The association between biocide tolerance and the presence or absence of qac genes among hospital-acquired and community-acquired MRSA isolates

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Objectives: The MBCs of three commonly used hospital biocides [containing quaternary ammonium compounds (QACs), chlorhexidine gluconate and triclosan] were determined for clinical isolates of Staphylococcus aureus, which were also screened for genes encoding Qac efflux pumps.

Methods: MBCs were determined by broth microdilution for 94 clinical isolates of S. aureus, including 38 hospital-acquired methicillin-resistant S. aureus (HA-MRSA), 25 community-associated methicillin-resistant S. aureus (CA-MRSA), 25 methicillin-susceptible S. aureus (MSSA) and 6 with intermediate resistance to vancomycin (VISA). All isolates were screened by PCR for the presence of qacA, B, C, G, H and J.

Results: Biocides had MBCs 10–1000-fold lower than the concentration recommended for use by the manufacturer. HA-MRSA isolates developed significantly enhanced tolerance to QACs following repeat exposure to subinhibitory concentrations. Ten HA-MRSA and four VISA isolates carried qacA. Two HA-MRSA isolates, one MSSA isolate and one VISA isolate carried qacC. One VISA isolate carried qacA and qacC. The CA-MRSA isolates did not carry qac genes. qacG, H and J were not detected in any HA-MRSA. Isolates with qac genes had significantly (P < 0.0001) higher MBCs for biocides containing QACs and chlorhexidine gluconate. These biocides induced expression of qac genes when assayed with a luciferase reporter.

Conclusions: Biocides commonly used in the hospital environment should be effective against clinical isolates of S. aureus if used at concentrations recommended by the manufacturer. However, isolates have the potential to develop increased tolerance to these agents and the expression of Qac efflux pumps results in isolates with a selective advantage when challenged with biocides containing QACs and chlorhexidine gluconate.

Keywords: Staphylococcus aureus, antimicrobials, efflux pumps

Introduction

Methicillin-resistant Staphylococcus aureus (MRSA) has become established as a major nosocomial pathogen infecting vulnerable individuals in hospitals and healthcare facilities worldwide.¹,² The glycopeptide vancomycin has been vitally important in the treatment of MRSA infections. However, MRSA isolates with reduced susceptibility to vancomycin have now been reported in many countries.³,⁴ Recently, there has also been an increasing incidence of infection by MRSA in young, otherwise healthy, community-dwelling individuals who have no association with healthcare facilities.⁵ Community-associated isolates of MRSA (CA-MRSA) can carry an array of genes encoding toxins, such as the Panton-Valentine leucocidin toxin,⁶,⁷ which increases the virulence of these strains and intensifies the threat to public health. Therefore, there is an ongoing need to control the spread of MRSA and limit sources of infection.

Considerable efforts have been made in recent years to improve practices of infection control within hospitals, leading to the increased use of disinfectants and antiseptics.⁸ Quaternary ammonium compounds (QACs), cationic biocides (such as chlorhexidine) and biocides containing the bisphenol ether, triclosan, are used widely in disinfectant preparations in healthcare...
settings to decontaminate surfaces, disinfect the hands of hospital personnel and treat patients colonized by S. aureus. Despite the implementation of standard practices for infection control, current measures have failed to control the spread of MRSA, which has been isolated from numerous locations in hospitals including catheters and disinfectant soap dispensers. This has raised concerns that, as for antibiotics, intensive exposure of hospital pathogens to biocides may result in the emergence of resistance to these agents.

Efflux-mediated resistance to QACs and cationic biocides has been reported in staphylococcal isolates from numerous sources. Clinical isolates of S. aureus carry a number of plasmid-borne qac genes, qacA, B and C, which encode proton-motive force-dependent export pumps. The QacA and QacB pump genes were maintained on tryptone soy agar (Oxoid, Basingstoke, UK) supplemented with 2% (w/v) NaCl. Two colonies of each strain were inoculated into 500 mL of overnight culture. The cultures were incubated at 37°C for 24 h and colony counts were performed. An aliquot of 20 μL was also removed from each culture and added to a dilution of biocide that was 2-fold greater than the original concentration. These cultures were then incubated for a further 24 h at 37°C with shaking. The process was repeated until the cells had been exposed to increasingly higher concentrations of biocides, up to 100-fold greater than the MBC.

Materials and methods

Bacterial strains

Bacterial strains were provided by the Scottish MRSA Reference Laboratory (Stobhill Hospital, Glasgow, UK). Ninety-four clinical strains of S. aureus were selected from a large library of clones and subclones based on differences in their PFGE banding patterns. There were 38 HA-MRSA isolates, 25 CA-MRSA isolates, 25 methicillin-susceptible S. aureus isolates (MSSA) and 6 isolates with intermediate resistance to vancomycin (VISA). Two VISA strains were isolated in Scotland, two originated in the USA and two were isolated in Japan. HA-MRSA, CA-MRSA and MSSA isolates were maintained on tryptone soy agar (Oxoid, Basingstoke, UK) and were incubated under aerobic conditions at 37°C for 24 h. VISA isolates were maintained on Mueller–Hinton agar (Oxoid, Basingstoke, UK) containing a subinhibitory concentration of vancomycin. S. aureus strains were stored in 80% (v/v) glycerol at −70°C and freshly subcultured before each experiment.

