Agr typing of *Staphylococcus aureus* species isolated from clinical samples in training hospitals of Isfahan and Shahrekord

Saeid Javdan¹, Tahmine Narimani², Milad Shahini Shams Abadi³ and Abolfazl Gholipour¹,4*

**Abstract**

**Objective:** As an opportunistic pathogen, *Staphylococcus aureus* is associated with serious nosocomial infections and growing antimicrobial resistance against beta-lactams among *S. aureus* strains has become a global challenge. The current study was designed to investigate the presence of *agr* genes among *S. aureus* strains recovered from clinical samples in university hospitals of Isfahan and Shahrekord.

**Results:** A total of 150 *S. aureus* isolates were screened by Disk diffusion method (DDM) and conventional PCR. The minimum (17.3%) and maximum (46%) antibiotic resistance rates were found in vancomycin and cefoxitin, respectively. The majority of our isolates were classified as *agr* type I followed by type II, type IV, and type III. The statistical analysis showed a significant correlation between *agr* type I and antibiotic resistance against cefoxitin and erythromycin (p = 0.04 and p = 0.03, respectively). Based on our findings, the *agr* typing could be considered an effective approach for molecular tracking of *S. aureus* infections.

**Keywords:** *Staphylococcus aureus*, Agr type, meC gene, Antibiotic resistance, Methicillin

**Introduction**

As a part of microflora of skin and mucous membranes of healthy individuals, *Staphylococcus aureus* is also an opportunistic pathogen and associated with hospital acquired infections such as septicemia, pneumonia, septic arthritis, osteomyelitis, toxic shock syndrome after surgery, folliculitis, endocarditis, and urinary tract infections (UTIs) [1, 2]. Antibiotic resistance by affecting more than two million people annually is one of the biggest global challenges. The increasing antimicrobial resistance among *S. aureus* species against beta-lactam antibiotics has led to serious problems with the treatment of their related infections. Despite considerable efforts in controlling antibiotic resistance, methicillin-resistance *S. aureus* (MRSA) is raising worldwide, in addition geographical and local variations influence its dynamic and crisis [1, 3]. The methicillin resistance development in *S. aureus* is related to the several Staphylococcal Cassette chromosome mec elements (SCCmec) encoding meC gene for a penicillin binding protein (PBp2a) [4]. MRSA strains are usually multi-drug resistant (MDR) and show resistancy to other antibiotics such tetracyclines, aminoglycosides, lincoysamides etc. [1, 5, 6]. Rapid and precise typing of *S. aureus* is really crucial to transmission identification of this pathogen. In this regard, Pulsed-Field gel electrophoresis and spa typing (*Staphylococcal protein A*) are common typing methods. The spa gene is one of the most distinctive factors related to this organism, and various patterns of it have been identified by several studies [7]. One of the major regulatory and control factors in the virulence gene expression of *S. aureus* is the accessory gene regulatory (agr) system. Indeed, agr operon including agrA, agrB, agrC, and agrD genes regulate over 70 genes in *S. aureus* 23 of which control its pathogenicity and invasive infections [8]. Moreover, *S. aureus* can be stratified into 4 different groups (agr I, agr II, agr III, and agr IV) according to the sequences of agrC (auto inducing peptide) and agrD (cyclic AIP) genes. It is stated that
agr types are different in their properties and prevalence in various geographical areas thus, identification of predominant types in each region may well be functional [9]. Given to the critical roles of agr genes, the current study was designed to detect and identify the agr groups of S. aureus strains isolated from clinical samples in training hospitals of Isfahan and Shahrekord cities.

**Main text**

**Materials and methods**

**Samples and bacterial isolates**

This cross sectional study was conducted in microbiology department of Shahrekord University of Medical Sciences. During May to November 2017, a total of 150 isolates of S. aureus were collected from clinical samples (wound, blood, urine, tissue etc.) of patients attending university hospitals in Isfahan (Alzahra and Kashani) and Shahrekord (Kashani and Hajar).

**Characterization assays**

The isolates were identified using Gram staining, catalase test, slide or tube coagulase test, DNase test, and growth on Mannitol Salt Agar (MSA) as a differential growth medium [10].

