DOI: 10.7860/JCDR/2014/6971.4219

Original Article

Microbiology Section

Accessory Gene Regulator Types of *Staphylococcus aureus* Isolated in Gorgan, North of Iran

MEYSAM HASANNEJAD BIBALAN[1], FATEMEH SHAKERI[2], NAEME JAVID[3], AMIR GHAEMI[4], EZZAT ALLAH GHAEMI[5]

ABSTRACT

Background: *Staphylococcus aureus* is a gram-positive bacterium that has remained a persistent pathogen, causing infections such as endocarditis, meningitis, and toxic shock syndrome in humans. The accessory gene regulator (agr) system of *Staphylococcus aureus* is responsible for controlling the expression of many genes that code for virulence factors. In this study, we assessed the *S. aureus* agr Group, based on their source of isolation, in Gorgan, North of Iran.

Materials and Methods: DNA of 194 *S. aureus* isolates was extracted by lysozyme-phenol chloroform method, which included 85 clinical samples, 58 samples which were isolated from noses of health care workers and 51 cases which were obtained from food products in Gorgan, northern Iran. PCR-based assays were used to evaluate agr locus nucleotide polymorphism for the identification of agr specificity Group. Distributions of each agr Group were determined and comparison between different sources was assessed by X^2. A p-value of <0.05 was considered as significant.

Results: The majority of isolates belonged to agr Group I (43.3%), followed by agr Group III (28.87%), agr Group II (22.68%), and agr Group IV (5.15%). In our study, a majority of *S. aureus* isolates were recovered from health care workers and food product specimens were of agr Group I and isolates which were recovered from patients were of agr Group III. These differences were statistically significant (P=0.005). There was no statistical difference between the source of isolation of clinical samples of *S. aureus* and agr type.

Conclusion: Agr Group I was predominant among health care workers and food product specimens in Gorgan, North of Iran, but in strains which were isolated from patients, agr Group III was predominant. Investigating the possible role of agr Group III in *Staphylococcus aureus* infection in future studies is recommended.

Keywords: *S. aureus*, Agr Group genes, PCR

INTRODUCTION

Staphylococcus aureus is a major cause of both community and hospital-acquired infections; it is a member of the human microbial flora which is responsible for infections which range from subcutaneous abscesses or furuncles, scalded skin syndrome, sepsis, necrotizing pneumonia, pyogenic arthritis and Toxic Shock Syndrome (TSS) [1]. Morbidity/mortality which results from *S. aureus* infection widely varies, depending on the clinical entity, with an incidence rate which ranges from 20 to 50 cases/100,000 population per year, which leads to 10% and 30% deaths [2].

To cause so many human diseases, the accessory gene regulator (agr) globally controls the coordinated production of virulence factors. This system is based on a two-component module which is known as the agr-locus (accessory-gene-regulator), that, in a cell density-dependent manner and through a secreted auto-Inducing-Peptide (AIP), allows a bacterial population to respond in concert when a critical cell-density is reached. The agr locus is composed of two divergent transcriptional units, RNAII and RNAIII, which are driven by the P2 and P3 promoters [3,4].

The P2 operon encodes four proteins (agrA, agrB, agrC, and agrD) and P3 promoter in the opposite direction, encoding the agr system effector molecule (RNAIII). agrD encodes a cyclic AIP that is processed and secreted into the extracellular space via the gene product of agrB. When a critical bacterial cell density is reached, concentrations of AIP bind to the receptor histidine kinase, AgrC, resulting in its activation and subsequent phosphorylation of AgrA. Phosphorylated AgrA then activates transcription of RNAIII at the P3 promoter. RNAIII serves as a transcription factor, turning on expression of genes which encode secreted virulence factors and down regulating expression of cell-associated virulence factors. An AIP with a thiolactonic ring structure, in the early exponential phase, causes immediate activation of the two promoters [4-6].

S. aureus isolates can be divided into four agr groups on the basis of the agrC gene which encodes the receptor of the auto inducing peptide and agrD gene, which are responsible for encoding cyclic AIP [7].

Several studies have shown that there was a link between type of agr and the *Staphylococcal* disease. Jarraud and colleagues [8], showed that *Staphylococcus aureus* TSST-1-producing isolates belonged to agr specificity Group III and that most of the exfoliatin-producing strains which were responsible for SSSS belonged to agr Group IV. Ben Ayed and colleagues [9], showed that agr Group III strains were associated with non invasive infections and that agr Group I strains were associated with invasive infections, especially bacteraemia. Chini and colleagues [10] found that TSS toxin 1-producing isolates belonged to agr specificity Groups I and III.

