Difference in *agr* Dysfunction and Reduced Vancomycin Susceptibility between MRSA Bacteremia Involving SCCmeC Types IV/IVa and I–III

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Abstract

**Background:** Dysfunction of *agr*, with reduced susceptibility or hetero-resistance to vancomycin, is thought to be associated with a worse outcome of methicillin-resistant *Staphylococcus aureus* (MRSA) bacteremia (MRSAB). However, the difference in *agr* dysfunction according to the SCCmeC type in MRSA infection is undetermined. We compared the prevalence of *agr* dysfunction, reduced vancomycin susceptibility and the outcomes of SCCmeC IV/IVa and I–III MRSAB.

**Methods:** The study included 307 cases of MRSAB. SCCmeC types were determined by multiplex PCR. The clinical and microbiological features and outcomes of 58 SCCmeC IV/IVa MRSAB were compared with those of 249 SCCmeC I–III MRSAB.

**Results:** Compared with SCCmeC I–III MRSAB, SCCmeC IV/IVa MRSAB was associated with lower rates of *agr* dysfunction (3% vs. 43%), vancomycin minimum inhibitory concentration (MIC) = 2 μg/mL (3% vs. 15%), and hetero-resistance to vancomycin (0% vs. 8%) (all *P* < 0.05). However, the 30-day and *S. aureus*-related mortality in patients with SCCmeC IV/IVa MRSAB were not different from those in patients with SCCmeC I–III MRSAB in multivariate analyses (HR 1.168, 95% CI 0.705–1.938; HR 1.025, 95% CI 0.556–1.889).

**Conclusions:** SCCmeC IV/IVa MRSAB was associated with lower rates of *agr* dysfunction and hetero-resistance to vancomycin and a lower vancomycin MIC, compared with SCCmeC I–III MRSAB. However, the outcomes of SCCmeC IV/IVa MRSAB did not differ from those of SCCmeC I–III MRSAB.

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Introduction

Accessory gene regulator (*agr*) is a global regulator gene of *Staphylococcus aureus* that controls the expression of major virulence factors, such as cytotoxins, enzymes, and superantigens [1]. Moreover, *agr* is the main quorum-sensing operon in *S. aureus* that regulates cell to cell signaling [2,3]. Traditionally, most human *S. aureus* isolates are considered *agr*+ and to have *agr* function; however, *S. aureus* with diminished or absent δ-hemolysin expression (*agr* dysfunction), the end-product of the *agr* system, has recently emerged and become prevalent in methicillin-resistant *S. aureus* (MRSA) [4].

Dysfunction of *agr* is thought to be associated with decreased susceptibility to vancomycin and vancomycin-intermediate *S. aureus* (VISA)/hetero-VISA [5–7], and some have suggested that *agr* dysfunction adversely affects the treatment outcomes of MRSA infection [8]. However, the prevalence of *agr* dysfunction according to the SCCmeC type in MRSA infection remains uncertain, although MRSA possessing SCCmeC type IV/IVa (SCCmeC type IV/IVa MRSA), known as a community-associated MRSA clone, has different antibiotic susceptibility patterns and toxin profiles from MRSA possessing SCCmeC types I–III (SCCmeC I–III MRSA). Moreover, it is still not known whether the outcomes of bacteremia caused by SCCmeC IV/IVa MRSA (SCCmeC IV/IVa MRSAB) are similar to that caused by SCCmeC I–III MRSA (SCCmeC I–III MRSAB), because clinical studies have obtained conflicting results [9–14].

This study compared the prevalence of *agr* dysfunction, hetero-VISA, and the vancomycin minimum inhibitory concentration (MIC) of SCCmeC IV/IVa MRSAB with those of SCCmeC I–III MRSAB, and investigated the impact of these factors on the outcomes of MRSA bacteremia.
Patients and Methods

Ethics

This Study was approved by the institutional review board of Chonnam National University Hospital. A waiver of consent was granted given the retrospective nature of the project.