**Antibiogram testing**

Disk diffusion method (DDM) as described by CLSI 2016 guideline [11] was performed for following antibiotics: erythromycin (15 mg), tetracycline (30 mg), vancomycin (30 mg), gentamicin (10 mg), rifampicin (5 mg), cefoxitin (15 mg), trimethoprim (5 mg), rifampicin (5 mg). In addition, all isolates were subjected to cefoxitin disc diffusion test to identify the methicillin sensitive S. aureus (MSSA) and MRSA.

**DNA extraction**

The nucleic acids of S. aureus isolates were extracted by phenol chloroform method followed by RNase treatment [12]. The purity of extraction was assessed using the A260/280 ratio and agarose gel electrophoresis.

**PCR amplification of the mecA gene**

molecular detection of mecA gene was carried out according to the following condition: initial denaturation at 95 °C for 3 min followed by 33 cycles of denaturation at 94 °C for 1 min, annealing at 53 °C for 30 s, and extension at 72 °C for 1 min and final extension step at 72 °C for 6 min.

**PCR detection of agr genes**

PCR assay for amplification of agr genes was set as follows: hot start at 95 °C/6 min, 32 cycles of 94 °C/45 s, 60 °C/1 min, 72 °C/70 s and a final extension step of 72 °C/8 min. All reactions performed in duplicate and along with the negative control (water) and positive (previously known positive-PCR products) control. The final products were detected by electrophoresis on 1% agarose gel containing DNA safe stain (Sinagene, Iran) and the sizes of the PCR products were estimated by the migration pattern of a 100-bp DNA ladder (Sinagene, Iran).

**Statistical analysis**

Statistical analysis was performed using SPSS version 22. The chi-square test was used to calculate statistical significance (p < 0.05).

**Results**

**Study population**

150 S. aureus isolates were collected from patients attending training hospitals in Isfahan (110 isolates from Alzahra hospital) and Shahrekord (25 cases from Kashani hospital and 15 isolates from Hajar hospital). The mean age of the participants was 47.6 years (SD: 21.5) and male/female ratio was 90/60. However, there was not any significant difference in sex and age of patients with S. aureus infection. S. aureus isolates were obtained from several clinical samples and different hospital wards.

**Antibiotic susceptibility**

According to our results the lowest (17.3%) and the highest (46%) antibiotic resistance rates were found in vancomycin and cefoxitin, respectively. In addition, MRSA strains were verified by PCR amplification of mecA gene. The antibiotic resistance distribution among different agr groups is shown in Table 1. The results of this study showed a significant correlation between agr type and antibiotic resistance against cefoxitin and erythromycin (p = 0.04 and p = 0.03, respectively).

**agr typing**

Molecular detection of 150 S. aureus isolates has indicated that agr type I was the predominant one (82/150) followed by type II (37/150), type IV (21/150), and type III (10/150) (Fig. 1). Table 2 is shown the frequency distribution of different agr types among different clinical samples.

**Discussion**

There is a dramatic increase in S. aureus infections, both with community-associated and hospital-acquired types, and development of antibiotic-resistant species, especially MRSA and vancomycin-resistant strains, is the major cause of the infections and further treatment complications [13]. Identification and typing of the isolates may imply a common source of infection; therefore, accurate analysis of these patterns can help to break the
Table 1  The antibiotic resistance profiles among 4 different agr types

