Frequency of Disinfectant Resistance Genes in Pediatric Strains of Methicillin-Resistant *Staphylococcus aureus* •

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Resistance to chlorhexidine gluconate (CHG) and quaternary ammonium compounds (QACs) is a potential public health threat, given widespread use of these agents for routine hospital cleaning, skin antisepsis, and patient decolonization.[1-3] Development of tolerance to CHG and QACs among methicillin-resistant *Staphylococcus aureus* (MRSA) isolates can be mediated by energy-dependent multidrug efflux proteins, which show increased expression in response to selective pressure from disinfectant use.[4] Plasmid-encoded efflux pump genes *qac A/B* and *smr* confer tolerance to both CHG and QACs, along with other compounds, including intercalating dyes and cationic biocides.[5,6] The efflux pump genes *qac A/B* and *smr* have been found in MRSA isolates with varying frequencies globally, mostly in tertiary care adult and pediatric populations.[6] Here we describe an evaluation of pediatric clinical MRSA isolates for the presence of disinfectant resistance genes *qac A/B* and *smr* and for tolerance to CHG.

Two hundred eighty-one clinical pediatric MRSA isolates from 2004 through 2009 were selected randomly from the Vanderbilt Children's Hospital MRSA Repository, a de-identified collection of unique MRSA isolates obtained from emergency room or hospitalized general pediatric patients with MRSA infection. The MRSA Repository is approved by the Vanderbilt Institutional Review Board and maintained as a de-identified data set with limited clinical information. All isolates were identified by the Vanderbilt University Hospital Laboratory according to Clinical and Laboratory Standards Institute standards prior to repository transfer.

Isolates were cultured overnight on blood agar at 37°C, and purified genomic DNA was used as a template for repetitive-element, sequence-based polymerase chain reaction to determine genetic classification of strains (DiversiLab System, BioMérieux). Plasmid-encoded *qac A/B* and *smr* genes were evaluated by polymerase chain reaction using previously published primers.[7,8] Minimum bactericidal concentrations (MBCs) of a randomly selected subset of 5 *qac A/B* and 5 *smr* positive MRSA strains, along with 5 randomly selected negative controls, were determined by broth microdilution methods using 20% w/v chlorhexidine digluconate solution (Sigma-Aldrich).[1] A Fisher exact test was performed to determine statistical significance.

Of the 281 isolates identified in the repository, 201 isolates (71.5%) belonged to USA300, the current epidemic clone in the United States. Of the remainder, 31 isolates (11.0%) belonged to USA100, 31 isolates (11.0%) belonged to USA500, and 18 isolates (6.4%) were other pulse types. Genes for *qac A/B* or *smr* were detected in 18.5% of isolates (52/281); 13.9% contained *smr* only, 4.3% harbored *qac A/B*, and 1 isolate contained both *smr* and *qacA/B*. Non-USA300 MRSA isolates were significantly more likely to harbor *qac A/B* or *smr* genes than USA300 MRSA isolates (Figure 1; $P = .0175$). MBC testing of 15 MRSA isolates (5 negative controls, 5 *qac A/B* positive, and 5 *smr* positive) in serial dilutions of CHG showed that all 15 isolates had MBCs less than 16 μg/mL, well below the recommended in-use concentration of 2,000 μg/mL.[6] No significant differences in MBC were noted between *qac A/B* or *smr* positive isolates and negative controls.

We found a moderate prevalence of plasmid-encoded disinfectant resistance genes (18.5%) in this random sample of pediatric MRSA isolates, similar to other studies in US pediatric populations.[9] In our study, pediatric isolates belonging to USA300, the pulse type associated with the community-associated MRSA epidemic, were less likely than non-USA300 MRSA isolates to possess disinfectant resistance genes. Nearly 15% of the USA300 strains, however, also harbored genes for efflux pumps capable of conferring tolerance to CHG. This was an unexpected finding, given the USA300 clone's predom-

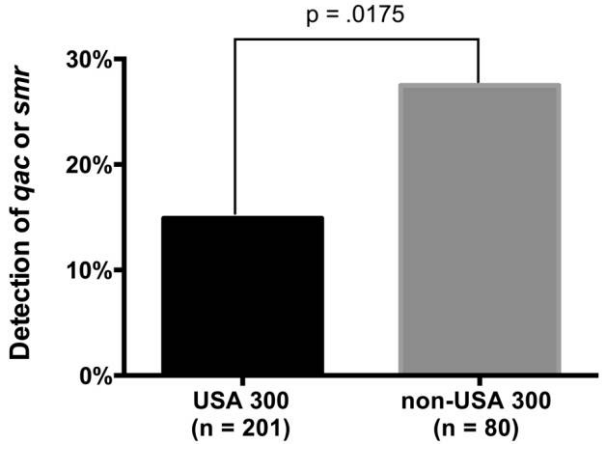

FIGURE 1. Presence of disinfectant resistance genes *smr* and *qac A/B* by methicillin-resistant *Staphylococcus aureus* pulse type. Non-USA300 isolates were more likely than USA300 isolates to harbor *smr* or *qacA/B* ($P = .0175$).

inant association with community-onset MRSA infection, since CHG is considered a healthcare-associated exposure.

This study has limited generalizability because it represents a single-center study that may not apply to other geographic regions. The de-identified nature of the data set attached to the MRSA repository did not allow for evaluation of the emergence of disinfectant resistance genes over time or evaluation of the relationship of disinfectant resistance genes to specific MRSA infections, such as device-associated infection.

CHG has gained an increasing role in the infection prevention arsenal for reducing healthcare-associated infections, and CHG resistance threatens current infection prevention efforts directed against multidrug-resistant organisms. CHG is widely used for surgical antisepsis, and daily bathing of critically ill patients with CHG is commonplace. The Randomized Evaluation of Decolonization vs Universal Clearance to Eliminate (REDUCE) MRSA trial showed that universal decolonization using nasal mupirocin and bathing with CHG significantly reduced rates of bloodstream infections in the community intensive care unit setting.[3] The popularity of universal decolonization strategies will further increase the use of CHG in hospital settings. Thus, it is important to consider the mechanisms by which MRSA might survive CHG exposure in the clinical setting.

S. aureus possesses both chromosomal and plasmid-mediated efflux pumps capable of targeting a wide range of compounds, from antimicrobials to disinfectants. Previous evaluations of CHG bactericidal activity against *qac A/B* gene-positive MRSA strains have shown evidence of increased tolerance to CHG with elevated MBC in those strains.[1,6] Another study showed increased tolerance and overexpression of *S. aureus* efflux pumps in association with exposure to disinfectants.[4] Our limited evaluation of bactericidal activity showed no evidence of increased MBC in *qac A/B* or *smr* positive isolates. Reassuringly, no MRSA isolates harboring phenotypic CHG resistance have been reported to date.

In summary, this report demonstrates a moderate prevalence of disinfectant resistance genes in a pediatric population in both USA300 and non-USA300 MRSA isolates. Non-USA300 isolates were significantly more likely to harbor disinfectant resistance genes. Ongoing evaluation of genotypic and phenotypic CHG resistance will help gauge the effectiveness of future infection prevention efforts utilizing CHG.

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