In this study, we investigated the prevalence of agr Groups in *S. aureus* isolates obtained from patients, health care workers and food products, to detect predominant type of isolate, according the source of *S. aureus* and to assess the possible relationship between agr Groups and infection types.

MATERIALS AND METHODS

Bacterial isolates: One hundred ninty four isolates of *Staphylococcus aureus* were studied in Golestan University of Medical Sciences. Study samples were collected from health care workers (58 samples), patients (85 samples) and food products (51 samples) between 2009 and 2012, at Gorgan which is located in the north of Iran. The isolates were identified by their growths on Manitol Salt Agar media, Gram staining, catalase test, slide or tube coagulase test, Dnase test and the presence of glutamate synthetase gene [11].

Genomic DNA Extraction: Bacterial DNA lysates were prepared as follows; 1-ml overnight culture of each *S. aureus* isolate was lysed by using lysozyme-phenol chloroform method and it was treated

with N-lauroyl sarcosine sodium salt -2% (300 µL), Proteinase K-100 µg(30 µl), and RNase A- 5 µl. DNA was extracted by phenol chloroform, isoamilalcohol, chloroform, and cold ethanol methods.

Agr typing: The agr specificity Groups were determined by PCR by using specific primers, which has been shown in [Table/Fig-1] [7].

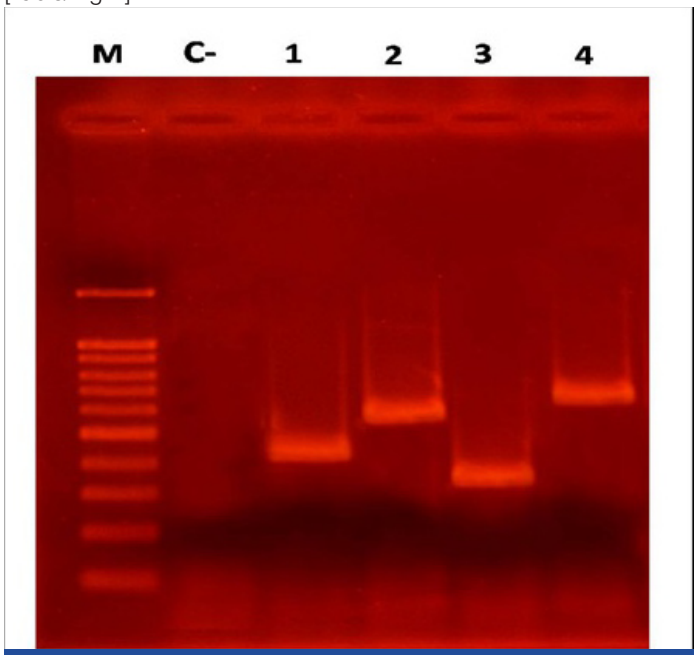
Gene	Primers	Product size
agr I	PanF 5-ATG CAC ATG GTG CAC ATG C-3	441-bp
	R 5-GTC ACA AGT ACT ATA AGC TGC GAT-3	
agr II	R 5-TAT TAC TAA TTG AAA AGT GGC CAT AGC-3	575-bp
agr III	R 5-GTA ATG TAA TAG CTT GTA TAA TAA TAC CCA G-3	323-bp
agr IV	R 5-CGA TAA TGC CGT AAT ACC CG-3	659-bp

[Table/Fig-1]: The genes and related primers used in this study

The PCR assay was performed in 25 µL of reaction mixture which contained: 1.5 U of Taq DNA polymerase , 200 µM of dNTPs , 5 mM of MgCl2, 2.5 µL of 10 × PCR buffer, 5 µl of the purified nucleic acid solutions and a 1 µM concentration of each primer.The thermal profile involved an initial denaturation step which was carried out at 94°C for 6 min, followed by 32 cycles of denaturation at 95°C for 45 s, primer annealing at 56°C for 1 min, and primer elongation at 72°C for 70 s. The cycling was followed by a final extension step which was carried out at 72°C for 8 min [7]. PCR products were electrophorezed in a 1.5% agarose gel and they were stained with ethidium bromide. Statistical analyses were done by using SPSS software, version 16 and X2 Statistical test.

