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High Rate of *qacA*- and *qacB*-Positive Methicillin-Resistant *Staphylococcus aureus* Isolates from Chlorhexidine-Impregnated Catheter-Related Bloodstream Infections

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Chlorhexidine has been widely used for infection control. Although the use of chlorhexidine-impregnated catheters has reduced catheter-related infections, chlorhexidine-resistant *Staphylococcus aureus* has emerged. The correlation between the existence of the chlorhexidine-resistant genes *qacA* and *qacB* (*qacA/B*) in methicillin-resistant *Staphylococcus aureus* (MRSA) isolates and the effectiveness of chlorhexidine-impregnated catheters in the prevention of MRSA infections is unknown. Sixty methicillin-sensitive *Staphylococcus aureus* (MSSA) and 96 MRSA isolates from the blood cultures of different patients were collected, and a case-control study was conducted to determine whether more clinical *S. aureus* isolates from chlorhexidine-impregnated catheter-related bloodstream infections (CRBSI) have the biocide-resistant genes (*qacA/B* or *smr*) than those from other infections. The chlorhexidine MIC_{50}s of MSSA and MRSA isolates were 1 μg/ml and 2 μg/ml, respectively. Results of PCR analyses showed that 3.3% ($n = 2$) of MSSA and 43.8% ($n = 42$) of MRSA isolates harbored *qacA/B* and 5% ($n = 3$) of MSSA and 25% ($n = 24$) of MRSA isolates contained *smr*. With multivariate logistic regression analyses, the significant risk factors for definite CRBSI with chlorhexidine-impregnated catheters were determined to be *S. aureus* isolates with *qacA/B* and a chlorhexidine MIC of ≥2 μg/ml (odds ratios [OR], 9.264 and 8.137, respectively, in all 156 *S. aureus* isolates and 6.097 and 4.373, respectively, in the 96 MRSA isolates). Further prospective studies are needed to investigate the transmission of these biocide-resistant genes.

Chlorhexidine gluconate, a water-soluble cationic bisbiguanide, has been widely used as an antiseptic agent since 1954 (12). It destroys the cell membrane and causes coagulation of intracellular contents of a variety of microorganisms, including Gram-positive and Gram-negative bacteria, lipophilic virus, protozoa, and fungus (10). Chlorhexidine has been approved by the U.S. Food and Drug Administration for infection control in various applications, including surgical hand scrub, general skin cleaning, preoperative scrub, central venous catheter site preparation, and vascular catheter dressings (12). The use of chlorhexidine-impregnated vascular catheters has been found to reduce catheter colonization of bacteria and catheter-related infections (14, 20). However, chlorhexidine-resistant bacteria, especially methicillin-resistant *Staphylococcus aureus* (MRSA), have been reported (8, 17, 18). In Taiwan, chlorhexidine gluconate was introduced for clinical use in 1973, and chlorhexidine-resistant MRSA started to emerge in 1990 (19). The correlation between the use of chlorhexidine-impregnated catheters and the existence of chlorhexidine-resistant genes is unknown. In this study, we conducted a case-control study to answer this question by comparing the prevalence of biocide-resistant genes (*qacA* and *qacB* [*qacA/B*] or *smr*) in isolates from chlorhexidine-impregnated catheter-related bloodstream infections (CRBSI) to that in other infections.

MATERIALS AND METHODS

Clinical MSSA and MRSA isolates. Sixty methicillin-sensitive *Staphylococcus aureus* (MSSA) and 96 MRSA isolates were collected from blood cultures of different patients from July 2008 to December 2009. Identification of these clinical isolates was achieved by the Bactec 9000 system (Becton, Dickinson, Sparks, MD), and the susceptibility of each isolate to oxacillin was determined by the BD Phoenix Automated Microbiology System (Becton, Dickinson). The basic and clinical information of each patient was obtained from medical records. Patients with community-acquired MRSA (CA-MRSA) infection were those without histories of surgery, long-term-care facility residence, dialysis, indwelling device or catheter usage within the recent 1 year, or hospitalization for less than 48 h before positive MRSA culture (1). Other MRSA infections were considered hospital acquired (HA-MRSA). The definitions for HA- or CA-MSSA isolates were the same as those for the MRSA isolates. The clinical (age, gender, hospital- or community-acquired, and clinical diagnosis) and molecular (SCC*mec*, *agr*, and *spa*) characteristics of the 96 MRSA isolates had been reported (5). The MIC of chlorhexidine of each isolates was determined by the agar dilution (Mueller-Hinton agar) method developed by the Clinical and Laboratory Standards Institute (4). Chlorhexidine digluconate solution (20%) was purchased from Sigma-Aldrich.