Patients

All patients ≥16 years old with MRSA bacteremia who were treated between January 2005 and December 2008 at two university hospitals and referral center centers, Chonnam National University Hospital (1000 beds; Gwangju, Republic of Korea) and Chonnam National University Hwasun Hospital (700 beds; Hwasun, Republic of Korea), were included. Cases were identified using computerized records from the Clinical Microbiology Laboratory. Only the first episode of MRSA bacteremia in a patient was included. Demographic and clinical data were collected by reviewing the electronic medical records of the patients.

Microbiological Tests

*S. aureus* was identified and methicillin resistance was determined using the automated systems Vitek 2 (bioMérieux, Marcy l’Etoile, France) or Microscan (Dade Behring Inc., Deerfield, IL). MICs of vancomycin were determined by Etest (AB BIODISK, Solna, Sweden) using a 0.5 McFarland inoculum on Muller–Hinton agar plates. Modified population analyses for hetero-VISA detection were performed using brain–heart infusion agar (BHIA; BD Diagnostics, Sparks, MD) plates containing various concentrations of vancomycin [15]. ATCC 29213, Mu50 (a VISA strain), and Mu3 (a hetero-VISA strain) were used as controls for Etest and modified population analysis. agr dysfunction was determined by examining α-hemolysin expression on blood agar plates using *S. aureus* RN4220, as described previously [6].

Multiplex PCR was performed to determine SCCme type for MRSA isolates, as described previously [16–19]. Panton–Valentine leukocidin (pvl) genes were detected by PCR, as described previously [20].

Definitions

*S. aureus* bacteremia was considered to be hospital-onset if *S. aureus* was isolated from cultures of blood samples obtained from patients who had been hospitalized for 48 h or longer. Otherwise, *S. aureus* bacteremia was considered to have been community-onset. *S. aureus* bacteremia was defined as community-acquired if *S. aureus* were isolated from cultures of blood samples obtained within 48 h of hospital admission and the patient had no medical history of MRSA infection or colonization. This included no medical history in the past year of dialysis, surgery, hospitalization, admission to a nursing home, skilled nursing facility, or hospice, and no history of treatment between January 2005 and December 2008 at two university hospitals and referral center centers, Chonnam National University Hospital (1000 beds; Gwangju, Republic of Korea) and Chonnam National University Hwasun Hospital (700 beds; Hwasun, Republic of Korea), were included. Cases were identified using computerized records from the Clinical Microbiology Laboratory. Only the first episode of MRSA bacteremia in a patient was included. Demographic and clinical data were collected by reviewing the electronic medical records of the patients.

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*S. aureus* bacteremia was defined as catheter-related if the catheter tip grew more than 15 colonies for *S. aureus*, or inflammation was present at the insertion site and no alternative source of infection was identified [22]. *Infective endocarditis* was defined by the modified Duke criteria [23]. *Metastatic infection* was defined as the presence of microbiological or radiographic evidence of *S. aureus* infection caused by hematogenous seeding [22]. *Persistent bacteremia* was defined as consecutive blood cultures positive for 7 or more days despite appropriate antibiotic use for 5 or more days [24]. Mortality was defined as *S. aureus*-related in the absence of another definite cause of death [24].

Statistical Analyses

Categorical variables were compared using Fisher’s exact test or the Pearson χ² test as appropriate, and continuous variables were compared using Student’s t-test. Multivariate analyses were performed using the Cox-regression hazard model in the backward stepwise conditional manner. All tests of significance were two-tailed, and P values ≤0.05 were deemed to indicate statistical significance. Statistical analyses of the data were performed using the PASW statistics software (version 18.0; SPSS Inc., Chicago, IL).

Results

SCCme Type and pvl in MRSA Blood Isolates

We identified 307 cases of first-episode MRSA bacteremia during the study period. The most common SCCme type was II (67.4%) followed by III (13.4%), IVa (12.4%), and IV (6.3%). Only one SCCme type IVa isolate carried pvl. The prevalence of agr dysfunction and the MICs of vancomycin were significantly lower in SCCme IV/IVa MRSA than SCCme I–III MRSA (P<0.05, each; Table 1). Hetero-VISA was observed only in SCCme I–III MRSA clones (Table 1). SCCme type IV/IVa isolates presented lower resistance rates to non-β-lactam antibiotic agents (P<0.05, each; Table 1).

