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Distribution of Antiseptic Resistance Genes *qacA, qacB*, and *smr* in Methicillin-Resistant *Staphylococcus aureus* Isolated in Toronto, Canada, from 2005 to 2009▽

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Decreased susceptibility to chlorhexidine gluconate (CHDN) in methicillin-resistant *Staphylococcus aureus* (MRSA) is associated with the *qacA*, *qacB*, and *smr* genes, encoding efflux pumps. A total of 334 MRSA isolates were collected from two Canadian intensive care units between 2005 and 2009. We identified the *qacAB* genes in 7 strains (2%; 2 *qacA* genes and 5 *qacB* genes) and the *smr* gene in 23 (7%) strains. CHDN minimal bactericidal concentrations were slightly higher for strains harboring *smr* genes.

Chlorhexidine gluconate (CHDN) is a hexamethylene biguanide cationic biocide compound with a rapid bactericidal action against a variety of Gram-positive and Gram-negative microorganisms. It is widely used for hand hygiene and surgical antisepsis. It has also been evaluated for modulation of oropharyngeal colonization in critical care units (3, 16). The Canadian Practice Guidelines for hospital-acquired and ventilator-associated pneumonia in adults recommend use of oral chlorhexidine to prevent intensive care unit (ICU)-acquired pneumonia in selected patient populations (15). Decreased susceptibility to CHDN is mediated primarily through the multidrug efflux pumps encoded by the *qacA*, qac*B*, and *smr* (*qacC*) genes (12). There are marked geographic differences in the global distribution of methicillin-resistant *Staphylococcus aureus* (MRSA) containing CHDN resistance genes. The *qacAB* genes were detected in 10% to 20% of United Kingdom isolates (9, 10, 19, 20) and in up to 63% in a European study (9). High incidence rates were also identified in Brazil (80%) (10). Asian studies detected *qac* genes in MRSA at lower rates (32 to 41%) (1, 14, 17). In contrast, *smr* genes were detected in 31% of MRSA isolates from India but in 0 to 3% of Asian isolates (1, 14, 17). The clinical significance of MRSA carrying the *qacA*, *qacB*, and *smr* genes is still controversial, and methods to determine CHDN susceptibility have not been standardized in microdilution or in agar dilution by organizations such as CLSI or EUCAST. However, a recent study described what seems to be the first clinically significant emergence of CHDN resistance in MRSA, since an antiseptic protocol in an ICU was associated with a replacement of the usual MRSA by a *qacA*-containing MRSA strain (designated ST239 TW20) with a 3-fold-higher CHDN minimal bactericidal concentration (MBC) (2, 6). We carried out a surveillance study of two Canadian ICUs to determine the frequency of these genes in MRSA isolates and to determine the effect of the presence of these genes on *in vitro* susceptibility.

MRSA strains were provided by Mount Sinai Hospital (MSH) and Sunnybrook Health Sciences Centre (SHSC), Toronto, Ontario, Canada. We collected the initial strain from each patient colonized or infected with MRSA within their ICU stay during 2005 to 2009 (SHSC) and in 2008 and 2009 (MSH). Isolates were cultured on Columbia agar with 5% sheep blood and were incubated under aerobic conditions at 37°C for 24 h. We performed PCR detection of the *qacAB* and *smr* genes in all strains using primer sequences published previously (13). *qacA*, *qacB*, and *smr* amplicons were sequenced to confirm identity. We subsequently sequenced the *qacA* and *qacB* whole genes in order to subtype the efflux pump, as described elsewhere (1). Detection of *mecA* was also included as an internal control to confirm the identity of MRSA (8). MICs and MBCs were determined for all strains positive for *qacA*, *qacB*, or *smr.* Control strains, negative for these genes, matched for month and location of primary isolation, were selected in a 1:2 ratio ($n = 58$) and also underwent determination of MICs and MBCs. We also included three *S. aureus* positive controls (TS77, TPS162, and L20), respectively, for *qacA*, *qacB*, and *smr* (N. Noguchi, personal communication). MBCs were determined as was described by Batra and collaborators with minor modifications (2, 11). Briefly, MBCs were determined by serial 2-fold dilution of chlorhexidine digluconate solution (Sigma-Aldrich, Oakville, Canada) in Mueller-Hinton broth as described previously. Each dilution was inoculated with 5×10^4 CFU of fresh culture in Mueller-Hinton broth set up in a microdilution plate, which was then incubated at 37°C for 24 h. MICs were determined by observing the presence or absence of growth in each dilution. An aliquot of 100 μl of each culture in the microdilution plates was used to determine the MBCs. The Mueller-Hinton agar plates inoculated with these aliquots were incubated for 24 h at 37°C, and colony counts were performed as described previously (11). MBCs were calculated as the concentration that produced