Catheter-related bloodstream infection and antimicrobial-impregnated catheters. The organism which caused catheter-related bloodstream infection (CRBSI) was defined as the same organism isolated from at least 1 percutaneous blood culture and from the catheter tip (>15 CFU per catheter segment) with concomitant clinical manifestations, such as fever, chills, or hypotension (11). The same organism in this study was defined as the *S. aureus* isolate with an identical antimicrogram isolated from the same patient during the same episode of an infection. The isolates not meeting the above-described criteria but suspected as the cause of a catheter-related infection by medical staff were categorized as possible CRBSI. The antimicrobial-impregnated catheters used in this study were

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TABLE 1 Chlorhexidine MIC of 156 *S. aureus* isolates

S. aureus type (no.)	No. of isolates with chlorhexidine MIC in indicated μg/ml (no. of isolates with *qacA/B*)						
	≤0.125	0.25	0.5	1	2	4	≥8
MSSA (60)	0	0	22 (0)	36 (0)	1 (1)	1 (1)	0
MRSA (96)	1 (0)	0	0	39 (16)	26 (11)	30 (15)	0
Total (156)	1 (0)	0	22 (0)	75 (16)	27 (12)	31 (16)	0

the Arrow-Howes Multi-Lumen central venous catheterization set with Blue FlexTip ARROWg^{+}ard blue catheters (Asheboro, NC), which contained chlorhexidine acetate and silver sulfadiazine. Chlorhexidine was also used for infection control interventions or procedures such as hand scrubbing and surgical site skin cleaning in our hospital. During this period, chlorhexidine was not used for the central and peripheral venous catheter site preparations, arterial catheter site preparations, or daily bathing for ICU patients. There was no other catheter containing chlorhexidine.

DNA extraction. Isolates were grown on BAP agar plates (BBL Microbiology Systems, Becton, Dickinson). Three to five bacterial colonies were suspended in 600 μl of TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0). Bacterial cells in the suspension were then pelleted by centrifugation, and genomic DNA of the pelleted cells was extracted by the Genomic DNA minikit (Geneaid, Taiwan).

Biocide-resistant genes. Detection of the biocide- and disinfectant-resistant genes was performed by PCR as previously reported (18) with the following primers: qacA/B-F, 5′-GCTGCATTTATGACAATGTTTG-3′, and qacA/B-R, 5′-AATCCCACCTACTAAAGCAG-3′; smr-F, 5′-ATAAG TACTGAAGTTATTGGAAGT-3′, and smr-R, 5′-TTCCGAAAATGTTT AACGAAACTA-3′. The amplification of the *qacA/B* gene was carried out for 5 min at 94°C, followed by 30 cycles of 30 s at 94°C for denaturation, 30 s at 55°C for annealing, and 30 s at 72°C for extension and 5 min at 72°C for the final extension. The process for amplification of the *smr* gene was the same as that for *qacA/B* except that 50°C was used as the annealing temperature. The PCR buffer contained 10 mM Tris-HCl (pH 8.8 at 25°C), 1.5 mM $MgCl_2$, 50 mM KCl, and 0.1% Triton X-100.

Statistical analyses. This case-control study was conducted to correlate the presence of antiseptic-resistant genes (*qacA/B* or *smr*) with chlorhexidine-impregnated catheter-related bloodstream infections. The cases were *S. aureus* isolates with *qacA/B* or *smr*, and controls were *S. aureus* isolates without *qacA/B* or *smr*.

Pearson's chi-square test or Fisher's exact test (when the expected number in any cell was less than five) was used to determine whether differences in clinical characteristics or molecular genotypes exist among case or control groups. Odds ratios (OR) and 95% confidence intervals (CI) of various factors for *S. aureus* septicemia with definite chlorhexidine-impregnated CRBSI were analyzed by the multivariate logistic regression. All statistics were calculated using the Statistical Package for the Social Sciences (SPSS) for Windows (Version 17.0; Chicago, IL). A *P* value of 0.05 or less was considered statistically significant. All tests of significance were two-tailed.