Clinical Features and Outcome of SCCme IV/IVa MRSA as Compared with SCCme I–III MRSA

The clinical features of SCCme IV/IVa MRSA and SCCme I–III MRSA are shown in Table 2. SCCme IV/IVa MRSA was significantly more associated with community-acquired and community-onset infection than SCCme I–III MRSA (P<0.05, each). Skin and soft-tissue infections (SSTIs) were significantly more common; however, vascular catheter-related infection was significantly less common in SCCme IV/IVa MRSA than SCCme I–III MRSA compared with SCCme I–III MRSA (P<0.05, each). Metastatic infection was more commonly observed in SCCme IV/IVa MRSA than in SCCme I–III MRSA (P<0.05). However, APACHE II score did not differ statistically between two groups (P=0.729). The use of glycopeptides as a definitive therapy of MRSA was more common in SCCme I–III MRSA than SCCme IV/IVa MRSA (P=0.004).

Outcomes of SCCme IV/IVa MRSA Compared with SCCme I–III MRSA

Univariate and multivariate analysis for risk factors associated with 30-day mortality in patients with MRSA are shown in Table 3. In the univariate analysis, age, cancer, chronic obstructive lung disease, and APACHE II score were all significantly associated with increased mortality; but eradication of infection foci was negatively related to 30-day mortality (P<0.05, each). Increased vancomycin MIC (2 μg/mL), hetero-VISA, and agr dysfunction were not associated with increased 30-day mortality in the univariate analysis. In the multivariate analysis, cancer and APACHE II scores were independent risk factors for 30-day mortality, and the eradication of infective foci was negatively related to 30-day mortality in patients with MRSA.

Thirty-day crude and 30-day *S. aureus*-related mortalities were not significantly different between patients with SCCme IV/IVa MRSA and those with SCCme I–III MRSA (Table 2, Fig. 1). Thirty-day crude and 30-day *S. aureus*-related mortalities also did not differ between patients with SCCme IV/IVa MRSA and SCCme I–III MRSA in multivariate analyses, despite
adjustment of independent risk factors using a Cox-regression model (Fig. 1).

Discussion

In the present study, we found that SCCmec IV/IVa MRSA were associated with low rates of agr dysfunction, compared with SCCmec I–III MRSA. However, outcomes of SCCmec IV/IVa MRSA were not different from those of SCCmec I–III MRSA.

Although agr dysfunction was suggested as contributing to increased mortality related to S. aureus bacteremia, little is known of the prevalence in CA-MRSA clones possessing SCCmec type IV/IVa as compared with HA-MRSA clones possessing SCCmec type I–III. In our previous study, the frequency of agr dysfunction in MSSA blood isolates was 14% and this rate was significantly lower than that in MRSA isolates [25]. In this study, we found similar results; the prevalence of agr dysfunction was significantly lower in SCCmec IV/IVa MRSA than SCCmec I–III MRSA. SCCmec IV/IVa MRSA clones were more similar to MSSA than SCCmec I–III MRSA clones in terms of the prevalence of agr dysfunction.

Previous studies demonstrated a limited vancomycin resistance potential in SCCmec IV/IVa MRSA clones [26,27]. However, recently, a SCCmec IV/IVa MRSA clone with an hetero-VISA or VISA phenotype was described [28–30], suggesting that hetero-VISA is not limited to typical “hospital” clones of S. aureus. Han et al. [31] recently showed that the reduced vancomycin susceptibility was lower in SCCmec IV MRSA blood isolates than SCCmec II MRSA isolates, in concordance with the current study. However, the prevalence of hetero-VISA and agr dysfunction of SCCmec IV MRSA isolates were not directly compared with those of SCCmec II MRSA isolates in that study. In this study, the hetero-VISA phenotype developed only in SCCmec I–III MRSA and vancomycin MICs were significantly lower in SCCmec IV/IVa MRSA. Our data suggest that although hetero-VISA or MRSA with vancomycin MIC = 2 µg/mL can be found in all MRSA lineages, their prevalence was still significantly lower in SCCmec IV/IVa MRSA.

