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Accessory Gene Regulator Polymorphism and Vancomycin Minimum Inhibitory Concentration in Methicillin-Resistant *Staphylococcus aureus*

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Background: Methicillin-resistant *Staphylococcus aureus* (MRSA) bacteremia with a vancomycin minimum inhibitory concentration (MIC) of 2 μg/mL presents a high rate of therapeutic failure in response to vancomycin. In addition, polymorphism in accessory gene regulator (*agr*) is associated with vancomycin therapeutic effects. The association between *agr* polymorphism and vancomycin MICs was investigated in MRSA isolates.

Methods: *Agr* group-specific PCR was conducted on 118 MRSA bloodstream isolates. Vancomycin susceptibility tests were conducted, while E-test GRD (bioMérieux SA, France) was used to detect heterogeneous vancomycin-intermediate *S. aureus* (hVISA).

Results: Of the 118 MRSA isolates, 59 (50.0%), 43 (36.4%), and 10 (8.5%) isolates belonged to *agr* group I, II, and III, respectively. Six isolates could not be classified. Twenty-six, 73, and 19 isolates presented a vancomycin MIC of 2, 1, and 0.5 μg/mL, respectively. Nine (34.6%), 14 (53.8%), and 2 (7.7%) isolates with MICs of 2 μg/mL belonged to *agr* group I, II, and III, respectively. Thirty-seven (50.6%), 26 (35.6%), and 6 (8.2%) isolates with MICs of 1 μg/mL belonged to *agr* group I, II, and III, respectively. Thirteen (68.4%), 3 (15.8%), and 2 (10.5%) isolates with MICs of 0.5 μg/mL belonged to *agr* group I, II, and III, respectively. The *agr* group II presented more isolates with MIC of 2 μg/mL (32.6%) than the *agr* non-group II (16%). Four isolates tested positive for hVISA. Three of them belonged to *agr* group II.

Conclusions: MRSA isolates with vancomycin MIC of 2 μg/mL were more common in *agr* group II than in *agr* non-group II.

Key Words: *Agr* polymorphism, *Staphylococcus aureus*, Vancomycin

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INTRODUCTION

In *Staphylococcus aureus* vancomycin susceptibility test, a minimum inhibitory concentration (MIC) of 16 μg/mL or more is categorized as resistant, 4-8 μg/mL as intermediate, and 2 μg/mL or less as susceptible according to CLSI criteria [1]. Glycopeptides such as vancomycin and teicoplanin are used as primary therapeutic options for methicillin-resistant *S. aureus* (MRSA) infection [2, 3]. Although MRSA infection with a vancomycin MIC of 2 μg/mL is categorized as susceptible according to the criteria of antibiotic susceptibility test, treatment using glycopeptides presents a high rate of failure [4]. Furthermore, MRSA with a vancomycin MIC of 2 μg/mL and MRSA with a vancomycin MIC of 1 μg/mL need to be distinguished [4, 5]. Even though vancomycin MICs are mainly determined by using automated equipment, differences in measurements may be observed when using theautomated antibiotic susceptibility test versus using CLSI reference method [6].

Accessory gene regulator (*agr*) operon participates in the regulation of virulence factors of *S. aureus*. *S. aureus* is divided into four *agr* groups based on its amino acid sequence polymorphism [7, 8]. It has been reported that *agr* polymorphism is related to vancomycin intermediate *S. aureus* (VISA) [9] and to failure of glycopeptide treatment for MRSA infections [8, 10].

VISA that shows heteroresistance to vancomycin (hVISA) has been increasing in frequency, since it was first reported in Japan in 1997 [11, 12]. The automated antibiotic susceptibility test used in most clinical microbiology laboratories can not detect hVISA [12] and vancomycin MICs higher than 1 μg/mL are related to hVISA [11, 13], thereby, necessitating additional tests for the detection of hVISA.

In MRSA infections, vancomycin MIC of 2 μg/mL, *agr* group II polymorphism, and hVISA are factors that may be related to treatment failure. This study used MRSA bloodstream isolates to compare vancomycin MICs obtained by CLSI broth microdilution (BMD) to those obtained with an automated susceptibility test and to determine the association between *agr* polymorphism and vancomycin MIC of 2 μg/mL. Further more, the distribution of hVISA was investigated.

METHODS

1. Bacterial isolates

A total of 118 MRSA strains isolated from blood cultures between September 2012 and August 2013 were used in this study. Thirty-six strains were isolated from blood cultures in two teaching hospitals in Seoul and 82 strains were isolated from blood cultures in a teaching hospital in Gyeonggi province in Korea. The isolates were stored at -70°C and then cultured on blood agar plates. The study was exempt from review by institution review board (IRB) of Hallym University Sacred Heart Hospital.

