) in Tunis (8) showed that out of a total of 57 S. aureus strains isolated from the patients were collected, 9 (15.7%) belonged to group I, 2 (3.5%) belonged to group II and 23 (40.3%) belonged to group III, which is similar to our findings. However, in a recent study, Chen and colleagues (2012) in Taiwan found that out of a total 134 S. aureus strains isolated from nasal carriage and patients were collected, agr group I was the most common type for both (nasal carriage 65% and patients 74%) (18).

Based on the some studies conducted it is obvious that a particular type of disease is associated with agr specific types. For example, Jarraud and colleagues in 2000 in America (7), showed that Staphylococcus aureus TSST-1-producing isolates belong to agr specificity group III and the majority of exfoliative-producing strains responsible for SSSS belong to agr group IV. However, Chini and colleagues (2006) in Greek (9) found that TSS toxin 1-producing isolates belonged to agr specificity group I and III. In a recent study, Cotar and colleagues (2012) in Romania (19) showed that agr group I was prevalent among the strains isolated from blood cultures, whereas agr group III had prevailed among strains isolated from respiratory tract specimens.

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.

Acknowledgments

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Authors’ Contributions

Meymas 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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References