| AGR type | FOX S (%) | FOX R (%) | E S (%) | E R (%) | T S (%) | T R (%) | VA S (%) | VA I (%) | VA R (%) | RP S (%) | RP I (%) | RP R (%) | TM S (%) | TM I (%) | TM R (%) | GM S (%) | GM I (%) | GM R (%) |
|----------|-----------|-----------|---------|---------|---------|---------|----------|---------|----------|----------|---------|---------|----------|---------|---------|---------|---------|---------|
| I        | 37 (45.1%) | 45 (54.9%) | 38 (46.3%) | 21 (25.6%) | 23 (28) | 47 (57.3%) | 7 (8.5%) | 28 (34.1%) | 70 (85.4%) | 12 (14.6%) | 49 (59.8%) | 3 (3.7%) | 30 (36.6%) | 52 (63.4%) | 6 (7.3%) | 24 (29.3%) | 58 (70.7%) | 2 (2.4%) | 22 (26.8%) |
| II       | 22 (59.5%) | 15 (40.5%) | 12 (32.4%) | 5 (13.5%) | 20 (54.1%) | 20 (54.1%) | 1 (2.7%) | 16 (41.2%) | 28 (75.7%) | 9 (24.3%) | 27 (73.9%) | 2 (5.4%) | 8 (21.6%) | 18 (48.6%) | 5 (13.5%) | 14 (37.8%) | 23 (62.2%) | 0 | 14 (37.8%) |
| III      | 7 (70%) | 3 (30%) | 3 (30%) | 1 (10%) | 6 (60%) | 7 (70%) | 0 | 30 (30%) | 8 (80%) | 2 (20%) | 8 (80%) | 1 (10%) | 1 (10%) | 4 (40%) | 1 (10%) | 5 (50%) | 7 (70%) | 1 (10%) | 2 (2%) |
| IV       | 15 (71.4%) | 6 (28.6%) | 6 (28.6%) | 7 (33.3%) | 8 (38.1%) | 10 (47.6%) | 1 (4.8%) | 10 (47.6%) | 18 (85.7%) | 3 (14.3%) | 14 (66.7%) | 1 (4.8%) | 6 (28.6%) | 14 (66.7%) | 1 (4.8%) | 6 (28.6%) | 13 (61.9%) | 0 | 8 (38.1%) |
| Total    | 81 (54%) | 69 (46%) | 59 (39.3%) | 34 (22.7%) | 57 (38%) | 84 (56%) | 9 (6%) | 57 (38%) | 124 (82.7%) | 26 (17.3%) | 98 (65.3%) | 7 (4.7%) | 45 (30%) | 88 (58.7%) | 13 (8.7%) | 49 (32.7%) | 101 (67.3%) | 3 (2%) | 46 (30.7%) |
chain of transmission. Accordingly, the present study was designed to identification of \textit{agr} types among \textit{S. aureus} isolates and possible association of these pathogens with some phenotypic characteristics such as antibiotic resistance and pathogenesis.

Dufour et al. [14] used \textit{agr} typing method for the first time to stratify \textit{S. aureus} isolates and affirmed that these bacteria can be divided into four groups I, II, III, IV by this system. Ever since, many researches have been applied the \textit{agr} typing approach and in several studies such as those by Lee et al. and Shopsin et al. [15, 16], the \textit{agr} group I was the most dominant \textit{S. aureus} type. Our findings indicated that \textit{agr} type I was the most predominant type among \textit{S. aureus} isolated from Isfahan and Shahrekord cities. Similarly, in several previous studies such as those by Cheraghi, Bibalan, Peerayeh, Khoramrooz, Mohsenzadeh, and Goudarzi \textit{agr} type I has been reported as the most dominant isolate of \textit{S. aureus} in different regions of Iran [17–22]. It is declared that certain \textit{agr} groups of \textit{S. aureus} are involved in some particular disease and infections, for example \textit{agr} type I isolates are associated with bacteremia and invasive infections [21]. In the present study, wound and tracheal aspirates, were sequentially the most frequent clinical samples and as it is summarized in Table 2, the \textit{agr} group I was the major \textit{agr} type among these sample. However, we couldn’t find any significant difference or correlation between \textit{agr} types and certain clinical specimen.

In the current study, the antimicrobial susceptibility testing revealed that the highest antibiotic resistance rate was against cefoxitin (46%) followed by erythromycin and tetracycline (both 38%). Several studies that have reported erythromycin and tetracycline as the antimicrobial agents with lowest susceptibility among \textit{S. aureus-agr} group I isolates [20, 23, 24]. As it is summarized in Table 1, the \textit{agr} types III and I showed the maximum and minimum resistance rates against tetracycline, respectively. In the present study, \textit{agr} type I isolates had the highest sensitivity to vancomycin; however, the smallest resistance rate against this agent was related to \textit{agr} type IV (Table 1). The greatest susceptibility and resistance to rifampin were found among \textit{agr} types IV and I of \textit{S. aureus} strains, respectively (Table 1). As it could be seen in Table 1, \textit{agr} types IV and III have shown the highest and the lowest susceptibility to trimethoprim, respectively. The maximum percentage of gentamicin susceptibility was related to \textit{agr} type I, while type III isolates had the highest resistance against this antimicrobial agent (Table 1). We found a significant correlation between \textit{agr} type and antibiotic resistance against cefoxitin and erythromycin (\(p = 0.04\) and \(p = 0.03\), respectively). Indeed, in this report, the \textit{agr} types never implied the sensitivity or resistance to antibiotics, but in the case of cefoxitin and erythromycin the \textit{agr} group I isolates showed the highest resistance against these agents.

