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Clinical and Molecular Characteristics of *qacA*- and *qacB*-Positive Methicillin-Resistant *Staphylococcus aureus* Causing Bloodstream Infections

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ABSTRACT The increasing use of chlorhexidine for methicillin-resistant *Staphylococcus aureus* (MRSA) decolonization has raised concerns about the emergence of resistance to these agents. However, the clinical significance of MRSA positive for the *qacA* and *qacB* chlorhexidine tolerance genes has not been established. We investigated the clinical features and predictive factors of MRSA bloodstream infection (BSI) isolates, caused by *qacA*- and *qacB*-positive MRSA, from 2010 to 2016 at a tertiary hospital in South Korea. A total of 246 MRSA BSI isolates were included; 71 (28.9%) isolates carried *qacA/B*. The annual frequency of *qacA*- and *qacB*-positive MRSA bacteremia did not change significantly over the study period. Patients infected with *qacA*- and *qacB*-positive MRSA had common risk factors for health care-associated infections, including prior antibiotic use, central venous catheterization *in situ*, intensive care unit-acquired bacteremia, and nosocomial infection. The *qacA*- and *qacB*-positive isolates were also associated with an increasing chlorhexidine MIC and resistance to non-β-lactam antibiotics. The *qacA*- and *qacB*-positive isolates were more likely to belong to sequence type 5 (ST5), which is a common health care-associated MRSA strain in South Korea. In multivariable analyses, *qacA*- and *qacB*-positive MRSA isolates were found to be associated with *agr* dysfunction (adjusted odds ratio [aOR], 6.45; 95% confidence interval [CI], 2.59 to 16.10), ST5 MRSA strain (aOR, 4.96; 95% CI, 1.85 to 13.26), nosocomial infection (aOR, 4.88; 95% CI, 2.20 to 10.83), and antibiotic use within the previous 3 months (aOR, 2.59; 95% CI, 1.20 to 5.59). These findings suggest that the microbiological features of *qacA* and *qacB* carriage provide a selective advantage for specific MRSA strains in hospital environments.

KEYWORDS *Staphylococcus aureus*, chlorhexidine, *qacA*, *qacB*

Methicillin-resistant *Staphylococcus aureus* (MRSA) has long been a major cause of health care-associated infections (HAIs), and it is associated with increased morbidity, mortality, and excess hospital costs (1–3). Chlorhexidine gluconate (CHG) is widely applied to minimize MRSA transmission between hospitalized patients as a part of infection control programs, being used in central venous catheter dressings and CHG-based body washes (4, 5). However, the widespread use of these topical antimicrobials has raised concerns regarding the emergence of MRSA strains resistant or tolerant to CHG (6). In *S. aureus*, resistance to or tolerance of chlorhexidine is associated with the *qacA* and *qacB* genes, which encode multidrug efflux pumps (7, 8). Moreover,

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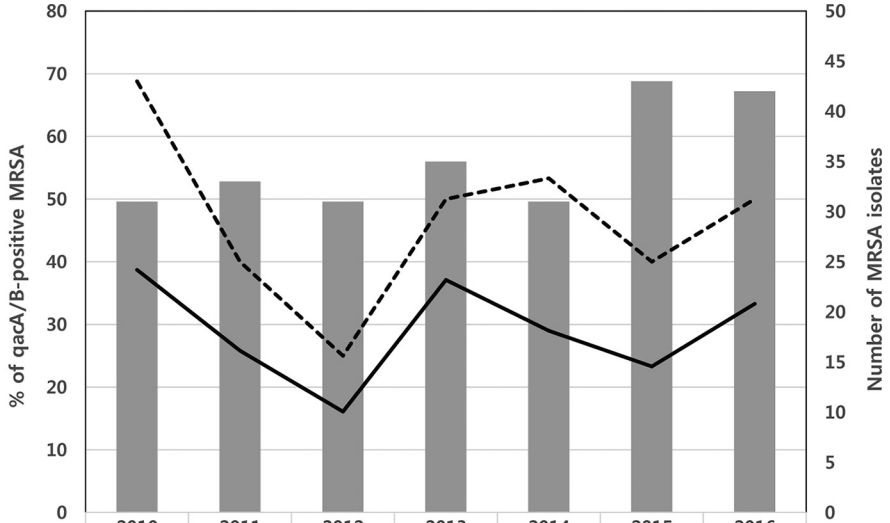

