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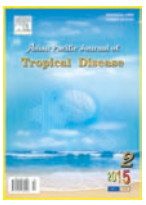

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Detection of accessory gene regulator groups genes and cassette chromosome *mec* types among *Staphylococcus aureus* isolated from intensive care unit patients

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PEER REVIEW

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Comments

This is an interesting study in which the authors detected antibiotic resistance pattern, MRSA, SCC*mec* types and *agr* groups among isolates of *S. aureus*. Further investigations are needed to find the role of *agr* on expression of antibiotic resistance genes.
Details on Page 156

ABSTRACT

Objective: To detect antibiotic resistance pattern, methicillin resistant *Staphylococcus aureus* (*S. aureus*), staphylococcal cassete chromosome *mec* (SCC*mec*) types and accessory gene regulator (*agr*) groups among isolates of *S. aureus*.
Methods: Of 78 *S. aureus* isolates, we performed antibiotic susceptibility test and then, and detected *mecA* gene, SCC*mec* types and *agr* specificity group genes by PCR assay.
Results: All the isolates were susceptible to vancomycin and linezolid. The majority of (94.2%) of methicillin resistant *S. aureus* harbored SCC*mec* type III. We detected *agr* group I in 45% and group II in 34.6% of the isolates. The other strains belonged to *agr* specificity groups III and IV (3.27% and 22.9%, respectively).
Conclusions: The majority of (45%) *S. aureus* isolates belonged to *agr* specificity group I. There was no statistical significant relationship between *agr* and antibiotic resistance and/or clinical signs.

KEYWORDS

Staphylococcus aureus, Drug resistance, *Agr* groups, MRSA, SCC*mec* types

1. Introduction

Staphylococcus aureus (*S. aureus*) isolates cause a wide spectrum of clinical signs, ranging from mild and requiring no treatment to systemic, severe and fatal infections that occur through invasion and toxin production[1]. *S. aureus* infections occur more commonly among hospitalized and/or immunocompromised individuals[2]. Methicillin resistant *S. aureus* (MRSA) isolates can resist a laundry of antibiotics, which can make treatment of infections much more difficult.

MRSA isolates are resistant to beta-lactam antibiotics by producing penicillin-binding protein 2a with significantly reduced affinity to beta-lactam[3]. All the staphylococcal cassete chromosome *mec* (SCCmec) types encode penicillin-binding protein 2a enzyme. Eleven SCC*mec* types (I-XI) have been reported, but the globally more predominant types include SCC*mec* I-V[4]. Moreover, MRSA isolates can be acquired from nosocomial or community origins [healthcare-associated or community-acquired (HA/CA)-MRSA][5]. CA-MRSA strains contain highly diverse and unique virulence factors.

Pathogenesis of *S. aureus* strains depends on various virulence factors which are transcriptionally controlled by a network of virulence regulators[6]. The *agr* operon plays an

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important role in expression of virulence genes, by encoding a specific peptide called autoinducing peptide (AIP). The *agr* operon comprises of two transcripts called RNAII and RNAIII. RNAII operon (or P2 promoter), encodes *agr* A, B, C and D. The *agr* D gene encodes AIP, which is modified by protein product of *agr* B. AIP high concentrations downregulate the expression of adhesive surface proteins, but upregulate extracellular enzymes and toxins. Furthermore, *agr* C component is a histidine kinase that acts as a sensor of AIP concentrations, and then activates *agr* A via phosphorylation mechanism. *Agr* A component is a response regulator that activates P2 or P3 promoters, either of which subsequently causes RNAII or RNAIII overexpression[7]. This two component signal transduction systems (*agr* C/A) downregulate the surface proteins and upregulate those secreted[8]. Several authors have suggested that there is a relationship between *agr* specificity group's characteristics and site of infections[3,4]. Moreover, there are reports with *agr* defective strains, because of *agr* loss during infection period[9]. The goals of this study were to determine the prevalence of MRSA, SCC*mec* types and *agr* specificity groups among *S. aureus* clinical isolates.

2. Materials and methods

2.1. Bacterial isolates

We collected a total of 78 *S. aureus* clinical isolates from trachea (58 isolates), blood cultures (10 isolates), lesion (6 isolates) and sputum (4 isolates) of intensive care unit patients (48 males and 30 females) from July 2012 to January 2013. In addition, the isolates were identified with catalase, slide and tube coagulases, and acid production from mannitol salt agar and DNase test.

2.2. Antibiotic susceptibility testing

Antimicrobial susceptibility test (AST) was conducted based on the Kirby Bauer assay (disk diffusion method), according to Clinical and Laboratory Standards Institute guidelines. We used *S. aureus* ATCC 25923 strain for quality control of antibiotic susceptibility test. Several disks were used in AST, including oxacillin (1 μg), tetracycline (30 μg), clindamycin (2 μg), erythromycin (15 μg), vancomycin (2 μg), linezolid (30 μg), trimethoprim-sulfamethoxazole (25 μg), amoxicillin (10 μg), gentamicin (10 μg) and ciprofloxacin (5 μg) (Mast Group Ltd., UK Corporation).