S. aureus was identified by using MicroScan Pos Combo 28 Panel (Siemens, West Sacramento, CA, USA) and conventional methods such as coagulase test, mannitol fermentation, and DNAse test. The methicillin resistance was determined by resistance to cefoxitin and by PCR to detect the *mecA* gene [14].

2. Antibiotic susceptibility testing

According to the manufacturer's instructions, antibiotic susceptibility test was conducted by using MicroScanPos Combo 28 Panel. Using the BMD method suggested by CLSI [1], MICs were measured for vancomycin concentrations of 0.25-16 μg/mL and were compared with those measured by the MicroScan panel. The rates of vancomycin MIC of 2 μg/mL determined by using the MicroScan panel and those determined by using BMD were compared.

3. Multiplex PCR of *agr* groups

Multiplex PCR of *agr* group I to IV was conducted on MRSA isolates, as previously described [15].

4. hVISAscreeing

Using E-test GRD (bioMérieux SA, Marcy l'Etoile, France) and Mueller-Hinton agar with 5% sheep blood, hVISA screening was conducted according to the manufacturer's instructions [16].

5. Statistical analysis

The STATA 12 (STAT Corp., College Station, TX, USA) software was used for statistical analysis. The rates of vancomycin MIC of 2 μg/mL in *agr* group II and those in *agr* non-group II were compared by using chi-square test, with a significance level of 0.05.

RESULTS

Agr group-specific PCR showed a total of 118 isolates, 59 (50.0%) isolates belonged to *agr* group I, 43 (36.4%) isolates belonged to *agr* group II, and 10 (8.5%) isolates belonged to *agr* group III. *Agr* group IV was not detected. Six isolates tested negative for all *agr* groups. Of the 36 isolates from two hospitals in Seoul, 17 (47.2%) isolates belonged to *agr* group I, 14 (38.9%) isolates belonged to *agr* group II, and four (11.1%) isolates belonged to *agr* group III. Of the 82 isolates from a hospital in Gyeonggi province, 42 (51.2%) isolates belonged to *agr* group I, 29 (35.4%) isolates belonged to *agr* group II, and six (7.3%) isolates belonged to *agr* group III.

Vancomycin susceptibility results from both BMD and MicroScan panel showed that the MICs of all 118 isolates were 2 μg/mL or less, corresponding to the susceptible category. Vancomycin susceptibility test using BMD showed that the MIC of 26 isolates was 2 μg/mL and that of 92 isolates was 1 μg/mL or less. On the other hand, vancomycin susceptibility test using the MicroScan panel showed that the MIC of 48 isolates was 2 μg/mL and that of 70 isolates was 1 μg/mL or less (Fig. 1). The number of isolates with MIC of 2 μg/mL measured by vancomycin susceptibility test using the MicroScan panel was significantly higher than that determined using BMD (P=0.002).

Of the 26 isolates with vancomycin MIC of 2 μg/mL measured by BMD, nine (34.6%) isolates belonged to *agr* group I, 14 (53.8%) isolates belonged to *agr* group II, and two (7.7%) iso-

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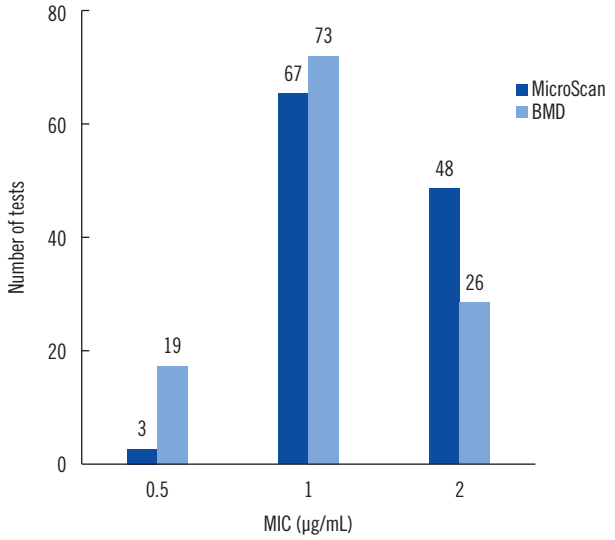

Fig. 1. Distribution of vancomycin MICs by methods.
Abbreviations: MIC, minimum inhibitory concentration; BMD, broth microdilution.

lates belonged to *agr* group III. Of the 73 isolates with vancomycin MIC of 1 μg/mL measured by BMD, 37 (50.6%) isolates belonged to *agr* group I, 26 (35.6%) isolates belonged to *agr* group II, and six (8.2%) isolates belonged to *agr* group III. Of the 19 isolates with vancomycin MIC of 0.5 μg/mL measured by BMD, 13 (68.4%) isolates belonged to *agr* group I, three (15.8%) isolates belonged to *agr* group II, and two (10.5%) isolates belonged to *agr* group III. The percentage of isolates with vancomycin MIC of 2 μg/mL measured by BMD was 15.3% (9/59) in *agr* group I, 32.6% in *agr* group II, and 20.0% (2/10) in *agr* group III (Table 1). The percentage of isolates with vancomycin MIC of 2 μg/mL in *agr* group II (32.6%)was significantly higher than that in *agr* non-group II (16%) (P=0.04).

