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 85clinical 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 *mec*A 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 strains can be 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 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 (30µL), and RNase A (5µL). (11).

3.3. The agr and mecA typing

Identification of the *agr* groups and *mec*A 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 $MgCl_2$ (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), X^2 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).
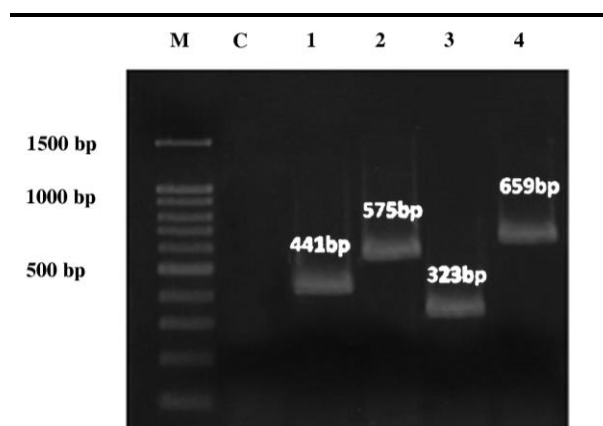

Figure 1. PCR product of four agr groups.
M: 100 bp DNA size marker, C⁻: negative control, line 1 through 4 respectively represent *agr* group I to IV.

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 methicillin (Table 4).

Table 1. The specific primers used in this study.

Gene	Primers	Product size (bp)	Reference
agr I	Forward: 5-ATG CAC ATG GTG CAC ATG C-3 Reverse: 5-GTC ACA AGT ACT ATA AGC TGC GAT-3	441	12
agr II	Reverse: 5-TAT TAC TAA TTG AAA AGT GGC CAT AGC-3	575	12
agr III	Reverse: 5-GTA ATG TAA TAG CTT GTA TAA TAA TAC CCA G-3	323	12
agr IV	Reverse: 5-CGA TAA TGC CGT AAT ACC CG-3	659	12
mec A	Forward: 5-AAAATCGATGGTAAAGGTTGGC-3 Reverse: 5-AGTTCTGCAGTACCGGATTTGC-3	533	12

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

Place of isolation	*agr* I	*agr* II	*agr* III	*agr* IV	Total
patient	29(34.1%)	15(17.6%)	35 (41.2%)	6(7.1%)	85(43.8%)
health worker	28(48.3%)	13(22.4%)	16(27.6%)	1(1.7%)	58(29.9%)
food product	27(52.9%)	16(31.4%)	5(9.8%)	3(5.9%)	51(26.3%)
Total	84*(43.3%)	44(22.7%)	56(28.9%)	10(5.1%)	194

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

Specimens	agr I N (%)	agr II N (%)	agr III N (%)	agr IV N (%)	Total N (%)
urine	8(28.6%)	6(21.4%)	11(39.3%)	3(10.7%)	28(33%)
wound	9(40.9%)	1(4.5%)	10(45.5%)	2(9.1%)	22(25.8%)
blood	6(37.5%)	4(25.0%)	5(31.2%)	1(6.2%)	16(18.8%)
others	6(31.6%)	4(21.1%)	9(47.4%)	0	19(22.4%)
Total	29(34.1%)	15(17.6%)	35(41.2%)	6(7.1%)	85

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

agr group	MRSA	MSSA	Total
agr I	18	50	68
agr II	12	28(70%)	40
agr III	40(61.5%)	25	65
agr IV	9	12	21
Total	79	115	194

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 subcutaneous 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, Shopsin 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 Najar Peerayeh 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 responses 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 Momtaz 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.

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.

Authors' Contributions

Meysam 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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