In this study, SSTI was significantly more common; however, vascular catheter-related infection was significantly less common in SCCmec IV/IVa MRSA compared with SCCmec I–III MRSA. Some investigators have shown that the agr system and α-hemolysin play essential roles in pathogenesis of S. aureus SSTI [32,33] in animal models. However, these roles have not been evaluated in human diseases. Our observational clinical findings regarding the association between SCCmec IV/IVa MRSA, which expresses agr and α-hemolysin, with SSTI in human disease consistently provide further evidence of the important role of the agr system and α-hemolysin in the pathogenesis of S. aureus SSTI. Although agr positively regulates cytotoxins and enzymes, it negatively regulates the biofilm-producing ability of S. aureus [2,34] and biofilm-producing ability of agr-dysfunctional MRSA blood isolates are higher compared to agr-functional MRSA blood isolates in our previous study [25]. SCCmec I–III MRSA showing high rate of agr dysfunction was a more common cause of catheter-related infection than SCCmec IV/IVa MRSA in this study. These findings suggest that the higher biofilm-producing ability of agr-dysfunctional MRSA might contribute to catheter-colonization and subsequent catheter-related infections, compared to agr-functional MRSA.

The outcomes of MRSA bacteremia are poorer than those of MSSA bacteremia [35]. However, studies on the outcomes of SCCmec IV/IVa MRSA as compared with SCCmec I–III MRSA show conflicting results. Chen et al. reported that mortalities in patients with SCCmec IV/IVa MRSA were significantly lower in SCCmec I–III MRSA [9]. However, these results were derived only from selected patients (those with community-onset bacteremia in the emergency department) and used 90-day mortality (instead of the more commonly applied 30-day mortality) as an outcome measure, which can be more affected by underlying conditions than S. aureus bacteremia itself. Note that in another study performed by the same group, the 14- and 30-day mortalities were not significantly different between patients with nosocomial SCCmec IV/IVa MRSA and SCCmec I–III MRSA [14], as well as data from the current study and those of another group [10–12].
We initially hypothesized that SCC\textit{mec} IV/IVa MRSAB was associated with better outcomes than SCC\textit{mec} I–III MRSAB because we thought SCC\textit{mec} IV/IVa MRSA might be associated with lower rates of \textit{agr} dysfunction and hetero-VISA phenotype and decreased vancomycin MICs than SCC\textit{mec} I–III MRSAB clones. A recent study suggested that \textit{agr} dysfunction was associated with higher mortality in MRSA bacteremia [8], and some data show an association between vancomycin MICs and the hetero-VISA phenotype and higher mortality rates [36–38]. However, in this study, the mortality rate in patients with SCC\textit{mec} IV/IVa MRSAB was not different from that in patients with SCC\textit{mec} I–III MRSAB, even though SCC\textit{mec} IV/IVa MRSA clones had lower rates of \textit{agr} dysfunction, hetero-VISA, and lower vancomycin MICs. In this study, \textit{agr} dysfunction was not associated with increased mortality in MRSA bacteremia, in contrast to a previous report [8]. Neither vancomycin MICs nor the hetero-VISA phenotype was associated with higher mortality rates in this study, in agreement with previous reports [39–46].

Two possible explanations exist for this result. One is that \textit{agr} dysfunction, vancomycin MICs, and the hetero-VISA phenotype did not themselves adversely influence the outcome of MRSA bacteremia in vivo. The second is that the virulence attenuation caused by \textit{agr} dysfunction might compromise the adverse influence on mortality of decreased sensitivity to glycopeptides in patients with MRSA bacteremia. Peleg \textit{et al.} showed that in MRSA with \textit{agr} dysfunction that had developed increased vancomycin MIC and the hetero-VISA/VISA phenotype, virulence toward \textit{Galleria mellonella} was attenuated [47]. This latter hypothesis might be