	2010	2011	2012	2013	2014	2015	2016
No. of MRSA	31	33	31	35	31	43	42
qacA/B_overall (%)	38.7	25.8	16.1	37.1	29	23.3	33.3
qacA/B_nosocomial (%)	68.8	40.0	25.0	50.0	53.3	40.0	50.0

FIG 1 Annual frequency of *qacA*- and *qacB*-positive isolates in overall and nosocomial MRSA bloodstream infection isolates that were available for microbiological tests.

some plasmids harboring the *qacA* and *qacB* genes may cotransmit with other antibiotic resistance genes as a result of selective pressure in certain *S. aureus* strains (9).

The clinical implications of *qacA*- and *qacB*-positive MRSA have not been established. A large clinical trial of CHG-based decolonization for the control of MRSA revealed the prevalence of *qacA* and *qacB* genes to be low and the impact of chlorhexidine tolerance on decolonization failure to be minimal (5, 10). However, some studies found that *qacA* and *qacB* carriage in MRSA was not uncommon in hospital environments, and the prevalence of certain MRSA clones, such as CC22 or ST239 (where CC stands for clonal complex and ST stands for sequence type), increased after the implementation of a chlorhexidine-based decolonization (11, 12). Another study reported that *qacA* and *qacB* genes in combination with low-level mupirocin resistance was related to failure of MRSA decolonization (13). Although a recent study reported that health care exposure, specifically nosocomial *S. aureus* acquisition and underlying medical conditions, was associated with chlorhexidine tolerance genes, factors predictive of the presence of *qacA* and *qacB* genes have not been well described (14). In the present study, we investigated the molecular epidemiology of *qacA*- and *qacB*-positive MRSA in bloodstream infection (BSI) isolates, and we describe the clinical significance of these isolates.

(These data were presented in part at IDWeek 2017 in San Diego, CA [15].)

RESULTS

Frequency of chlorhexidine tolerance genes in MRSA BSI isolates. Of 246 MRSA BSI isolates, 77 (31.3%) possessed one or both chlorhexidine tolerance genes. Seventy-one isolates (28.9%) were positive for *qacA* and *qacB*, and seven (2.8%) were positive for *smr*, including one isolate (0.4%) that was positive for all three genes. The annual frequencies of *qacA*- and *qacB*-positive isolates of the overall and nosocomial MRSA BSIs did not change significantly during the 7-year study period ($P = 0.808$ and $P = 0.578$, respectively) (Fig. 1). Most BSIs caused by *qacA*- and *qacB*-positive MRSA were nosocomial (78.9%) or community-onset health care-associated (CO-HCA) (18.3%) infections. Two cases (2.8%) were community acquired.

Clinical and microbiological characteristics of patients infected with *qacA*- and *qacB*-positive MRSA BSI isolates. The clinical characteristics of 246 patients with

TABLE 1 Comparison of clinical characteristics of patients with MRSA bacteremia based on *qacA* and *qacB* status

Variable[a]	Value(s) by *qacA* and *qacB* status		*P* value
	Positive ($n = 71$)	Negative ($n = 175$)	
Age, median (IQR) yr	72 (61.5–79.5)	69 (57–75)	0.043
Male gender	45 (63.4)	106 (60.6)	0.773
Mode of acquisition			
Community onset	15 (21.1)	109 (62.3)	<0.001
Community acquired	2 (2.8)	42 (24.0)	<0.001
Healthcare associated	13 (18.3)	67 (38.3)	0.003
Nosocomial	56 (78.9)	66 (37.7)	<0.001
Comorbidity			
Diabetes mellitus	25 (35.2)	77 (44.0)	0.253
Solid cancer	10 (14.1)	27 (15.4)	0.847
Hematologic malignancy	1 (1.4)	7 (4.0)	0.444
Liver cirrhosis	6 (8.5)	23 (13.1)	0.385
End-stage renal disease	8 (11.3)	20 (11.4)	0.999
Chronic pulmonary disease	6 (8.5)	10 (5.7)	0.408
Charlson comorbidity score, median (IQR)	4 (3 – 5)	4 (3 – 6)	0.554
Polymicrobial BSI	8 (11.3)	15 (8.6)	0.481
Hospitalization in the prior 1 yr	30 (42.3)	86 (49.1)	0.398
Surgery in the prior 3 months	20 (28.2)	29 (16.6)	0.052
Antibiotic use within the previous 3 months	49 (69.0)	72 (41.1)	<0.001
Central venous catheter in place	49 (69.0)	55 (31.4)	<0.001
Chlorhexidine-impregnated catheter[b]	24/46 (52.2)	25/54 (46.3)	0.688
ICU-acquired bacteremia	20 (28.2)	14 (8.0)	<0.001
Primary site of infection			
Central venous catheter-related infection	45 (63.4)	49 (28.0)	<0.001
Pneumonia	3 (4.2)	14 (8.0)	0.409
Surgical wound infection	4 (5.6)	5 (2.9)	0.285
Infective endocarditis	0	8 (4.6)	0.109
Soft-tissue, bone, and joint infection	5 (7.0)	43 (24.6)	0.001
Unknown	15 (21.1)	40 (22.9)	0.866
Others	2 (2.8)	15 (8.6)	0.164
Septic shock on bacteremia presentation	16 (22.5)	36 (20.6)	0.733
Median length of hospital stay after bacteremia, days (IQR)	24 (9–45.5)	20 (10–34.5)	0.265
30-Day mortality	19 (26.8)	54 (30.9)	0.543
In-hospital mortality	25 (35.2)	56 (32.0)	0.655