RESULTS

Forty-nine (81.7%) of the 60 MSSA isolates were from patients <65 years old, and 27 were from males. Forty-two isolates were from hospital-acquired infections, but 66.7% ($n = 28$) of these were isolated within 48 h after admission. Infections or diseases caused by these MSSA isolates included primary bacteremia ($n = 31$), soft tissue, joint, or bone infections ($n = 11$), endocarditis ($n = 5$), catheter-related infections ($n = 5$), pneumonia ($n = 3$), arteriovenous shunt infections ($n = 3$), complicated urinary tract infection ($n = 1$), and ventriculoperitoneal shunt-related meningitis ($n = 1$). The *qacA/B* genes were found in 2 isolates (3.3%) from soft tissue, joint, or bone infections. Only two isolates from primary bacteremia and one from infective endocarditis were found to carry *smr* (5%). Among the 96 MRSA isolates, 42 (43.8%) carried *qacA/B* and 24 (25%) harbored *smr*. Four isolates contained both *qacA/B* and *smr*. The characteristics of the 96 MRSA isolates have been described elsewhere (5). The chlorhexidine MIC of each isolate is shown in Table 1. No MSSA or MRSA isolates with a chlorhexidine MIC of <1 μg/ml had *qacA/B*. Only 2 of the 60 MSSA isolates with a chlorhexidine MIC of ≥1 μg/ml carried *qacA/B*, whereas 43 (45.3%) of the 96 MRSA isolates with a chlorhexidine MIC of ≥1 μg/ml had *qacA/B*.

TABLE 2 Clinical characteristic of the 156 *S. aureus* (96 MRSA + 60 MSSA) isolates with or without *qacA/B*

Parameter	No. (%)		*P* value[a]
	qacA/B positive ($n = 44$)	*qacA/B* negative ($n = 112$)	
Gender			
Male ($n = 85$)	24 (54.5)	61 (54.5)	1.000
Female ($n = 71$)	20 (45.5)	51 (45.5)	
Age (yr)			
≥65 ($n = 63$)	24 (54.5)	39 (34.8)	0.03*
<65 ($n = 93$)	20 (45.5)	73 (65.2)	
Chlorhexidine-impregnated catheter insertion[b]			
Yes ($n = 41$)	19 (43.2)	22 (19.6)	0.004*
No ($n = 115$)	25 (56.8)	90 (80.4)	
Clinical HA or CA			
HA ($n = 127$)	40 (90.9)	87 (77.7)	0.068
CA ($n = 29$)	4 (9.1)	25 (22.3)	
MIC, μg/ml			
≥2 ($n = 58$)	28 (63.6)	30 (26.8)	<0.001*
≤1 ($n = 98$)	16 (34.4)	82 (73.2)	
Clinical diagnosis			
Definite CRBSI with chlorhexidine-impregnated catheters ($n = 18$)	13 (29.5)	5 (4.5)	<0.001*
Possible CRBSI or CRBSI with non-antimicrobial-impregnated catheters ($n = 17$)	5 (11.4)	12 (10.7)	
Others[c] ($n = 121$)	26 (59.1)	95 (84.8)	

[a] *P* value by chi-square test or Fisher's exact test when the cell expectation was less than five. *, $P \leq 0.05$.
[b] Within 15 days before *S. aureus* septicemia.
[c] Primary bacteremia ($n = 75$), soft tissue, joint, or bone infections ($n = 23$), infective endocarditis ($n = 11$), pneumonia ($n = 7$), arteriovenous shunt infection in hemodialysis patients ($n = 3$), complicated urinary tract infection ($n = 1$), and ventriculoperitoneal shunt-related meningitis ($n = 1$).

Clinical characteristics of all 156 *S. aureus* isolates are shown in Table 2. There was no significant difference in gender or clinical HA/CA in relation to the numbers of isolates with or without *qacA/B*, but more isolates with *qacA/B* were from patients ≥65 years of age or who received chlorhexidine-impregnated catheter

TABLE 3 Determination of risk factors between the clinical diagnosis of definite CRBSI with chlorhexidine-impregnated catheters ($n = 18$) and other diagnoses ($n = 138$)[a]

Factor	β	OR[b]	95% CI[b]
qacA/B (ref. negative)	2.226	9.264	2.961–28.980*
smr (ref. negative)	−0.510	0.600	0.128–2.823
MIC (ref. ≤1 μg/ml)	2.096	8.134	2.425–27.282*