Biocides

Commonly used hospital biocides were obtained in commercial preparations. These were: Trigene, a product containing a mixture of the QACs (alkyl dimethyl benzyl ammonium chloride and didecyl dimethyl ammonium chloride); Mediscrub, containing 1% (w/v) triclosan; and the cationic biocide MediHex-4, containing 4% (w/v) chlorhexidine gluconate (all supplied by Medichem International, Queensborough, Kent).

MBCs of biocides

MBCs of the three biocides were determined by serial 2-fold dilution of the biocides in Mueller–Hinton broth (Oxoid, Basingstoke, UK) supplemented with 2% (w/v) NaCl. Each dilution was inoculated with 2 × 10⁶ cfu of overnight culture in fresh Mueller–Hinton broth. The cultures were incubated at 37°C with shaking for 24 h. An aliquot of 100 μL of each culture was used to inoculate a Petri dish of Mueller–Hinton agar supplemented with 2% (w/v) NaCl. The plates were incubated at 37°C for a further 24 h and colony counts were performed. The MBC was calculated as the concentration of biocide that produced 99.9% killing of cells. The MBC of each biocide for all 94 strains of S. aureus was determined using this method. Each dilution series was performed in triplicate and the entire experiment was repeated once. The results were analysed using Prism® software (GraphPad Software, Inc.) and a one-way ANOVA was used to compare the MBC results for HA-MRSA, CA-MRSA and VISA isolates.

Increased tolerance

A single colony of each strain was cultured for 24 h in Mueller–Hinton broth supplemented with 2% (w/v) NaCl. A dilution of biocide 1/10 MBC was prepared in Mueller–Hinton broth and inoculated with 2 × 10⁵ cfu of overnight culture. The cultures were incubated at 37°C for 24 h with shaking. After 24 h, 100 μL was removed from each culture and used to inoculate a Petri dish of Mueller–Hinton agar [supplemented with 2% (w/v) NaCl]. Plates were incubated at 37°C for 24 h and colony counts were performed. An aliquot of 20 μL was also removed from each culture and added to a dilution of biocide that was 2-fold greater than the original concentration. These cultures were then incubated for a further 24 h at 37°C with shaking. The process was repeated until the cells had been exposed to increasingly higher concentrations of biocides, up to 1000-fold greater than the MBC.

Template for PCR

Two colonies of each strain were inoculated into 500 μL of sterile water, and the suspension was heated to 100°C for 15 min. The tube was then centrifuged at 10 000 g for 2 min and the supernatant containing the bacterial DNA was removed and used in the PCR.

PCR of qac genes

PCR was carried out for detection of qacA, B and C using primer sequences published previously. A single primer pair amplified qacA and B: the DNA sequences of these two genes differ in only seven bases. Primer sequences were designed for the detection of qacG, H and J (Table 1).
The volume of each reaction in the PCR was 50 μL. Each reaction contained 5 μL of 10× Expand High Fidelity buffer (without MgCl2) (Roche, Lewes, UK), 4 μL of MgCl2, a final concentration of 2 mM (Roche, Lewes, UK), 8 μL of dNTPs (1.25 mM), 1 μL (50 pmol) of reverse primer, 1 μL (50 pmol) of forward primer and 20 μL of sterile H2O. Template bacterial DNA (10 μL) was added, and a negative control was included containing 10 μL of sterile water instead of template DNA. Finally, 1 U of Expand High Fidelity Enzyme mix (Roche, Lewes, UK), containing thermostable Taq DNA polymerase and Tgo DNA polymerase with proof-reading activity, was added to each reaction. The cycling conditions were as follows: DNA denaturation at 94°C for 3 min, followed by 25 cycles of 94°C for 30 s, 55°C for 30 s and 72°C for 1 min. This was completed with an extension step of 72°C for 5 min. PCR products were analysed by electrophoresis on a 2% (w/v) agarose/TBE gel.

Cloning and sequencing of PCR products

The amplified qacA/B PCR products were purified using the Wizard® SV Gel and PCR Clean-Up System (Promega, Southampton, UK) and cloned using the Novagen pT7Blue-3 Perfectly Blunt® Cloning Kit (Merck Biosciences, Nottingham, UK). Insert-containing vectors were sequenced using the GFXTM Micro Plasmid Prep Kit (Amersham, Little Chalfont, UK) and sequenced with the BigDye Terminator Kit (ABI Biosystems, Foster City, CA, USA) on an ABI 3730 sequencer (ABI Biosystems, Foster City, CA, USA) on an ABI 3730 sequencer (ABI Biosystems) to determine whether isolates carried the qacA or B gene. Sequence analysis and alignments were carried out using BioEdit software (www.mbio.ncsu.edu/BioEdit/bioedit.html) and additional searches for related sequences were performed online via the National Centre for Biotechnology BLAST server (www.ncbi.nlm.nih.gov/BLAST).