[a]Data are no. (%) of patients unless otherwise indicated.
[b]Among 104 patients with central venous catheters, four were excluded because of a lack of information on the catheter type.

MRSA BSIs are shown in Table 1. The most common cause of infection was central venous catheter infection (38.2%), followed by unknown primary bacteremia (22.4%) and soft-tissue, bone, and joint infection (19.5%). There were no significant differences in sex, underlying diseases, Charlson comorbidity score, or hospitalization within the previous year between the *qacA*- and *qacB*-positive and *qacA*- and *qacB*-negative MRSA isolates. However, *qacA*- and *qacB*-positive isolates were more likely to be associated with older age (72 versus 69 years; $P = 0.043$), antibiotic use within the past 3 months (69.0% versus 41.1%; $P < 0.001$), central venous catheter *in situ* (69.0% versus 31.4%; $P < 0.001$), and intensive care unit (ICU)-acquired bacteremia (28.2% versus 8.0%; $P < 0.001$). Patients with *qacA*- and *qacB*-positive isolates also had more central venous catheter-related infections (63.4% versus 28.0%; $P < 0.001$) but fewer soft-tissue, bone, and joint infections (7.0% versus 24.6%; $P = 0.001$) than did patients with *qacA*- and *qacB*-negative isolates. However, there were no significant differences in the incidence of septic shock on bacteremia presentation, the length of hospital stay after bacteremia, and mortality between the two groups.

The microbiological characteristics of the MRSA BSI isolates based on *qacA* and *qacB* status are shown in Table 2. The *qacA*- and *qacB*-positive MRSA isolates were significantly associated with higher chlorhexidine MICs (median, 4 versus 2 μg/ml; range, 2 to

TABLE 2 Microbiological characteristics of MRSA bloodstream infection isolates based on *qacA* and *qacB* status

Variable[a]	Value(s) by *qacA* and *qacB* status		*P* value
	Positive ($n = 71$)	Negative ($n = 175$)	
Chlorhexidine MIC (BMD)			<0.001
≤2 mg/liter	4 (5.6)	120 (68.6)	
4 mg/liter	64 (90.1)	44 (25.1)	
≥8 mg/liter	3 (4.2)	11 (6.3)	
Vancomycin MIC of ≥2 mg/liter (BMD)	18 (25.4)	26 (14.9)	0.066
Carriage of *smr*	1 (1.4)	6 (3.4)	0.677
Dysfunctional *agr*	60 (84.5)	45 (25.7)	<0.001
Resistance to			
Mupirocin	9 (12.7)	7 (4.0)	0.020
Clindamycin	70 (98.6)	113 (64.6)	<0.001
Ciprofloxacin	70 (98.6)	70 (40.0)	<0.001
Tetracycline	68 (95.8)	52 (29.7)	<0.001
Erythromycin	70 (98.6)	113 (64.6)	<0.001
Fusidic acid	63 (88.7)	29 (16.6)	<0.001
Gentamicin	67 (94.4)	47 (26.9)	<0.001
Rifampin	3 (4.2)	2 (1.1)	0.146
Trimethoprim/sulfamethoxazole	5 (7.0)	5 (2.9)	0.158
Multidrug resistance[b]	70 (98.6)	72 (41.1)	<0.001

[a]Data are no. (%) of patients unless otherwise indicated.
[b]Isolates were considered multidrug resistant if they were resistant to three or more different classes of non-β-lactam antimicrobials.