[a] Risk factors are based on analyses of all 156 *S. aureus* (96 MRSA and 60 MSSA) septicemia patients. Other diagnoses included primary bacteremia ($n = 75$), soft tissue, joint, or bone infections ($n = 23$), possible CRBSI or CRBSI with non-antimicrobial-impregnated catheter ($n = 17$), infective endocarditis ($n = 11$), pneumonia ($n = 7$), arteriovenous shunt infection in hemodialysis patients ($n = 3$), complicated urinary tract infection ($n = 1$), and ventriculoperitoneal shunt-related meningitis ($n = 1$). ref., reference group.
[b] OR (odds ratios) and 95% CI (confidence interval) of various factors for definite CRBSI with chlorhexidine-impregnated catheters calculated by multivariate logistic regression with adjustments for gender and age. *, $P \leq 0.05$.

insertion within 15 days of *S. aureus* septicemia. There were also more *S. aureus* isolates with a chlorhexidine MIC of ≥2 μg/ml and with a definite chlorhexidine-impregnated CRBSI in the case group than in the control group. There was no difference in any factors between case and control isolates with or without the *smr* gene. Based on results of multivariate logistic regression analyses, both the presence of *qacA/B* in *S. aureus* isolates and the presence of *S. aureus* isolates with a chlorhexidine MIC ≥ 2 μg/ml were found to be risk factors for definite CRBSI with chlorhexidine-impregnated catheters (odds ratios, 9.246 and 8.134, respectively, in Table 3). In contrast, the *smr* gene was not found to be a risk factor. Because all patients with definite chlorhexidine-impregnated CRBSI had hospital-acquired infections and received chlorhexidine-impregnated catheter insertions within 15 days before *S. aureus* septicemia, these two factors were not included in the multivariate logistic regression analysis.

For the 96 MRSA isolates, there was no difference in gender, age, or chlorhexidine-impregnated catheter insertion in relation to the prevalence of *qacA/B* ($P = 0.401$, 0.682, and 0.530, respectively) (Table 4). More SCC*mec* type IV and II MRSA isolates (72% and 47%, respectively) than SCC*mec* type III and V isolates (28.6% and 25%, respectively) were found to harbor *qacA/B* ($P = 0.015$). However, there was no difference in the prevalence of *qacA/B* between SCC*mec* II and III isolates ("molecular" hospital acquired) and SCC*mec* IV and V isolates ("molecular" community acquired). There was also no correlation between the prevalence of *qacA/B* and *agr* or *spa* molecular types. For chlorhexidine-impregnated CRBSI, more MRSA isolates carried *qacA/B* (76.5%; $P = 0.011$). The *qacA/B* carrier rate was similar between MRSA isolates from possible CRBSI or non-antimicrobial-impregnated CRBSI (38.5%) and other diagnoses (36.4%). There was no significant difference in any molecular or clinical factors between *S. aureus* isolates with or without *smr*. Multivariate logistic regression analyses of MRSA septicemia patients with definite chlorhexidine-impregnated CRBSI revealed that the existence of *qacA/B* and chlorhexidine MIC of ≥2 μg/ml were the risk factors (OR, 18.641 and 4.373, respectively). SCC*mec*, *agr*, and *spa* types and *smr* were not found to be risk factors for CRBSI (Table 5).

TABLE 4 Molecular and clinical characteristics of 96 MRSA isolates with or without *qacA/B*