### Table 2. Clinical features of 307 patients with SCC\textit{mec} IV/IVa MRSAB or SCC\textit{mec} I–III MRSAB.

| Characteristics                          | No. (%) of patients with | \(P\) value |
|------------------------------------------|--------------------------|-------------|
|                                          | SCC\textit{mec} IV/IVa MRSAB (n = 58) | SCC\textit{mec} I–III MRSAB (n = 249) |
| Age\textsuperscript{a}                  | 62.0 ± 15.1             | 59.5 ± 15.6 | 0.280 |
| Acquisition                              |                          |             |       |
| Community-onset                          | 17 (30)                  | 31 (12)     | 0.001\textsuperscript{b} |
| Community-acquired                       | 5 (9)                    | 6 (2)       | 0.038\textsuperscript{b} |
| Underlying disorder                      |                          |             |       |
| Diabetes                                 | 21 (36)                  | 76 (31)     | 0.402 |
| Cancer                                   | 16 (28)                  | 29 (12)     | 0.002\textsuperscript{b} |
| Cerebrovascular accident                 | 8 (14)                   | 57 (23)     | 0.127 |
| Liver cirrhosis                          | 6 (10)                   | 23 (9)      | 0.795 |
| Congestive heart failure                 | 6 (10)                   | 24 (10)     | 0.873 |
| Renal replacement therapy                | 5 (9)                    | 23 (9)      | 0.883 |
| Chronic obstructive lung disease         | 2 (3)                    | 16 (6)      | 0.542 |
| APACHE II score\textsuperscript{a}      | 19.5 ± 10.4              | 19.9 ± 8.9  | 0.729 |
| Primary site of infection                |                          |             |       |
| Skin and soft tissue                     | 22 (38)                  | 43 (17)     | 0.001\textsuperscript{b} |
| Bone and joint                           | 8 (14)                   | 18 (7)      | 0.118 |
| Intravascular catheter                   | 10 (17)                  | 91 (37)     | 0.005\textsuperscript{b} |
| Lung                                     | 6 (10)                   | 23 (9)      | 0.795 |
| Intra-abdominal                          | 2 (3)                    | 21 (8)      | 0.271 |
| Complicated bacteremia                   |                          |             |       |
| Infective endocarditis                   | 0 (0)                    | 2 (1)       | >0.999 |
| Persistent bacteremia                    | 9 (16)                   | 19 (8)      | 0.060 |
| Metastatic infection                     | 11 (19)                  | 6 (2)       | <0.001\textsuperscript{b} |
| Therapy                                  |                          |             |       |
| Adequate empirical antibiotics within 48 h | 26 (45)                  | 91 (37)     | 0.242 |
| Glycopeptides as definitive antibiotics   | 35 (60)                  | 196 (79)    | 0.004\textsuperscript{b} |
| Eradication of infection foci            | 21 (36)                  | 96 (39)     | 0.740 |
| Outcomes                                 |                          |             |       |
| 30-day crude mortality\textsuperscript{c} | 20/58 (35)               | 78/245 (32) | 0.698 |
| 30-day \textit{S. aureus}-related mortality\textsuperscript{c} | 18/58 (31)               | 58/245 (24) | 0.245 |

\textbf{NOTE.} SCC\textit{mec} IV/IVa MRSAB, bacteremia caused by MRSA possessing SCC\textit{mec} type IV or IVa; SCC\textit{mec} I–III MRSAB, bacteremia caused by MRSA possessing SCC\textit{mec} types I–III; APACHE, acute physiology and chronic health evaluation.

\textsuperscript{a}Continuous variables are expressed as means (±SD).

\textsuperscript{b}Statistically significant \(P≤0.05\).

\textsuperscript{c}Expressed as number of deaths/number of patients followed up (%).
supported by the findings of other clinical studies: the paradoxical relationship between increased vancomycin MIC and the decreased mortality and septic shock rates in MRSA bacteremia [37,41,44], and the similar outcomes of SCCmec IV/IVA MRSAB and SCCmec I–III MRSAB, despite the high prevalence of both complicated (this study) and severe infections in SCCmec IV/IVA MRSAB [10,11].