RESULTS

One hundred ninty four *S. aureus* isolates which were obtained from health care workers, patients and food products, were studied. A majority of isolates belonged to agr Group I (43.3%), followed by agr Group III (28.87%), agr Group II (22.68%), and agr Group IV (5.15%) [Table/Fig-2].



[Table/Fig-2]: PCR product of agr gene *S. aureus* isolated in Gorgan, North of Iran
M: 100 bp DNA ladder, C-: negative control, line 1 through 4 respectively represent agr Group I to IV

agr Group I was the main agr Group in *S. aureus* which was isolated from healthcare workers and food products, whereas in *S. aureus* which was isolated from clinical samples of patients, agr Group III was predominant. These differences were statistically significant (p=0.005). On the other hand, the frequency of agr Group IV was the least abundant in all the three sources [Table/Fig-3].

Although agr Group III was predominant in isolates obtained from patients, in blood samples, the frequencies of agr Groups I and III were more than those seen in other Groups. agr Group IV was detected from urine, wound and blood, with similar distributions [Table/Fig-4]. There was no significant differences between the agr Group and the source of clinical sample from which *Staphylococcus aureus* was isolated (p>0.05).

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/Fig-3]: Distribution of different *Staphylococcus aureus* agr types based on source of bacteria isolation p-values= 000.5

Specimens	agr I	agr II	agr III	agr IV	Total
Urine	8(28.6%)	6(21.4%)	11(39.3%)	3(10.7%)	28
Wound	9(40.9%)	1(4.5%)	10(45.5%)	2(9.1%)	22
Blood	6(37.5%)	4(25.0%)	5(31.2%)	1(6.2%)	16
Other	6(31.6%)	4(21.1%)	9(47.4%)	0	19
Total	**29(34.1%)**	**15(17.6%)**	**35(41.2%)**	**6(7.1%)**	**85**

[Table/Fig-4]: Distribution of different *Staphylococcus aureus* agr gene types isolated from patients p-values> 0.05 for agr group and the source of clinical sample

DISCUSSION

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

S. aureus isolates can be divided into four agr Groups on the basis of the agrC gene which encodes the receptor of the auto inducing peptide and agrD gene which encodes the auto inducing peptide [7]. In our study, *Staphylococcus aureus* was classified, based on agr locus in four agr Groups. Dufour and colleagues [12], first used this method for classification of *Staphylococcus aureus* and they showed that isolates of this bacterium could be divided into four Groups, I, II, III, IV. Although agr specific Group IV was absent in many previously reported studies [7,13,14], we detected agr Group IV in blood, wound and urine samples. As was seen in a majority of previous studies, in our region too, agr Group I was the most prevalent agr type. For example, Shopsin and colleagues [7] found that agr specific Group I (42%) was prevalent in children and their guardians. In the study done by van Leeuwen and colleagues' [13] on a collection of 192 *S. aureus* strains, 71% of strains were found to belong to agr Group I and in the study done by Najar Peerayeh and colleagues [15] on a collection of 212 *S. aureus* strains, 55.1% of strains were found to belong to agr Group I. In a more recent study done by Indrawattana and colleagues in 2013 in Thailand, it was found that agr specific Group I (58.7%) was predominant agr Group [16].

The predominant agr type which was isolated from food products in present study was agr I, followed by agr Group II, but in a study which was conducted by Momtaz in 2010, agr Group II was found to be most prevalent among *S. aureus* which was isolated from milk in Iran [17].

The predominant agr Group found in patients was Group III, but it was less frequent in healthcare workers and food products. Ben and colleagues, showed in Tunis [9] that among a total of 57 *S. aureus* strains which were isolated from patients, 9 (15.7%) belonged to Group I, 2 (3.5%) belonged to Group II and that 23

(40.3%) belonged to Group III, which were similar to our findings, but in a recent study done by Chen and colleagues, in Taiwan, they showed that among a total of 134 *S. aureus* strains which were isolated from nasal carriages and patients, agr Group I was the most common type found in both (nasal carriages- 65% and patients -74%) [18].