Parameter	No. (%) *qacA/B* positive ($n = 42$)	No. (%) *qacA/B* negative ($n = 54$)	*P* value[a]
Gender			
Male ($n = 58$)	23 (54.8)	35 (64.8)	0.401
Female ($n = 38$)	19 (45.2)	19 (35.2)	
Age (yr)			
≥65 ($n = 52$)	24 (57.1)	28 (51.9)	0.682
<65 ($n = 44$)	18 (42.9)	26 (48.1)	
Chlorhexidine-impregnated catheter insertion[b]			
Yes ($n = 39$)	19 (45.2)	20 (37)	0.530
No ($n = 57$)	23 (54.8)	34 (63)	
SCC*mec* type			
II ($n = 38$)	18 (42.9)	20 (37)	0.015*
III ($n = 28$)	8 (19)	20 (37)	
IV ($n = 18$)	13 (31)	5 (9.3)	
V ($n = 12$)	3 (7.1)	9 (16.7)	
Clinical HA or CA			
HA ($n = 86$)	38 (44.2)	48 (88.9)	0.750
CA ($n = 10$)	4 (40)	6 (11.1)	
Molecular HA or CA			
SCC*mec* II + III ($n = 66$)	26 (61.9)	40 (74.1)	0.268
SCC*mec* IV + V ($n = 30$)	16 (38.1)	14 (25.9)	
agr type			
I ($n = 55$)	22 (52.4)	33 (61.1)	0.633
II ($n = 36$)	17 (40.5)	19 (35.2)	
Others[c] ($n = 5$)	3 (7.1)	2 (3.7)	
spa type			
t002 ($n = 31$)	15 (35.7)	16 (29.6)	0.587
t037 ($n = 23$)	8 (19)	15 (27.8)	
t437 ($n = 21$)	11(26.2)	10 (18.5)	
Others[d] ($n = 21$)	8(19)	13 (24.1)	
MIC, μg/ml			
≥2 ($n = 56$)	26 (61.9)	30 (55.5)	0.677
≤1 ($n = 40$)	16 (38.1)	24 (44.4)	
Clinical diagnosis			
Definite CRBSI with chlorhexidine-impregnated catheters ($n = 17$)	13 (31)	4 (7.4)	0.011*
Possible CRBSI or CRBSI with non-antimicrobial-impregnated catheters ($n = 13$)	5 (11.9)	8 (14.8)	
Others[e] ($n = 66$)	24 (57.1)	42 (77.8)	

[a] *P* value by chi-square test or Fisher's exact test when the cell expectation was less than five. *, $P \leq 0.05$.
[b] Within 15 days before MRSA septicemia.
[c] *agr* III ($n = 1$), *agr* IV ($n = 3$), nontypeable ($n = 1$).
[d] t1081 ($n = 3$); t1094 ($n = 3$); t234 ($n = 2$); t138, t186, t214, t234, t441, t824, t932, t1212, t1751, t3527, t3528, new, nontypeable ($n = 1$).
[e] Primary bacteremia ($n = 44$), soft tissue, joint, or bone infections ($n = 12$), infective endocarditis ($n = 6$), pneumonia ($n = 4$).

DISCUSSION

According to results of nosocomial infection surveillance in Taiwan from 2008 to 2010, the percentages of CRBSI in 21 medical

TABLE 5 Determination of risk factors between the clinical diagnosis of definite CRBSI with chlorhexidine-impregnated catheters ($n = 17$) and other diagnoses ($n = 79$)[a]

Factor	β	OR[b]	95% CI[b]
SCC*mec* typing (ref. type II)	−0.261	0.770	0.449–1.321
Molecular HA or CA (ref. CA)	0.894	2.444	0.644–9.272
agr typing (ref. type 1)	0.146	1.157	0.582–2.300
spa typing (ref. t002)	−0.389	0.678	0.412–1.115
qacA/B (ref. negative)	1.808	6.097	1.796–21.007*
smr (ref. negative)	−1.148	0.317	0.066–1.527
MIC (ref. ≤1 μg/ml)	1.475	4.373	1.155–16.560*

[a] Risk factors are based on analyses of 96 MRSA septicemia patients. Other diagnoses include primary bacteremia ($n = 44$), possible CRBSI or CRBSI with non-antimicrobial-impregnated catheter ($n = 13$), soft tissue, joint, or bone infections ($n = 12$), infective endocarditis ($n = 6$), and pneumonia ($n = 4$). ref., reference group.
[b] OR (odds ratios) and 95% CI (confidence interval) of various factors for definite CRBSI with chlorhexidine-impregnated catheters calculated by multivariate logistic regression with adjustments for age and gender. *, $P \leq 0.05$.

centers are approximately 0.5% (2). Several factors may affect the rate of CRBSI, including types of intravascular devices, intended use of catheters, insertion sites, experience of the person who performs the insertion, duration of placement, underlying diseases of patients, and adoption of preventive strategies (11). Chlorhexidine preparations have been widely used for skin preparation, patient daily skin cleaning, and antimicrobial-impregnated catheters (14). However, exposure to these antiseptics has resulted in bacteria with reduced susceptibility to these agents through intrinsic or acquired mechanisms, such as acquiring plasmids or transposons that confer the resistance (15). The Qac multiple-drug-resistant pumps QacA, QacB, and QacC have been shown to confer resistance to various antimicrobial organic cations. The QacA pump is responsible for high-level resistance to both biguanidines (e.g., chlorhexidine) and diamidine (e.g., pentamidine) (13), and the QacC (Smr) pump is responsible for quaternary ammonium resistance (7). The difference between QacB and QacA is a single amino acid substitution at position 323, leading to a lower resistance level of biguanidines and diamidine in QacB (13).