2.3. Genomic DNA extraction

Total genomic DNA was extracted via preparation of a suspension of bacterial isolates in 200 μL of tris-ethylene diamine tetraacetic acid buffer and lysostaphin [comprising 200 μL of tris-ethylene diamine tetraacetic acid buffer and 20 μL of lysostaphin (2 μg/mL, Sigma)]. The DNA was isolated according to Straubinger method[10].

2.4. DNA amplification

DNA was amplified with specific primers (Table 1) to detect *mecA* gene, SCC*mec* types and *agr* specificity groups among the clinical isolates.

Table 1
Primers used for *mecA*, *agr* locus and SCC*mec* genes amplification.

Primer	Sequence: 3′→5′	Product size	Reference
mecA	F: GTG AAG ATA TAC CAA GTG ATT	146	11
	R: ATG CGC TATAGATTGAAA GGA		
SCC*mec*I	F: GCTTTAAAGAGTGTCGTTACAGG	613	11
	R: GTTCTCTCATAGTATGACGTCC		
SCC*mec*II	F: CGTTGAAGATGATGAAGCG	398	11
	R: CGAAATCAATGGTTAATGGACC		
SCC*mec*III	F: CCATATTGTGTACGATGCG	280	11
	R: CCTTAGTTGTCGTAACAGATCG		
SCC*mec*IV	F: GCCTTATTCGAAGAAACCG	776	11
	R: CTACTCTTCTGAAAAGCGTCG		
SCC*mec*V	F: GAACATTGTTACTTAAATGAGCG	325	11
	R: TGAAAGTTGTACCCTTGACACC		
*agr*I	F: ATGCACATGGTGCACATGC	440	12
	R: GTCACAAGTACTATAAGCTGCGAT		
*agr*II	F: ATGCACATGGTGCACATGC	572	12
	R: GTATTACTAATTGAAAAGTGCCATAGC		
*agr*III	F: ATGCACATGGTGCACATGC	406	12
	R: CTGTTGAAAAAGTCAACTAAAAGCTC		
*agr*IV	F: ATGCACATGGTGCACATGC	588	12
	R: CGATAATGCCGTAATAC CCG		

F: Forward; R: Reverse.

The annealing temperature was 55 °C (30 seconds) for *mecA* gene and 51 °C (1 min) for SCC*mec* types, according to Zhang *et al*[11]. We carried out duplex PCR with an annealing temperature of 51 °C (1 min) for *agr* specificity groups, according to the method of Shopsin *et al*[12]. Primers for *mecA*, SCC*mec* types and *agr* specificity groups have been shown in Table 1. For visualization of PCR products during electrophoresis, 5 μL of each product was mixed with 1 μL of each gel red and loading buffer dyes, and was run in 1% agarose gel electrophoresis and observed by ultraviolet transilluminator.

2.5. Statistical analysis

We used the *Chi*-square test to compare each pair of *agr* groups between methicillin-sensitive *S. aureus* (MSSA) and MRSA groups ($P<0.05$ was considered significant).

3. Results

In the antibiotic susceptibility testing, 50 isolates (64%)

were resistant to amoxicillin. For the other antibiotics such as tetracycline, ciprofloxacin, gentamicin, trimethoprim-sulfamethoxazole, erythromycin and clindamycin, rate of resistance were as 21 (23%), 14 (18%), 20 (26%), 10 (13%), 23 (29.4%) and 14 (18%) isolates, respectively. Seventeen (21.7%) isolates were resistant to oxacillin and phenotypically detected as MRSA. All the studied isolates were susceptible to vancomycin and linezolid. Sixteen (94.2%) MRSA isolates harbored SCC*mec* type III, and one MRSA (5.8%) harbored SCC*mec* type V (Figures 1 and 2).

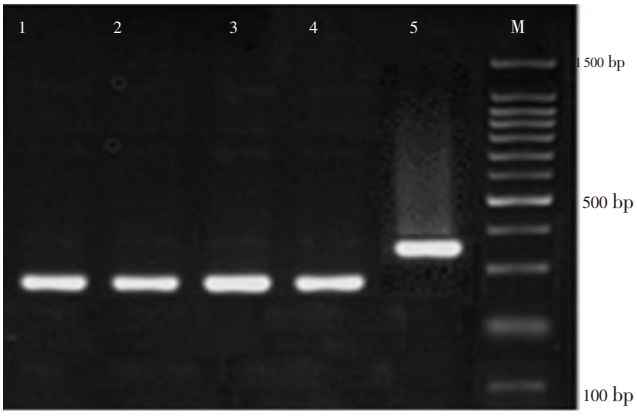

Figure 1. Electrophoresis of SCC*mec* PCR products detected (SCC*mec* types 3 and 5 PCR products).
Columns 1 to 4: SCC*mec* typeIII PCR products with 280 bp; Column 5: Product of SCC*mec* V with 325 bp; M: Marker (100 bp).