8 versus 1 to 32 μg/ml) ($P < 0.001$) and *agr* dysfunction (84.5% versus 25.7%; $P < 0.001$), and they were more often resistant to several non-β-lactam antibiotics, including mupirocin, clindamycin, ciprofloxacin, tetracycline, erythromycin, fusidic acid, and gentamicin. In addition, there was a significant association between *agr* dysfunction and higher chlorhexidine MICs in overall MRSA isolates (median, 4 versus 2 μg/ml; range, 1 to 16 versus 1 to 32 μg/ml) ($P < 0.001$). However, this association was not evident when the MRSA isolates were stratified by *qacA* and *qacB* status (see Fig. S1 in the supplemental material).

Genotypic characteristics of *qacA*- and *qacB*-positive MRSA BSIs. Table 3 shows the genotypic characteristics of MRSA BSIs based on *qacA* and *qacB* status. The *qacA*- and *qacB*-positive isolates were more likely to belong to the staphylococcal cassette chromosome *mec* II (SCC*mec* II) or SCC*mec* III groups, which are common health care-associated MRSA strains in South Korea, than the SCC*mec* IV group (97.1% versus 2.8%; $P < 0.001$). ST5 (88.7%) was the most common sequence type in the *qacA*- and *qacB*-positive MRSA isolates. The dominant *spa* types in the *qacA*- and *qacB*-positive isolates were t2460 (71.8%) and t9353 (11.3%).

Factors associated with the presence of *qacA* and *qacB* genes. When the clinical and microbiological factors that were significantly associated with the presence of *qacA* and *qacB* genes in univariate analyses were included in a multiple logistic regression analysis, *qacA*- and *qacB*-positive MRSA isolates were independently associated with *agr* dysfunction (adjusted odds ratio [aOR], 6.45; 95% confidence interval [CI], 2.59 to 16.10), ST5 MRSA strain (aOR, 4.96; 95% CI, 1.85 to 13.26), nosocomial infection (aOR, 4.88; 95% CI, 2.20 to 10.83), and antibiotic use within the previous 3 months (aOR, 2.59; 95% CI, 1.20 to 5.59) (Table 4).

Because of the high proportion of ST5 in *qacA*- and *qacB*-positive MRSA isolates, we further assessed the relationship between the specific ST of MRSA and two other important risk factors for the presence of *qacA* and *qacB* genes: *agr* dysfunction and nosocomial infection (Tables S1 and S2). When the study group was classified into ST5 and non-ST5 MRSA, *qacA* and *qacB* carriage was significantly associated with *agr* dysfunction in ST5 (93.7% versus 57.1%: $P < 0.001$) but not in non-ST5 MRSA (12.5% versus 13.5%; $P = 0.999$). There was also a significant association between the presence

TABLE 3 Genotypic characteristics of MRSA bloodstream infection isolates based on *qacA* and *qacB* status

Variable[a]	Value(s) by *qacA* and *qacB* status		*P* value
	Positive (*n* = 71)	Negative (*n* = 175)	
SCC*mec* type			<0.001
II	65 (91.5)	59 (33.7)	
III	4 (5.6)	4(2.3)	
IV	2 (2.8)	112 (64.0)	
MLST			<0.001
ST5	63 (88.7)	49 (28.0)	
ST72	1 (1.4)	104 (59.4)	
Other[b]	7 (9.9)	22 (12.6)	
spa type			<0.001
t2460	51 (71.8)	15 (8.5)	
t664	0	56 (32.0)	
t324	0	29 (16.6)	
t002	1 (1.4)	26 (14.9)	
Other[c]	19 (26.8)	49 (28.0)	