In this study, we found that fewer MSSA isolates harbor *qacA/B* (3.3%) or *smr* (5%) than MRSA isolates (43.8% and 25%, respectively), which is similar to the results of a previous report (9). The reason for more MRSA isolates with *qacA/B* or *smr* may be more selective pressures due to wide applications of chlorhexidine in clinical procedures. The coexistence of *qacA/B* with multiresistant genes such as those encoding β-lactamase and heavy metal-resistant enzymes on the same plasmid may also be a contributing factor (9). Concomitant resistance to a range of antimicrobial agents could make MRSA isolates a better fit in hospital environments. This fitness could explain the higher chlorhexidine MIC in MRSA isolates (MIC_{50} = 2 μg/ml) than in MSSA isolates (MIC_{50} = 1 μg/ml). The *qacA/B* carrier rate was found to be higher in *S. aureus* isolates with a higher chlorhexidine MIC (51.6%, 44.4%, 21.3%, and 0% in isolates with MICs of 4 μg/ml, 2 μg/ml, 1 μg/ml, and ≤0.5 μg/ml, respectively).

The correlation between the presence of *qacA/B* and chlorhexidine resistance has not been definitely determined. Some reports showed a high degree (>90%) of correlation (16), but others showed no relationship (8). In this study, we found that more *S. aureus* isolates carrying *qacA/B* caused chlorhexidine-impregnated CRBSI. One possibility for this phenomenon is that patients infected with *qacA/B*-positive *S. aureus* had more insertions with chlorhexidine-impregnated catheters ($P = 0.004$ in Table 2). This was not the case in patients with MSSA infection, as most (46 of 60) of them developed septicemia within 48 h of admission and only two patients received chlorhexidine-impregnated catheter insertions before septicemia. In addition, most MSSA isolates except two were *qacA/B* negative. Since there was no difference in the frequency of chlorhexidine-impregnated catheter insertion in patients with *qacA/B*-negative or *qacA/B*-positive MRSA infections ($P = 0.530$ in Table 4), our finding that *qacA/B*-positive *S. aureus* isolates caused more chlorhexidine-impregnated CRBSI is significant.

Because few MSSA isolates carried *qacA/B* ($n = 2$) and only one patient with CRBSI had chlorhexidine-impregnated catheter insertion, the 96 MRSA isolates were analyzed for their roles in CRBSI (Tables 4 and 5). The results showed no significant relationship between the existence of *qacA/B* and different clinical backgrounds (age, gender, frequency of chlorhexidine-impregnated catheter insertion, and hospital- or community-acquired infections), *agr* and *spa* genotypes, or chlorhexidine MIC, except that more SCC*mec* II and IV MRSA isolates (47.4% and 72.2%, respectively) were found to carry *qacA/B*. Multivariate logistic regression analyses with adjustments for gender and age revealed that the presence of *qacA/B* and chlorhexidine MIC of ≥2 μg/ml were the two risk factors for chlorhexidine-impregnated CRBSI caused by MRSA (OR, 6.097 and 4.373, respectively). This finding suggests that the transmission of *qacA/B* was not related to the clonal spreading of MRSA in our hospital but was related to the selective pressures in preventive procedures for nosocomial infections. The carrier rate of *qacA/B* in MRSA isolates determined in this study was 43.8%, higher than that of previous reports (19). The prevalence of the *smr* gene for quaternary ammonium resistance was determined to be 25%, in contrast to the negative result of a previous study in Taiwan (16), despite the fact that quaternary ammonium had not been used in our hospital for more than 10 years.

The clinical significance of the existence of these antiseptic-resistant genes remains to be investigated. Since there is no internationally standardized method for *in vitro* susceptibility tests of these antiseptics (15), the interpretation of susceptibility to these biocides may not be the same as that for systemic antibiotics. However, the possibility of increased CRBSI episodes as a result of more MRSA isolates containing *qacA/B* cannot be ignored. Thus, the threat of MRSA to infection control is not confined to glycopeptide resistance (3, 6) but also can affect resistance to the biocides commonly used in clinical procedures. Further investigations on the effects of *qacA/B* in chlorhexidine-integrated preventive procedures are warranted.

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