Four isolates were positive in hVISA screening using E-test GRD. Of those, three isolates belonged to *agr* group II and one isolate belonged to *agr* group I. All four isolates that were E-test GRD positive had vancomycin MICs of 2 μg/mL measured by the BMD method and the MicroScan panel.

DISCUSSION

When determining the vancomycin MIC of MRSA isolates, one needs to take into consideration the differences among the methods used for the antibiotic susceptibility test. E-test and MicroScan system estimate higher vancomycin MICs than the Vitek 2 system, which measures it at a lower level than BMD, the reference method [6, 17]. This study showed 100% categorical agreement, as all target isolates were vancomycin susceptible, using both the MicroScan panel and BMD. However, the number of isolates with a MIC of 2 μg/mL was significantly higher when measured using the MicroScan panel than when using BMD.

Table 1. *Agr* groups and vancomycin MIC smeasured by BMD in MRSA isolates

Agr group	Vancomycin MIC (μg/mL)			Total
	0.5	1	2	
I	13	37	9	59
II	3	26	14	43
III	2	6	2	10
Non-typable	1	4	1	6
Total	19	73	26	118

Abbreviations: MIC, minimum inhibitory concentration; BMD, broth microdilution; MRSA, methicillin-resistant *Staphylococcus aureus*.

A study that collected and analyzed isolates of *S. aureus* from different regions around the world reported that they belong primarily to *agr* group I [18]. In addition, a study conducted in Germany showed *agr* group I to be predominantly represented in epidemic MRSA [19]. On the other hand, *agr* group II is reported to be commonly detected in patients with chronic wounds [20]. Furthermore, a study conducted in a teaching hospital in Korea using MRSA isolated from various clinical specimens showed that 49.3% of isolates belonged to *agr* group I and 44.0% belonged to *agr* group II, showing a relatively high percentage of *agr* group II [21]. This study was conducted by using bloodstream isolates collected from three teaching hospitals in Korea and showed that 50.0% (59/118) of isolates belonged to *agr* group I and 36.4% (43/118) belonged to *agr* group II, demonstrating a high percentage of *agr* group II MRSA in Korea (Table 1). This was consistent with the results of a previous study [21].

Various studies showed an association between *agr* group and vancomycin susceptibility in MRSA. Some studies reported that MRSA infection associated with *agr* group II had a higher failure rate of vancomycin therapy [8], and that glycopeptide intermediate *S. aureus* (GISA) and *agr* group II are related [9]. On the other hand, some studies reported that reduced susceptibility to glycopeptides is related to both *agr* group I and II [15] and that there is no association between *agr* polymorphism and vancomycin resistance [22]. In this study, MRSA associated with *agr* group II showed a significantly higher percentage of isolates with vancomycin MIC of 2 μg/mL compared with *agr* non-group II. Considering the association between MRSA with vancomycin MIC of 2 μg/mL and the therapeutic failure of glycopeptides, the

present results support those of a previous study that reported an association between *agr* group II and failure of vancomycin therapy [8]. Several reports suggested that *agr* dysfunction is associated with worse outcomes among patients with *S. aureus* infections [10, 23]. This study investigated not the expression of *agr* genes but the presence of the *agr* groups.

hVISA is related to the therapeutic failure of vancomycin [24, 25] and shows vancomycin susceptible results with MIC higher than 1 μg/mL [13]. One study that investigated MRSA isolated from the blood showed that hVISA was not detected in MRSA with vancomycin MIC of 1 μg/mL or less, while it was detected in 18.1% of MRSA with vancomycin MICs higher than 1 μg/mL [26]. In this study, hVISA screening test was negative for all MRSA isolates with a vancomycin MIC of 1 μg/mL or less, but was positive for 15.4% (4/26) of isolates with a MIC of 2 μg/mL. As three out of four isolates that tested positive in the hVISA screening test belonged to *agr* group II, an association between hVISA and *agr* polymorphism could be inferred. However, this study has limitations. The number of hVISA isolates was small, and determination of population analysis profile was not conducted as a confirmatory test for hVISA.

In summary, *agr* groups I and II were the major groups represented in MRSA isolates from blood cultures in Korea. The percentage of MRSA isolates with vancomycin MIC of 2 μg/mL was the highest in *agr* group II (32.6%). All four isolates that tested positive in hVISA screening had vancomycin MIC of 2 μg/mL, and three of the four isolates belonged to *agr* group II.

Authors' Disclosures of Potential Conflicts of Interest

No potential conflicts of interest relevant to this article were reported.

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