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Accessory Gene Regulator Specificity Groups Among *Staphylococcus aureus* Isolated From Hospitalized Children

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Background: *Staphylococcus aureus (S. aureus)* especially those with methicillin resistance are human pathogens capable of causing a wide variety of diseases, ranging from mild skin lesions to systemic and fatal infections.
Objectives: The aim of this study was to detect the accessory gene regulator (*agr)* specificity groups among methicillin resistant and susceptible *S. aureus* isolated from children.
Materials and Methods: During July 2012 to January 2013, 22 *S. aureus*c linical isolates were collected from children aged between 2 to 11, in Loghman Hospital of Tehran. Antibiogram test was performed using disc diffusion method. Polymerase chain reaction (PCR) was conducted to detect mecA gene and *agr* specificity groups.
Results: Among 22 *S. aureus* clinical isolates collected from children, five isolates (22.7 %) were resistant to methicillin. fourteen isolates (63.6%) were resistant to amoxicillin and all were susceptible to vancomycin and linezolid. *Agr* specificity group I was detected in 12 (54.5%) isolates (in 2 MRSA and 10 MSSA isolates), *agr* group II in four (18%, in 3 MSSA and 1 MRSA), group III in 3 (9%, 2 in MSSA and one in MRSA), while *agr* specificity group IV was found in three (13.6%, 2 MSSA and 1 MRSA) isolates.
Conclusions: The *agr* specific group I had the highest rate of detection among pathogens isolated from hospitalized children in Tehran.

Keywords:Staphylococcus aureus; Drug Resistance, Microbial pathogenesis; *mecA* gene

1. Background

Staphylococcus aureus (*S. aureus)* is one of the most important infectious agents in nosocomial infections, causing a wide variety of clinical signs ranging from mild to systemic and potentially fatal disorders either from invasion or toxin production; especially in patients with compromised immune systems or disabilities (1, 2). Methicillin resistant *S. aureus* (MRSA) isolates are versatile pathogens with high resistance rates to antibiotic therapy. MRSA isolates are nosocomial and community acquired and considered as a threat to healthcare settings (3). Community associated MRSA isolates can cause virulent infections and may spread more easily from person to person or cause more skin disease (due to Panton- Valentine Leukocidin toxin) (4). MRSA isolates are resistant to the beta-lactam antibiotics by producing penicillin binding protein 2a (PBP2a) with significantly reduced affinity for these antibiotics (5). PBP2a is encoded by staphylococcal cassete chromosome *mec* (SCC*mec*). Colonization and pathogenicity of *S. aureus* is a complex process involving a diverse array of virulence factors controlled by a network of virulence regulators (6). Quorum sensing is a process of cell-cell communication that enables bacteria to recognize cell density and response to extracellular signaling molecules called autoinducers. The many processes controlled by quorum sensing include bioluminescence, biofilm formation, antibiotic production, competence, sporulation and virulence factor production (7). Quorum sensing in *S. aureus* is based on the *agr* system that encoded by the *agr* locus. The accessory gene regulator (*agr*) is an important gene regulator by encoding the specific peptide called auto-inducing peptide (AIP). As an important quorum sensing system, this gene regulator is a chromosomal locus comprising of two transcripts called RNAII and RNAIII. RNAII operon (or P2 promoter) which is sensitive to AIP concentration, encodes *agr* A, B, C and D. AIP is encoded by *agr* D gene, then post-translationally modified or processed by *agr* B product. However, *agr* C component encodes a histidine kinase (agr C), a sensor for auto inducer peptide and activates *agr* A. The response regulator that activates P2 or P3 promoters is the *agr* A component. Regulation of either of these promoters subsequently leads to RNAII or RNAIII

Implication for health policy/practice/research/medical education:
Staphylococcus aureus is a human pathogen with ubiquitous prevalence in hospital and community. Methicillin resistant isolates are becoming a threat to healthcare settings. The *agr* groups genes regulate *S. aureus* pathogenesis. The correlation between *agr* specific groups and/or MRSA and infection sources can help for detecting pathogenic strains.

transcription (8). The adhesive surface proteins are regulated positively by RNAIII transcript during logarithmic phase of growth. There are suggestions about the correlation between *agr* groups and the kind of infection (3, 4).