Some studies have shown that some particular type of disease was associated with agr specific types; for example, Jarraud and colleagues, showed in America [8], that *Staphylococcus aureus* TSST-1-producing isolates belonged to agr specificity Group III and that most of the exfoliatin-producing strains which were responsible for SSSS belonged to agr Group IV. But Spiliopoulou and colleagues, found in Greece [10] that TSS toxin 1-producing isolates belonged to agr specificity Groups I and III. Kolawole and colleagues, showed in Nigeria [19] that seb gene-positive isolates belonged to agr Groups I and IV and that seg-sei gene-positive isolates dominated in agr Groups IV and II. In another study done by Rasmussen and colleagues in Sweden, [20] they found that agr Group II was associated with invasive disease and that agr Group III was linked with carriage status. Ben and colleagues [9], showed that agr Group III strains were associated with non invasive infections and that agr Group I strains were associated with invasive infections, especially bacteraemia, which confirmed our findings, which showed that the frequency of agr Group I in bacteria which was isolated from blood cultures was higher than those seen in other Groups.

Our study could not show a distinction between certain types of diseases and agr types. So, studies which are done on strains which are isolated from patients with certain diseases can clear role of agr types in pathogenesis. Cotar and colleagues, showed in Romania [21] that agr Group I was prevalent among strains which were isolated from blood cultures, which was observed in our study too. One of the purposes behind using bacterial typing as a marker is to understand the epidemiologies of infectious diseases. agr typing and other methods such as spa typing, MLST, coa typing and PFGE can be used as tools to achieve this purpose. These findings suggest that agr type varies for each region and identifying predominant types are useful. In Golestan, North of Iran, the major *S. aureus* strain which was recovered from patients was agr Group III.

CONCLUSION

agr Group I was predominant among health care workers and food product specimens in Gorgan, north of Iran, but in strains which were isolated from patients, agr Group III was predominant, which indicated that the agr Group III was more virulent and invasive than other Groups, or perhaps this phenomenon was accidental? To answer this question, doing larger studies on *S. aureus* strains which are isolated from various infections and agr typing are recommended. Investigating the possible role of agr Group III in *Staphylococcus aureus* infections in future studies is recommended.

REFERENCES

[1] Jarraud S, Mougel C, Thioulouse J, Lina G, Meugnier H, Forey F, et al. Relationships between *Staphylococcus aureus* genetic background, virulence factors, agr Groups (alleles), and human disease. *Infection and Immunity*. 2002; 70(2):631-41.

[2] van Hal SJ, Jensen SO, Vaska VL, Espedido BA, Paterson DL, Gosbell IB. Predictors of mortality in *Staphylococcus aureus* Bacteremia. *Clin Microbiol Rev*. 2012 Apr;25(2):362-86.

[3] Novick RP, Projan S, Kornblum J, Ross H, Ji G, Kreiswirth B, et al. The agr P2 operon: An autocatalytic sensory transduction system in*Staphylococcus aureus*. *Molecular and General Genetics MGG*. 1995;248(4):446-58.

[4] Novick RP, Ross H, Projan S, Kornblum J, Kreiswirth B, Moghazeh S. Synthesis of *Staphylococcal* virulence factors is controlled by a regulatory RNA molecule. *The EMBO Journal*. 1993;12(10):3967.

[5] Sakoulas G. The accessory gene regulator (agr) in methicillin-resistant *Staphylococcus aureus*: Role in virulence and reduced susceptibility to glycopeptide antibiotics. Drug Discovery Today: *Disease Mechanisms*. 2006;3(2):287-94.

[6] Ji G, Beavis RC, Novick RP. Cell density control of *Staphylococcal* virulence mediated by an octapeptide pheromone. *Proceedings of the National Academy of Sciences*. 1995;92(26):12055-9.

[7] Shopsin B, Mathema B, Alcabes P, Said-Salim B, Lina G, Matsuka A, et al. Prevalence of agr specificity Groups among *Staphylococcus aureus* strains colonizing children and their guardians. *Journal of Clinical Microbiology*. 2003;4 1(1):456-9.

[8] Jarraud S, Lyon G, Figueiredo A, Gérard L, Vandenesch F, Etienne J, et al. Exfoliatin-Producing Strains Define a Fourth agr Specificity Group in *Staphylococcus aureus*. *Journal of Bacteriology*. 2000;182(22):6517-22.

[9] Ben Ayed S, Boutiba-Ben Boubaker I, Samir E, Ben Redjeb S.. Prevalence of agr specificity Groups among methicilin resistant *Staphylococcus aureus* circulating at Charles Nicolle hospital of Tunis. *Pathol Biol (Paris)*. 2006 Oct-Nov;54(8-9):435-8.

[10] Chini V, Dimitracopoulos G, Spiliopoulou I. Occurrence of the enterotoxin gene cluster and the toxic shock syndrome toxin 1 gene among clinical isolates of methicillin-resistant *Staphylococcus aureus* is related to clonal type and agr Group. *Journal of Clinical Microbiology*. 2006;44(5):1881-3.