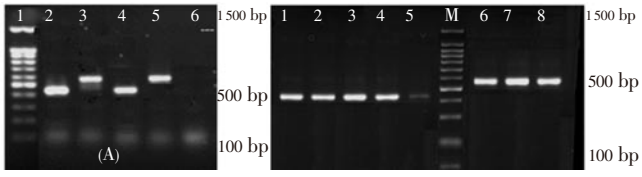

Figure 2. PCR products of *agr* specificity groups.
(A) Columns 1 and 6: Marker and control negative, respectively; Columns 2 and 4: Products of *agr* specificity group I (440 bp); Columns 3 and 5: *agr* specificity group IV (588 bp).
(B) Columns 1 to 4: *agr* group III (406 bp); Columns 5 to 8: Products of *agr* specificity group II (572 bp); M: Marker.

3.1. MRSA strains and agr groups

Nine (53.00%) MRSA isolates belonged to *agr* specificity group I, followed by *agr* group II in seven (41.17%) and *agr* specificity group IV in one (5.88%) MRSA isolate. None of them belonged to *agr* specificity group III (Tables 2 and 3).

Table 2
Prevalence of *agr* groups in MSSA and MRSA isolates. *n* (%).

Isolates	*agr*I	*agr*II	*agr*III	*agr*IV
MSSA (*n*=61)	26 (42.60)	20 (32.80)	2 (3.27)	13 (21.30)
MRSA (*n*=17)	9 (53.00)	7 (41.17)	0	1 (5.88)
Total (*n*=78)	35 (57.30)	27 (44.20)	2 (3.27)	14 (22.90)

Table 3
Agr specificity groups' prevalence in MRSA.

Isolates (MRSA)	Clinical sample	Genus	SCC*mec*	*agr*	Antibiotic resistance
1	Bronchus	F	III	I	T, A, SXT, CD, E, CIP, GM
2	Trachea	F	III	II	T, A, SXT, CD, E, CIP, GM
3	Trachea	M	III	II	T, A
4	Lesion	M	III	II	T, A, CD, E, CIP, GM
5	Trachea	F	III	I	T, A, CD, E, CIP
6	Blood	F	III	II	T, A, CD, E, CIP
7	Trachea	M	III	I	T, A, CD, E, CIP
8	Trachea	M	III	I	T, A, CD, E, CIP
9	Lesion	M	III	II	A
10	Trachea	F	III	I	T, A, SXT, CD, E, CIP,GM
11	Trachea	M	III	I	T, A, CD, E, CIP, GM
12	Trachea	M	V	II	T, A, E, CIP
13	Trachea	M	III	I	T, A, SXT, CD, E, CIP, GM
14	Trachea	M	III	II	T, A, SXT, CD, E, CIP, GM
15	Trachea	M	III	I	T, A, SXT, CD, E, CIP, GM
16	Bronchus	M	III	I	T, A, SXT, CD, E, CIP, GM
17	Sputum	F	III	IV	T, A, SXT, CD, E, CIP, GM

F: Female; M: Male; T: Tetracycline; A: Amoxicillin; SXT: Trimethoprim-sulfametoxazole; CD: Clindamycin; E: Erythromycin; CIP: Ciprofloxacin; GM: Gentamicin.

3.2. MSSA strains and agr groups

Twenty-six (42.60%) MSSA isolates belonged to *agr* specificity group I, and 20 (32.80%) isolates belonged to *agr* group II. Moreover, 2 (3.27%) and 13 (21.30%) MSSA isolates belonged to *agr* specificity group III and IV, respectively (Table 2). There was no significant difference regarding presence of *agr* groups between MRSA and MSSA strains.

4. Discussion

Accurate antibiotic susceptibility profile reporting is important for treatment of infections caused by *S. aureus*. In this study, we didn't determine any correlation between antibiotic resistance and origins of clinical isolates. All isolates showed susceptibility to vancomycin and linezolid, though the majority of them were resistant to amoxicillin. Seventeen (21.8%) isolates were resistant to oxacillin disk and harbored *mecA* gene. Two blood clinical isolates (2.5%) were resistant to methicillin and eight of these isolates (10.25%) were susceptible to this disk. Moreover, MRSA isolates with SCC*mec* type III were significantly more resistant to antibiotics, indicating isolates with hospital acquired infections[4]. Shittu's study results are similar to our findings, in which all of the isolates were susceptible to vancomycin and linezolid, with higher antibiotic resistance among MRSA strains[13]. In Sharma's study, all of *S. aureus* clinical isolates were susceptible to vancomycin and higher antibiotic resistance reported in MRSA isolates[14]. Similarly, Sharma *et al.* observed that all studied isolates were

susceptible to vancomycin and the antibiotic resistance was significantly higher in MRSA strains[15]. These results show that vancomycin and linezolid have been remained among few effective antibiotics to deal with *S. aureus* infections.