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TABLE 1. *spa* type, CHDN MBC level, and carriage of *qacAB* and *smr* genes in 88 MRSA isolates[a]

spa type (n^b)	No. of isolates with gene carriage and CHDN MBC (μg/ml) of[c]:								
	5			10			20		
	neg	*qac*	*smr*	neg	*qac*	*smr*	neg	*qac*	*smr*
t002 (43)	12	2	1	12	3	6	3	0	4
t003 (1)	0	0	0	1	0	0	0	0	0
t004 (1)	0	0	1	0	0	0	0	0	0
t007 (1)	0	0	0	0	0	1	0	0	0
t008 (18)	3	0	1	5	0	4	3	0	2
t018 (1)	1	0	0	0	0	0	0	0	0
t032 (1)	0	0	0	1	0	0	0	0	0
t037 (4)	1	0	0	1	2	0	0	0	0
t045 (6)	2	0	0	4	0	0	0	0	0
t059 (1)	0	0	0	0	0	0	1	0	0
t062 (1)	1	0	0	0	0	0	0	0	0
t064 (4)	0	0	1	0	0	2	0	0	1
t1610 (1)	0	0	0	1	0	0	0	0	0
t2235 (2)	1	0	0	1	0	0	0	0	0
t242 (1)	0	0	0	1	0	0	0	0	0
t437 (1)	0	0	0	1	0	0	0	0	0
t640 (1)	0	0	0	1	0	0	0	0	0
Total (88)	21 (78)	2 (7)	4 (15)	29 (62)	5 (11)	13 (28)	7 (50)	0 (0)	7 (50)

[a] International comparison of *spa* types commonly associated with Canadian MRSA (CMRSA) strains (4): t002 is CMRSA2, USA100, USA800, and EMRSA-3; t008 is CMRSA9, CMRSA10, and USA300; t037 is CMRSA 3; t064 is CMRSA5 and USA500.

[b] *n*, no. of isolates.

[c] neg, negative. A value in parentheses is the percentage of total isolates with the indicated CHDN MBC (for an MBC of 5 μg/ml, $n = 27$; for an MBC of 10 μg/ml, $n = 47$; for an MBC of 20 μg/ml, $n = 14$).

≥99.9% killing of cells. *spa* typing was performed as an epidemiological method for *qacAB*- and *smr*-positive strains and matched controls using primers as described previously (5). The Ridom StaphType software program was used for analysis and assignment of a *spa* type to each isolate.

A total of 334 strains were obtained from the two Toronto hospitals (93 from MSH and 241 from SHSC). Molecular amplification detected the *qacAB* genes in 7 strains (2%), whereas the *smr* gene was detected in 23 (7%) strains. No isolates contained both genes. Sequencing of the *qacAB* genes revealed that 2/7 strains carried *qacA* and 5/7 carried *qacB*. MIC and MBC values were similar for strains harboring *qacAB* or *smr* versus strains negative for these genes (Table 1). MBCs of positive controls TS77 (*qacA*), TPS162 (*qacB*), and L20 (*smr*) were 10 μg/ml, 10 μg/ml, and 5 μg/ml, respectively. Of the 27 strains with an MBC of 5 μg/ml, 21 (78%) were negative for the *qacA*, *qacB*, and *smr* genes, whereas of the 14 strains with an MBC of 20 μg/ml, 50% harbored the *smr* gene. Molecular typing revealed that the *spa* types t002 and t008 were the most frequent *spa* types identified in our study. Interestingly, all t064 strains carried the *smr* gene, but they had very diverse MBCs, ranging from 5 to 20 μg/ml. Twenty-seven isolates (90%) positive for the *qacAB* or *smr* gene were linked to these 3 *spa* types.

We found that the *qacA*, *qacB*, and *smr* genes are relatively infrequent in MRSA isolated from patients in two Toronto ICUs. *spa* typing revealed that our clones are consistent with Canadian MRSA epidemiology, so we do not expect a selection bias (4, 18). It is known that the global distribution of the *qac* and *smr* genes is highly variable (1, 9, 10, 13, 14, 17, 19, 20). The local utilization of chlorhexidine and other antiseptics could affect the distribution of resistance genes, but a relationship is difficult to infer. Interestingly, we did not witness a clinically significant increase in CHDN MBC to be associated with the presence of the *qacA* or *qacB* gene. The QacA pump confers a reduced susceptibility to a broad range of hydrophobic compounds, including CHDN. QacB has a similar action but has a limited impact on CHDN because of an amino acid substitution at position 323, and sequencing is needed to differentiate *qacA* from *qacB* (7, 19). Most studies did not make this differentiation, making comparison more difficult (14). The fact that we did not observe a significant increase in MBCs associated with the *qac* and *smr* genes is in line with the relatively small increase witnessed by other studies, usually within a 2 2-fold-dilution increase. In a United Kingdom study, isolates with the *qacAB* genes had significantly higher MBCs for CHDN than negative strains (73 μg/ml versus 18 μg/ml) (19). Sheng et al. identified the *qacA* and *qacB* genes more often in MRSA isolates with a CHDN MIC of ≥4 μg/ml than in strains with ≤2 μg/ml (93% versus 5%, respectively) (17). A more recent United Kingdom report found higher MBCs in strains possessing the *qacA* gene than in those that didn't (78 μg/ml versus 26 μg/ml) (2, 6). In addition, if our clones were to be slightly less active in CHDN efflux, the difference in MBCs would then be difficult to observe. Nonetheless, the clinical impact of such a small MBC increase is debated, since the achieved concentration of topically applied CHDN 0.1% (wt/vol) solution will yield 1,000 μg/ml, manyfold higher than the MBC of any *qac*- or *smr*-harboring strain.

In conclusion, we infrequently found the *qacA*, *qacB*, and *smr* genes in MRSA from two intensive care units in Canada. However, the increase in CHDN usage in routine patient care warrants periodic monitoring of susceptibility in order to detect any raise in either gene associated with resistance, as well as phenotypic testing to identify any other mechanisms of resistance.

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