[11] Shakeri F, Shojai A, Golalipour M, Rahimi Alang S, Vaez H, Ghaemi EA. Spa Diversity among MRSA and MSSA Strains of *Staphylococcus aureus* in North of Iran. *International Journal of Microbiology*. 2010;2010.

[12] Dufour P, Jarraud S, Vandenesch F, Greenland T, Novick RP, Bes M, et al. High genetic variability of the agr locus in Staphylococcus species. *Journal of Bacteriology*. 2002;184(4):1180-6.

[13] van Leeuwen W, van Nieuwenhuizen W, Gijzen C, Verbrugh H, van Belkum A. Population studies of methicillin-resistant and-sensitive *Staphylococcus aureus* strains reveal a lack of variability in the agrD gene, encoding a *Staphylococcal* autoinducer peptide. *Journal of Bacteriology*. 2000;182(20):5721-9.

[14] Yoon HJ, Choi JY, Lee K, Yong D, Kim JM, Song YG. Accessory gene regulator Group polymorphisms in methicillin-resistant *Staphylococcus aureus*: an association with clinical significance. *Yonsei Medical Journal*. 2007;48(2):176-83.

[15] Peerayeh SN, Azimian A, Nejad QB, Kashi M. Prevalence of agr Specificity Groups Among *Staphylococcus aureus* Isolates From University Hospitals in Tehran. *Lab Medicine*. 2009;40(1):27-9.

[16] Indrawattana N, Sungkhachat O, Sookrung N, Chongsa-nguan M, Tungtrongchitr A, Voravuthikunchai SP, et al. *Staphylococcus aureus* Clinical Isolates: Antibiotic Susceptibility, Molecular Characteristics, and Ability to Form Biofilm. *Bio Med Research International*. 2013;2013: Article ID 314654, 11 pages.

[17] Momtaz H, Tajbakhsh E, Abbasian B, Moumeni M. Investigation of accessory gene regulator (agr) in *Staphylococcus aureus* isolated from clinical and subclinical bovine mastitis in Iran. *African Journal of Agricultural Research*. 2010; 4(9):471-4.

[18] Chen FJ, Siu LK, Lin JC, Wang CH, Lu PL. Molecular typing and characterization of nasal carriage and community-onset infection methicillin-susceptible *Staphylococcus aureus* isolates in two Taiwan medical centers. *BMC Infect Dis*. 2012 Dec 10;12:343.

[19] Kolawole DO, Adeyanju A, Schaumburg F, Akinyoola AL, Lawal OO, Amusa YB, et al. Characterization of Colonizing *Staphylococcus aureus* Isolated from Surgical Wards' Patients in a Nigerian University Hospital. *PLoS One*. 2013;(7): e68721.

[20] Rasmussen G, Monecke S, Ehricht R, Söderquist B. Prevalence of Clonal Complexes and Virulence Genes among Commensal and Invasive *Staphylococcus aureus* Isolates in Sweden. *PloS One*. 2013;8(10).

[21] Cotar IA, Chifiriuc MC, Holban AM, Banu O, Lazar V. Prevalence of agr specificity Groups among *Staphylococcus aureus* strains isolated from different clinical specimens patients with cardiovascular surgery associated infections. *Biointerface Res Appl Chem*. 2012;2:264-70.

PARTICULARS OF CONTRIBUTORS:

1. Faculty, Department of Microbiology, Golestan University of Medical Sciences, Gorgon, Iran.
2. Faculty, Department of Microbiology, Golestan University of Medical Sciences, Gorgon, Iran.
3. Faculty, Department of Microbiology, Golestan University of Medical Sciences, Gorgon, Iran.
4. Assistant Professor, Department of Microbiology, Golestan University of Medical Sciences, Gorgon, Iran.
5. Professor, Department of Microbiology, Golestan University of Medical Sciences, Gorgon, Iran.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:

Dr. Ezzat Allah Ghaemi,
Professor, Department of Microbiology, Golestan University of Medical Sciences, Gorgon, Iran.
Phone: +989122835601, E-mail: eghaemi@yahoo.com

FINANCIAL OR OTHER COMPETING INTERESTS: None.

Date of Submission: **Jul 15, 2013**
Date of Peer Review: **Jan 13, 2014**
Date of Acceptance: **Feb 15, 2014**
Date of Publishing: **Apr 15, 2014**