[a]Data are no. (%) of patients unless otherwise indicated.
[b]The other MLSTs were ST239 (*n* = 5), ST518 (*n* = 1), and nontypeable (*n* = 1) in the *qac*-positive group and ST188 (*n* = 4), ST89 (*n* = 3), ST8 (*n* = 2), ST239 (*n* = 2), ST2084 (*n* = 1), and nontypeable (*n* = 10) in the *qac*-negative group.
[c]The other *spa* types were t9353 (*n* = 8), t037 (*n* = 3), t111 (*n* = 1), t264 (*n* = 1), t2029 (*n* = 1), t2139 (*n* = 1), t2703 (*n* = 1), and nontypeable (*n* = 2) in the *qac*-positive group and t148 (*n* = 5), t189 (*n* = 3), t375 (*n* = 3), t1368 (*n* = 3), t901 (*n* = 2), t4359 (*n* = 2), t9353 (*n* = 2), t008 (*n* = 1), t037 (*n* = 1), t045 (*n* = 1), t126 (*n* = 1), t242 (*n* = 1), t535 (*n* = 1), t601 (*n* = 1), t1767 (*n* = 1), t2431 (*n* = 1), t2461 (*n* = 1), t2882 (*n* = 1), t4331 (*n* = 1), t4705 (*n* = 1), t5071 (*n* = 1), t5229 (*n* = 1), t5440 (*n* = 1), t5716 (*n* = 1), t12703 (*n* = 1), and nontypeable (*n* = 8) in the *qac*-negative group.

of *qacA* and *qacB* genes and *agr* dysfunction in both nosocomial (83.9% versus 27.3%; $P < 0.001$) and community-onset MRSA infections (86.7% versus 24.8%; $P < 0.001$).

DISCUSSION

Although numerous studies have investigated the prevalence and clinical implications of the presence of *qacA* and *qacB* in *S. aureus*, few have addressed those issues in the context of bloodstream MRSA isolates (11, 16). Of about 250 samples of MRSA BSI isolates, we found that *qacA*- and *qacB*-positive MRSA isolates were not uncommon (28.9%) and were related to the ST5 strain, which is the most common health care-associated MRSA clone in South Korea (17). Although this study was conducted over a relatively short period, the frequency of *qacA*- and *qacB*-positive MRSA did not change significantly over 7 years. A study of MRSA isolates collected from 11 Asian countries between 1998 and 1999 showed that, of the isolates collected from South Korea, 32.4% (34/105) carried the *qacA* and *qacB* genes and 0% the *smr* genes (18). These findings imply that genotypic antiseptic tolerance is widely distributed in South Korea, despite

TABLE 4 Univariate and multivariable analyses of risk factors associated with the presence of *qacA* and *qacB* genes in MRSA bloodstream infection isolates

Variable	Univariate analysis		Multivariable analysis	
	OR (95% CI)	*P* value	aOR (95% CI)	*P* value
Increased age per 10 yr	1.17 (0.86–1.60)	0.320		
Surgery in the prior 3 mo	1.51 (0.54–4.25)	0.432		
Central venous catheter in place	1.17 (0.45–3.02)	0.746		
ICU-acquired bacteremia	1.34 (0.48–3.74)	0.579		
Soft-tissue, bone, and joint infection	0.95 (0.22–4.09)	0.949		
Vancomycin MIC of ≥2 μg/ml	1.13 (0.39–3.30)	0.823		
Nosocomial infection	3.88 (1.43–10.54)	0.008	4.88 (2.20–10.83)	<0.001
Antibiotic use within the previous 3 mo	2.43 (1.06–5.56)	0.036	2.59 (1.20–5.59)	0.016
Dysfunctional *agr*	6.41 (2.48–16.57)	<0.001	6.45 (2.59–16.10)	<0.001
MLST ST5	4.64 (1.70–12.67)	0.003	4.96 (1.85–13.26)	0.001

the lack of a nationwide longitudinal study reporting the prevalence of *qacA* and *qacB* genes in *S. aureus*.

The clonal association of *qacA* and *qacB* in MRSA has been controversial. Two UK studies reported an increasing prevalence of CC22 and ST239 clones during the implementation of the CHG-bathing protocol (11, 19), and some Asian studies have revealed a high frequency of *qacA* and *qacB* among the ST239, ST5, and ST241 clones (12, 20). Meanwhile, U.S. studies did not demonstrate this clonal predominance (10, 21). Our previous study, performed at a surgical ICU, revealed that most *qacA*- and *qacB*-positive MRSA isolates were identified as ST5-SCC*mec* II (69.2%) and ST239-SCC*mce* III (23.1%), which are common healthcare-associated MRSA strains in South Korea (22). This clonal association between the *qacA* and *qacB* genes and the ST5 clone was also demonstrated in this study. However, no association between *qacA* and *qacB* and ST239 was evident, because the number of ST239 MRSA BSIs was too low to be analyzed statistically.