2. Objectives

This study was conducted to detect the *agr* specificity groups among methicillin resistant and susceptible children *S. aureus* isolates.

3. Materials and Methods

From July 2012 to January 2013, a total number of 22 *S. aureus* clinical isolates were collected from trachea (14 isolates), blood cultures (4 isolates), skin lesions (2 isolates) and sputum (2 isolates) from hospitalized children (13 male and 9 female) with different clinical signs, aged between 2 to 11 years, in Loghman Hospital of Tehran. The identification tests were conducted by conventional tests (catalase, slide and tube coagulases, growth on mannitol salt agar and DNase tests). Antimicrobial susceptibility test was performed by Kirby Bauer assay (disk diffusion method), according to clinical and laboratory standards institute (CLSI) guidelines. *S. aureus* ATCC 25923 was used as control strain in this study. The discs used in this study included: oxacillin (1 μg), tetracycline (30 μg), clindamycin (2 μg), erythromycin (15 μg), vancomycin (2 μg), linezolid (30 μg), trimethoprim-sulfamethoxazole (10 μg), amoxicillin (10 μg), gentamycin (120 μg) and ciprofloxacin (5 μg) (from MAST, UK). DNA extraction was performed by boiling method (TE buffer [Tris-Hcl + EDTA, pH 8] and lysostaphin [2 μg/mL, 20 μL]) (9), and then the DNA was stored at -20°C. PCR assay was performed to detect *mecA* gene and *agr* specificity groups (by duplex PCR). Primers for *mecA* and *agr* specificity groups has been shown in Table 1 (3, 10, 11).

Table 1. Specific Primers Used for Detection of *mecA* Gene and *agr* Specificity Groups

Primer	Sequence 5′3′	Size (bp)
mecA		
	F: GTG AAG ATA TAC CAA GTG ATT	147
	R: ATG CGC TATAGATTGAAA GGA	
***agr*I**		
	F: ATGCACATGGTGCACATGC	440
	R: GTCACAAGTACTATAAGCTGCGAT	
***agr*II**		
	F: ATGCACATGGTGCACATGC	572
	R: GTATTACTAATTGAAAAGTGCCATAGC	
***agr*III**		
	F: ATGCACATGGTGCACATGC	406
	R: CTGTTGAAAAAGTCAACTAAAAGCTC	
***agr*IV**		
	F: ATGCACATGGTGCACATGC	588
	R: CGATAATGCCGTAATAC CCG	

3.1. Data Analysis

Data were analyzed by Pearson Chi-Square. A P value less than 0.05 was considered as statistically significant.

4. Results

The antibiotic susceptibility pattern was as follows: resistance to amoxicillin was observed in 14 isolates (63.6%), tetracycline in eight (36.3%), ciprofloxacin in five (22.7%), gentamycin in four (18%), trimethoprim-sulfamethoxazole in four (18%), erythromycin in six (27.2%) and clindamycin in four (18%) isolates. All o isolates were susceptible to vancomycin and linezolid. MRSA isolates were detected with oxacillin disk (1 μg) during disk diffusion and then confirmed by detection of *mecA* gene. In 12 (54.5%) isolates which had 440 bp in electrophoresis, *agr* group I was found. Among these, 10 isolates (55.6%) were MSSA and two (50% of MRSA isolates) were MRSA. Three MSSA isolates (16.7%) and one MRSA (25%) belonged to *agr* group II. In three isolates (11.11% of MSSA and 25% of MRSA) *agr* specificity group III was detected, while *agr* specificity group IV was detected in four (18%) isolates, involving two MSSA (11.7%) and one MRSA (25%). Table 2 shows the characteristics of five MRSA isolates all were resistant to at least 6 antibiotics of which two resistant to the all, except for linezolid and vancomycin. Four isolates belonged to *agr* specific group I and one isolate to *agr* group IV. Prevalence of *agr* specific groups in MRSA and MSSA is presented in Table 3. There was no statistical significant difference between methicillin resistant and susceptible clinical isolates regarding presence of *agr* groups (P = 0.13). Furthermore, there was no relationship between infection types and *agr* groups (Figures 1 and 2).