The majority of \textit{S. aureus} isolates in this study were classified as \textit{agr} group I and our results suggest a probable correlation between this type and antibiotic resistance to cefoxitin and erythromycin. Here we can conclude that \textit{agr} typing is a suitable and effective approach for molecular tracking of \textit{S. aureus} infection.
Limitations
The lack of investigation on others typing methods in S. aureus isolates can be mentioned as one of the main limitations of the present study.

Abbreviations
MRSA: methicillin-resistance Staphylococcus aureus; MDR: multi-drug resistant; SCCmec: Staphylococcal Cassette chromosome mec elements; PCR: polymerase chain reaction.

Acknowledgements
Not applicable.

Authors’ contributions
SA, TN, MSSA, AG: design of study. MSSA, SA, AG: acquisition of data. TN, SJ, AG: evaluation of data, preparation of the manuscript. MSSA, AG: assessment of data. All authors read and approved the final manuscript.

Funding
This research was supported by the budget of research projects of the Shahrekord University of medical sciences (Number of project: 2443). Funding body were used to purchase equipment and tools.

Availability of data and materials
All relevant data are included in the manuscript.

Ethics approval and consent to participate
This study was approved by the Ethics Committee of Shahrekord University of Medical Sciences. The informed consent was obtained from all the participants, and informed consent obtained was written.

Consent to publish
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Author details
1 Cellular and Molecular Research Center, Basic Health Sciences Institute, Shahrekord University of Medical sciences, Shahrekord, Iran. 2 Department of Microbiology, Isfahan University of Medical Sciences, Isfahan, Iran. 3 Department of Bacteriology & Virology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran. 4 Department of Microbiology and Immunology, Cellular and Molecular Research Center, Shahrekord University of Medical Sciences, Shahrekord, Iran.

Received: 19 May 2019 Accepted: 18 June 2019
Published online: 27 June 2019

References
1. Lowy FD. Antimicrobial resistance: the example of Staphylococcus aureus. J Clin Invest. 2003;111(9):1265–73.
2. Zouhir A, Taieb M, Lamine MA, Cherif A, Jridi T, Mahjoubi B, et al. ANTISTAPHYBASE: database of antimicrobial peptides (AMPs) and essential oils (EOs) against methicillin-resistant Staphylococcus aureus (MRSA) and Staphylococcus aureus. Arch Microbiol. 2017;199(2):215–22.
3. Medina Cruz D, Mi G, Webster TJ. Synthesis and characterization of biogenic selenium nanoparticles with antimicrobial properties made by Staphylococcus aureus, methicillin-resistant Staphylococcus aureus (MRSA), Escherichia coli and Pseudomonas aeruginosa. J Biomed Mater Res Part A. 2018;106:1400–12.

4. García-Álvarez L, Holden MT, Lindsay H, Webb CR, Brown DF, Curran MD, et al. Metcillin-resistant Staphylococcus aureus with a novel mecA homologue in human and bovine populations in the UK and Denmark: a descriptive study. Lancet Infect Dis. 2011;11(8):595–603.

5. Gorak EJ, Yamada SM, Brown JD. Community-acquired methicillin-resistant Staphylococcus aureus in hospitalized adults and children without known risk factors. Clin Infect Dis. 1999;29(4):797–800.

6. Herold BC, Immerslueck LC, Maranan MC, Launderdale DS, Gaskin RE, Boyle-Vavra S, et al. Community-acquired methicillin-resistant Staphylococcus aureus in children with no identified predisposing risk. JAMA. 1998;279(8):595–8.