In the present study, patients infected with *qacA*- and *qacB*-positive MRSA had common risk factors for health care-associated infections, including prior antibiotic use, central venous catheter *in situ*, ICU-acquired bacteremia, and nosocomial infection. These findings are consistent with previous studies revealing an association between the presence of *qacA* and *qacB* and health care exposure and provide evidence that the widespread use of CHG in hospital settings selects for genotypic chlorhexidine-tolerant strains that are able to survive in the presence of this antiseptic (14, 19, 23). In addition, as several previous studies have reported, we also found an association between chlorhexidine tolerance genes and resistance to non-β-lactam antibiotics and a higher chlorhexidine MIC (11, 14, 23). Although prior antibiotic use was independently associated with *qacA*- and *qacB*-positive MRSA in the present study, we could not determine whether there was antibiotic pressure on the selection of these microorganisms or whether this finding was simply a reflection of the coexistence of antimicrobial resistance genes on mobile genetic elements (24).

We also found that the presence of *qacA* and *qacB* genes in MRSA BSIs was independently associated with *agr* dysfunction, consistent with our previous study (22). Loss of *agr* function in *S. aureus* has been reported to have certain advantages in hospital environments, in that the strain may be associated with attenuated vancomycin activity, vancomycin heteroresistance, persistent bacteremia, and increased biofilm production (25, 26). Since the concentration of chlorhexidine used in practice (10,000 to 40,000 μg/ml) is much higher than the higher MICs (4 to 16 μg/ml) of some isolates *in vitro*, chlorhexidine tolerance in *S. aureus* is regarded as an insignificant clinical problem (11). However, if MRSA strains with *agr* dysfunction form a biofilm, chlorhexidine tolerance could matter, because bacteria within biofilms can be more resistant or tolerant to this antiseptic than their planktonic counterparts, as reported by Bonez et al. (27, 28). In the present study, we could not draw a conclusion about whether MRSA with *agr* dysfunction was independently associated with higher chlorhexidine MICs, and we could not determine the causal relationship between *qacA* and *qacB* genes and *agr* dysfunction in MRSA. However, our data appear to provide evidence supporting the selection of *qacA*- and *qacB*-positive MRSA strains in hospital environments under chlorhexidine exposure.

Our study had some limitations. First, considering the high proportion of ST5 MRSA isolates in this study, it is possible that the predictive factors of *qacA* and *qacB* genes are merely reflecting the characteristics of the ST5 phenotype itself. Second, since it was conducted on only MRSA isolates in a single institution, our findings should not be generalized to methicillin-susceptible strains or to other hospital settings. Third, we did not perform microbiological tests on MRSA isolates from paired surveillance cultures, so we could not evaluate whether patients colonized with *qacA*- and *qacB*-positive MRSA strains were more vulnerable to BSIs, especially to central venous BSIs, than those with *qacA*- and *qacB*-negative strains under the pressure of chlorhexidine. Fourth, caution should be exercised in interpreting the results of chlorhexidine MICs, because there was no internationally agreed methodology for the detection of reduced chlorhexidine

susceptibility until now (11). Fifth, we did not measure RNA expression of *qacA* and *qacB*, and the presence of these genes does not guarantee phenotypic chlorhexidine tolerance or resistance (24).

In conclusion, the present study demonstrated that *qacA*- and *qacB*-positive isolates were not uncommon among MRSA BSI isolates collected over a 7-year period. The *qacA*- and *qacB*-positive MRSA isolates were independently associated with *agr* dysfunction, nosocomial infection, antibiotic use within the previous 3 months, and the ST5 MRSA strain. Our data support the hypothesis that the microbiological features of *qacA*- and *qacB*-positive MRSA isolates, such as increased resistance to non-β-lactam antibiotics, higher MIC of chlorhexidine, and *agr* dysfunction, provide these strains with an advantage when exposed to chlorhexidine in hospital settings (12, 16, 22).