Table 2. Characteristics of MRSA Isolated From Children [a]

Isolates (MRSA)	Clinical Sample	Sex	*agr* Groups	Antibiotic Resistance
1	Blood	M	I	T, A, CD, E, CIP, GM
2	Lesion	F	IV	T, A, CD, E, CIP, GM
3	Trachea	M	I	T, A, CD, SXT, E, CIP, GM
4	Lesion	M	I	T, A, CD, SXT, E, CIP, GM
5	Trachea	M	I	T, A, CD, SXT, E, CIP

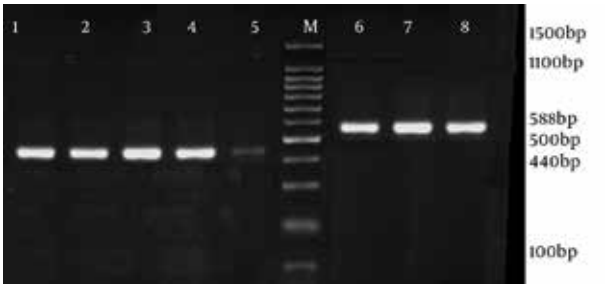
[a] Abbreviations: A, amoxicillin; CD, clindamycin; CIP, ciprofloxacin; E, erythromycin; GM, gentamycin; SXT, trimethoprim-sulfamethoxazole; T, tetracycline.

Table 3. Prevalence of *agr* Specificity Groups in MRSA and MSSA[a]

Isolates	*agr* I	*agr* II	*agr* III	*agr* IV
MSSA (n = 17)	10 (55.6)	3 (16.7)	2 (11.11)	2 (12)
MRSA (n = 5)	2 (40)	1 (20)	1 (20)	1 (20)
Total (n = 22)	12 (54.5)	4 (18)	3 (9)	3 (18)

[a] Data are presented as No. (%).

Figure 1. Electrophoresis of *agr* groups I and IV PCR Products (Duplex PCR)



M: marker. Line 1-5: agr specificity groups I with 440 bp, and Line 6-8: *agr* group IV by 588 bp.

Figure 2. Electrophoresis of *agr* groups II and III

Line1: marker, 2, 4: *agr* groups III (406 bp), and line 3 and 5: *agr* group II (572 bp).

5. Discussion

Community acquired methicillin resistant *S. aureus* (CA-MRSA) can affect healthy children with high morbidity and mortality (12). In the present study, all isolates were susceptible to vancomycin and linezolid and had high resistance to amoxicillin, suggesting the presence of plasmids encoding beta-lactamases, easily transmitted to other strains. There was higher antibiotic resistance in MRSA isolates, such as aminoglycosides and tetracycline. This is especially common in hospital associated isolates (13). These findings were also confirmed by Adebayo's study, in which all isolates were susceptible to vancomycin and linezolid, and also antibiotic resistance was more prevalent in MRSA strains (14). Armin reported resistance to vancomycin and linezolid (15). Quorum sensing system regulates staphylococcal virulence factors. This system participates in staphylococcal scaled skin syndrome (*agr* specificity groups III), exfoliative toxin production (*agr* group IV), and encoding surface proteins (16-18). Also, *agr* group I was the most prevalent specific group (54.5%), due to present study's findings, confirming previous surveys (19). In the Ho CM study, 91.6% of the clinical isolates had *agr* group I (20). Also in the Barbara study, 45.7% of the clinical isolates were *agr*I positive (21). This group has been detected mostly in suppurated infections such as endocarditis (3). The second prevalent groups were *agr* groups II and IV (18% and 13.6%, respectively). Group II *agr* has been more associated to respiratory infections, especially in community acquired MRSA (3, 22). The rate of group III prevalence was 9% which has been shown to be prevalent in isolates with active vancomycin resistance mechanisms (7). Quorum sensing system leads *Staphylococcus aureus* to express virulence factors (23), alter the antibiotic susceptibility pattern, resist the immune system and form biofilm. It was shown that *agr* group I is the most frequent specific group among children, also the most prevalent group in MSSA and MRSA isolates and there was no statistically significant difference between two groups, while MRSA strains had significantly higher antibiotic resistance.

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Authors' Contribution

Abdolmajid Ghasemian performed the microbiological and molecular studies. Shahin Najar Peerayeh designed the research, Bita Bakhshi and Mohsen Mirzaee advised the research.

Financial Disclosure

There is no conflict of interest.

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