7. Afrough P, Pourmand MR, Zeinalinia N, Yousefi M, Abdossamadi Z, Bagherzadeh Yazdchi S. Molecular typing of clinical and nasal carriage isolates of Staphylococcus aureus by spa gene patterns. J Mazandaran Univ Med Sci. 2012;22(94):28–34.

8. Thompson TA, Brown PD. Association between the agr locus and the presence of virulence genes and pathogenesis in Staphylococcus aureus using a Caenorhabditis elegans model. Int J Infect Dis. 2017;54:72–6.

9. Bibalan MH, Shakeri F, Javid N, Ghaemi A, Ghaemi EA. Accessory gene regulator types of Staphylococcus aureus isolated in Gorgan, North of Iran. J Clin Diagn Res JCDR. 2014;8(4):DC07.

10. Mahon CR, Lehman DC, Manuselis G. Textbook of diagnostic microbiology-E-Book. Maryland Heights: Elsevier Health Sciences; 2014.

11. P. W. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing. CLSI guideline M100S.2016.

12. Sambrook J, Fritsch E, Maniatis T. Molecular cloning: a laboratory manual—cold Spring Harbor. New York: Cold spring harbor laboratory press; 1989.

13. Trinh TD, Zasowski EJ, Claeyts KC, Casapaio AM, Compton M, Lagnf A, et al. Role of vancomycin minimum inhibitory concentrations by modified population analysis profile method and clinical outcomes in high inoculum methicillin-resistant Staphylococcus aureus infections. Infect Dis Ther. 2018;7:1–9.

14. Dufour P, Jarraud S, Vandenesch F, Greenland T, Novick RP, Bes M, et al. High genetic variability of the agr locus in Staphylococcus species. J Bacteriol. 2002;184(4):1180–6.

15. 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 staphyloccocal autoinducer peptide. J Bacteriol. 2000;182(20):5721–9.

16. Shopsin B, Matherna A, 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. J Clin Microbiol. 2003;41(1):456–9.

17. Cheraghi S, Pourgholi L, Shafaraki SH, Jalali A, Nosrati R, et al. Analysis of virulence genes and accessory gene regulator (agr) types among methicillin-resistant Staphylococcus aureus strains in Iran. J Glob Antimicrob Resist. 2017;10:315–20.

18. Hasannejad Bibalan M, Ghaemi E, Shakeri F, Javid N. The relation between accessory gene regulator (agr) types of S. aureus and some phenotypic criteria. Relat. 2014;17(87):1–8.

19. Khosrehrooz SS, Mansouri F, Marashifard M, Hosseini SAAM, Chenar-estane-Olia FA, Ganavehei B, et al. Detection of biofilm related genes, classical enterotoxin genes and agr typing among Staphylococcus aureus isolated from bovine with subclinical mastitis in southwest of Iran. Microb Pathog. 2016;97:45–51.

20. Goudarzi M, Bahramian M, Tabrizi MS, Udo EE, Figueiredo AMS, Fazeli M, et al. Genetic diversity of methicillin resistant Staphylococcus aureus strains isolated from burn patients in Iran: ST239-SCCmec III/037 emerges as the major clone. Microb Pathog. 2017;105:1–7.

21. Peerayeh SN, Azimian A, Najdi QH, Kashi M. Prevalence of agr specificity groups among Staphylococcus aureus isolates from university hospitals in Tehran. Lab Med. 2015;40(1):27–9.

22. Mohsenzadeh M, Ghazvini K, Azimian A, editors. Frequency of specific agr groups and antibiotic resistance in Staphylococcus aureus isolated from bovine mastitis in the northeast of Iran. Veterinary Research Forum, 2015. Urmia: Faculty of Veterinary Medicine, Urmia University.

23. Ghasemian A, Peerayeh SN, Bakhshi B, Mirzaee M. High frequency of icaAD, clumping factors A/B, fib and eno Genes in Staphylococcus aureus species isolated from wounds in Tehran, Iran during 2012–2013. Arch Clin Infect Dis. 2015;10(4):e23033.

24. Arabestani MR, Kazemian H, Tabar ZK, Hosseini SM. Prevalence of virulence genes, agr and antimicrobial resistance of Staphylococcus aureus isolated from food and dairy products in Hamadan Iran. Der Pharm Lett. 2016;8(8):62–7.

Publisher’s Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.