MATERIALS AND METHODS

Study setting, patients, and MRSA isolates. This study was conducted at Gyeongsang National University Hospital (GNUH), an 890-bed community-based tertiary hospital in Jinju, South Korea, between March 2010 and December 2016. Because an increase in the incidence of MRSA infection in ICUs was noted, a CHG bathing protocol was introduced at a surgical ICU beginning in December 2012 and at a medical ICU beginning in January 2015. Chlorhexidine-impregnated catheters have been used as one type of central venous catheter since 2009. The detailed infection control programs at our institution have been described previously (22).

In total, 365 patients with MRSA bacteremia were identified at GNUH during the 7-year study period. Of these, 246 (67.4%) nonduplicate BSI isolates were available for laboratory tests and were therefore included in this study. Clinical data were reviewed for the patients whose MRSA BSI isolates were available for the tests. We compared the clinical and microbiological characteristics of MRSA isolates between *qacA*- and *qacB*-positive and *qacA*- and *qacB*-negative MRSA bacteremia.

Clinical data and definitions. The following data were retrospectively reviewed for this study: age, sex, mode of acquisition of infection, comorbidity, Charlson comorbidity score, hospitalization within the previous year, antibiotic use within the previous 3 months, central venous catheter in place during the bacteremia, ICU-acquired bacteremia, primary site of bacteremia, septic shock on bacteremia presentation, length of hospital stay after bacteremia, 30-day mortality, and in-hospital mortality. The modes of acquisition of infection were classified as follows (29). Community-onset (CO) infection was defined as an infection diagnosed within 48 h of hospitalization that did not satisfy the criteria for nosocomial infection. CO-HCA infection was considered if any of the following criteria were present: hospitalization for >48 h in the previous 12 months; hemodialysis, intravenous medication, or home wound care in the previous 3 months; or residence in a nursing home or long-term-care facility. Nosocomial infections were those in which the patient developed signs/symptoms of infection ≥72 h after hospital admission. ICU-acquired bacteremia was defined as a bacteremia infection ≥72 h after ICU admission.

Microbiological methods. All *S. aureus* isolates were identified by standard methods. Antimicrobial susceptibility was identified using a Vitek-2 system (bioMérieux, Marcy l'Etoile, France), and the MICs of vancomycin were determined using the broth microdilution (BMD) method according to Clinical and Laboratory Standards Institute guidelines (30). The MICs of chlorhexidine were determined using a broth microdilution method and a 20% (wt/vol) CHG solution (Sigma-Aldrich, St. Louis, MO, USA); the final concentrations of the antiseptic ranged from 0.5 to 32 mg/liter (22). We used δ-hemolysin activity to determine *agr* functionality, as described elsewhere (31).

Detection of the *qacA* and *qacB* genes and *smr* gene was carried out by PCR using previously published primers (18). Three *S. aureus* strains (TS77, TPS162, and L20) were used as positive controls for *qacA, qacB,* and *smr* (Riken BRC, Ibaraki, Japan), respectively. Staphylococcal protein A (*spa*) typing, multilocus sequence typing (MLST), and staphylococcal cassette chromosome *mec* (SCC*mec*) typing were conducted according to previously published methods (32–34).

Statistical analysis. The χ^2 test for trend was used to compare the frequency of *qacA*- and *qacB*-positive MRSA BSI isolates by year during the study period. Continuous and categorical variables were compared using the Mann-Whitney *U* test and Fisher's exact test, respectively. When multivariable logistic regression was performed, the risk factors of *qacA* and *qacB* carriage included in the model were selected according to the following steps. First, the risk factors were chosen based on the *P* value being less than 0.15 in univariate analysis. Second, to identify the multicollinearity problem, the correlation between the risk factors was confirmed using Pearson or Spearman correlation analysis. Finally, a model with a stepwise method was selected for combinations of risk factors included in the multivariable analysis. In this case, to prevent overfitting problems due to insufficient *qacA*- and *qacB*-positive cases, the number of risk factors to be included in the final model was limited to fewer than six. Multivariable logistic regression and model selection were performed by SAS, version 9.4 (SAS Institute, Cary, NC, USA), software. All other analyses were performed using IBM SPSS software (ver. 20.0; IBM Corporation, Armonk, NY, USA). All tests were two tailed, and a *P* value of <0.05 was considered statistically significant. The GNUH Institutional Review Board approved this study.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/AAC.02157-18.

SUPPLEMENTAL FILE 1, PDF file, 0.2 MB.

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