Our study has some limitations. First, only one MRSA isolate included in this study possessed pvl. For this reason, our results are limited to pvl-negative SCCmec IV/IVA MRSA clones. Further investigation is needed, including more common SCCmec IV/IVA MRSA clones such as US300. Second, serum glycopeptide levels could affect the outcomes of SAB and act as a confounding factor, but these values were not included in the analysis because serum vancomycin levels were not measured in all patients. Third, because only one isolate per patient was examined, there is some possibility that the results may not reflect the agr status of all the bloodstream MRSA population but only reflect the predominant population within each patient. Fourth, only agr status, not the

| Table 3. Univariate and Multivariate analyses for risk factors associated with 30-day mortality in patients with MRSA bacteremia. |
|-----------------|-----------------|-----------------|-----------------|
| Risk Factor            | Univariate analysis | Multivariate analysis |
|                        | No.(%) of patients | p-value | HR | 95% CI | p-value |
|-----------------|
| Survival (n = 209) | Death (n = 98) |
|-----------------|-----------------|-----------------|-----------------|
| Agea            | 58.6±16.0       | 63.1±14.1       | 0.017b         |
| Acquisition     |                 |                 |                 |
| Hospital-onset  | 178 (85)        | 81 (83)         | 0.572          |
| Health care-acquired | 203 (97)    | 93 (95)         | 0.327          |
| Underlying diseases |                 |                 |                 |
| Diabetes        | 63 (30)         | 34 (35)         | 0.424          |
| Cancer          | 24 (12)         | 21 (21)         | 0.022b         |
| Liver cirrhosis | 19 (9)          | 10 (10)         | 0.756          |
| Renal replacement therapy | 20 (10) | 8 (8)         | 0.690          |
| Congestive heart failure | 18 (9)   | 12 (12)         | 0.324          |
| Cerebrovascular accident | 44 (21) | 21 (21)         | 0.940          |
| Chronic obstructive lung disease | 7 (3)   | 11 (11)         | 0.006b         |
| Primary site of infection |                 |                 |                 |
| Skin and soft tissue | 46 (22)    | 19 (19)         | 0.600          |
| Bone and joint   | 19 (9)          | 7 (7)           | 0.568          |
| Lung             | 16 (8)          | 13 (13)         | 0.117          |
| Intravascular catheter-related | 74 (35) | 27 (28)         | 0.172          |
| Intra-abdominal infection | 18 (0) | 5 (5)           | 0.276          |
| Primary bacteremia | 38 (18)    | 27 (28)         | 0.061          |
| Complicated bacteremia |                 |                 |                 |
| Infective endocarditis | 1 (0)     | 1 (1)           | 0.537          |
| Other metastatic infection | 9 (4)    | 8 (8)           | 0.168          |
| Persistent bacteremia | 16 (8)    | 12 (12)         | 0.193          |
| APACHE II scorea | 16.5±6.9       | 27.0±9.3        | <0.001b        |
| Treatment         |                 |                 |                 |
| Adequate antibiotics within 48 hours | 81 (39)  | 36 (37)         | 0.734          |
| Glycopeptides as definitive antibiotics | 163 (78) | 68 (69)        | 0.104          |
| Eradication of infection foci | 95 (46) | 22 (22)        | <0.001b        |
| Microbiological characteristics |                 |                 |                 |
| SCCmec IV/IVA MRSA | 38 (18) | 20 (20)         | 0.642          |
| hetero-VISA       | 13 (6)          | 6 (6)           | 0.974          |
| agr dysfunction   | 72 (34)         | 36 (37)         | 0.696          |
| Vancomycin MIC = 2 μg/mL | 27 (13) | 12 (12)         | 0.869          |

NOTE. HR, hazard ratio; CI, confidence interval; APACHE, acute physiology and chronic health evaluation; SCCmec IV/IVA MRSA, MRSA possessing SCCmec type IV or IVA; hetero-VISA, hetero-vancomycin-intermediate S. aureus; MIC, minimum inhibitory concentration.

aContinuous variables are expressed as means (±SD).
bStatistically significant (P<0.05).
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overall virulence gene expression of the individual strains, was
examined in this study.

In conclusion, the rates of agr dysfunction, hetero-VISA
phenotype, and increased vancomycin MICs were lower in
SCCmec IV/IVa MRSAB than in SCCmec I–III MRSAB in this
study. However, the outcomes of SCCmec IV/IVa MRSAB did
not differ from those of SCCmec I–III MRSAB.

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Author Contributions

Conceived and designed the experiments: HCJ. Performed the experi-
ments: HCJ SJK SMC. Analyzed the data: HCJ SJK. Contributed
reagents/materials/analysis tools: KHP JHS HEC SIJ HBK. Wrote the
paper: HCJ SJK SIJ.

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