In this study, SCC*mec* type III was detected in 16 (98.2%) of MRSA isolates, and one isolate (5.8%) harbored SCC*mec* type V. These results are slightly higher than Japoni's study in south of Iran, that reported SCC*mec* type III in 74.3% of isolates[16]. Likewise, Fatholahzadeh reported SCC*mec* type III in 78% of the isolates[17]. In the study of Azimian *et al*, SCC*mec* type III has been reported as the major SCC*mec* type[18]. In Reiter's study, all of patients with cystic fibrosis harbored SCC*mec* type III[19]. It seems that the clinical origin of isolates, epidemiological varieties, and the time of every study may be pivotal factors interfering in results obtained. Hospital associated MRSA isolates are resistant to a wide spectrum of antibiotics, and usually carry SCC*mec* type III[20]. We detected SCC*mec* type V in one MRSA isolate. It is believed that SCC*mec* type V can be acquired from community and cause severe diseases (due to Panton-Valentine leukocidin toxin production) traditionally associated to CA-MRSA[21]. There are various reports of SCC*mec* typeV prevalence from some areas[18,19,22]. In our study, the majority of MRSA isolates (11 isolates, 65%) were from trachea, among which nine (53%) and seven (41.1%) isolates belonged to *agr* group I and II, respectively.

Various pathogenesis factors from the nosocomial strains of *S. aureus* cause the staphylococcal clinical symptoms, especially MRSA isolates containing factors that may enhance their virulence[23]. MRSA strains can affect healthy people, sometimes with high morbidity and mortality results[24]. Virulence factors of *S. aureus* are regulated by various mechanisms, such as *agr* system[25]. In this study, the majority of the isolates (54.5%) belonged to *agr* group I, similar to previous survey performed by Azimian *et al*[26]. In addition, Indrawattana's study showed that the majority of isolates belonged to *agr* specificity group I (58.7%) and in the Ho's study, 91.6% of the clinical isolates belonged to *agr* group I[27,28]. Kahl reported that 45.7% of the clinical isolates were *agr* I positive[29]. However, Kolawole *et al.* reported that 20 of 192 isolates belonged to *agr* group I[30]. These findings indicate that *agr* group I may have an indispensible role in regulation of staphylococcal virulence. We detected no significant relationship between antibiotic resistance patterns and *agr* specificity groups. Prevalence of *agr* groups II and IV were the same (each was 18%) in this study. The *agr* group II has been more associated to respiratory infections, especially in CA-MRSA[3,31], but our study confirmed no relationship between the origin of clinical specimens and presence of any *agr* group. The results from several studies in various areas are related potentially to some factors, such as geography, origin of specimens, and the time of conduction of studies. The rate of group III was 9% in this study, being slightly higher than Kolawole's study results that detected in 7.2% (14 of 192 isolates). There was not any significant relationship between each specific *agr* group prevalence and antibiotic susceptibility pattern of the isolates or correlation with clinical signs, similar to Indrawattana's study[27]. Further investigations are needed to find the role of *agr* on expression of antibiotic resistance genes.

The majority of MSSA and MRSA isolates belonged to *agr* group I. There was no statistical significant relationship between the *agr* groups and antibiotic resistance and/or clinical signs.

Conflict of interest statement

We declare that we have no conflict of interest.

Comments

Background

S. aureus isolates cause a wide spectrum of clinical signs, ranging from mild and requiring no treatment to systemic, severe and fatal infections that occur through invasion and toxin production. MRSA isolates can resist a laundry of antibiotics, which can make treatment of infections much more difficult.

Research frontiers

This study aimed to determine the prevalence of MRSA, SCC*mec* types and agr specificity groups among *S. aureus* clinical isolates.

Related reports

Kahl reported that 45.7% of the clinical isolates were *agr* I positive. While Kolawole *et al.* reported that 20 of 192 isolates belonged to *agr* group I.

Innovations & breakthroughs

This study confirmed no relationship between the origin of clinical specimens and presence of any *agr* group.

Applications

The study found that there was not any significant relationship between each specific *agr* group prevalence and antibiotic susceptibility pattern of the isolates or correlation with clinical signs. The results may be helpful to researchers to do further investigations.

Peer review

This is an interesting study in which the authors detected antibiotic resistance pattern, MRSA, SCC*mec* types and *agr* groups among isolates of *S. aureus*. Further investigations are needed to find the role of *agr* on expression of antibiotic resistance genes.

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