Detection of biocide induction of QacR-regulated genes by luciferase assay

The biosensing reporter plasmid, pQacLux, was used to determine whether any of the hospital biocides used in this study induced expression of QacR-regulated genes. pQacLux carries the regulatory region and the divergent coding sequence of qacR from the staphylococcal multiresistance plasmid pSK1, coupled to the modified bacterial luciferase operon luxCDABE from Photobacterium luminescens.26 Competent cells of the non-pathogenic S. aureus strain RN4220 were prepared and transformed with 0.5 μg of pQacLux DNA by electroporation, as described previously.27 One colony of RN4220/pQacLux was used to inoculate 5 mL of L-broth containing 0.5% (w/v) glucose and 10 μg/mL chloramphenicol (pQacLux carries chloramphenicol resistance) and incubated for 18 h at 37°C with shaking. Following incubation, 100 μL of this culture was diluted in 5 mL of fresh L-broth containing 0.5% glucose (w/v) and 10 μg/mL chloramphenicol and grown until the OD reached 0.06. An aliquot of 190 μL of this culture was then combined with 10 μL of a dilution of Trigene, MediHex-4 or Mediscrub and added to the wells of a white-walled transparent-bottomed 96-well plate (Greiner Bio-one, Gloucestershire). The plate was incubated at 37°C at 200 rpm and light emission and OD400 were measured every 30 min on a luminometer (BMG LUMItstar® Galaxy, Offenberg, Germany) for 6 h. Each reaction was carried out in triplicate. Cells with no biocide (positive control) and a negative control containing media only were included for reference. The entire experiment was repeated once. The maximum relative light value was divided by the OD of the cells at each time point.

Results

MBCs of biocides

The MBCs of Trigene, MediHex-4 and Mediscrub for 38 HA-MRSA isolates, 25 CA-MRSA isolates, 6 VISA isolates (exposed to vancomycin) and 25 MSSA isolates were determined by broth microdilution. The MBC varied between isolates and differed between the four groups of CA-MRSA, HA-MRSA, VISA and MSSA isolates.

The manufacturer suggests that a 0.1% (w/v) solution of Trigene is used to disinfect surfaces. In this study, all isolates had MBCs of <0.01% (w/v) Trigene (Figure 1). CA-MRSA isolates showed a mean MBC of 0.0032% Trigene, whereas HA-MRSA isolates had a mean MBC of 0.0069% and VISA strains had a mean MBC of 0.0086%. MSSA isolates had a mean MBC of 0.0036% Trigene. The mean MBC of Trigene for CA-MRSA and MSSA isolates was significantly lower than the mean MBC of HA-MRSA isolates (P < 0.0001), whereas VISA isolates had a mean MBC that was significantly higher than that for HA-MRSA isolates (P < 0.01).

MediHex-4 is recommended for use without dilution, giving a final concentration of chlorhexidine gluconate of 4% (w/v). CA-MRSA isolates had a mean MBC of 0.0004%, HA-MRSA isolates had a mean MBC of 0.0033%, VISA strains had a mean MBC of 0.0024% and MSSA isolates had a mean MBC of 0.0069%.
of 0.00037% (Figure 2). The mean MBC for VISA isolates was lower than the mean MBC for HA-MRSA isolates but this difference was not statistically significant. CA-MRSA and MSSA isolates had a significantly lower ($P < 0.05$ and $P < 0.01$, respectively) mean MBC of MediHex-4 than HA-MRSA or VISA isolates.

Mediscrub is recommended for use at a final concentration of 1% (w/v) triclosan. CA-MRSA isolates had a mean MBC of 0.00037%, HA-MRSA isolates had a mean MBC of 0.001%, VISA strains had a mean MBC of 0.00055% and MSSA isolates had a mean MBC of 0.0005% Mediscrub (Figure 3). The mean MBC for HA-MRSA isolates was significantly higher than for CA-MRSA and MSSA isolates ($P < 0.05$). VISA isolates appeared to have a lower mean MBC than HA-MRSA isolates, and CA-MRSA and MSSA isolates seemed to have a lower mean MBC than VISA isolates but these differences were not statistically significant ($P = 0.2$, $P = 0.37$ and $P = 0.25$, respectively).

**Detection of qac genes by PCR**

$qacA/B$ were found in 10 of the 38 HA-MRSA isolates screened and 4 VISA isolates. $qacC$ was detected in two HA-MRSA isolates, one VISA isolate and one MSSA isolate (Table 2). The HA-MRSA isolates and the MSSA isolate that carried $qacC$ did not carry the $qacA/B$ genes. One VISA isolate carried $qacA/B$ and $qacC$, although it is not known whether the genes were carried on the same plasmid. None of the clinical isolates of $S. aureus$ produced PCR amplicons with the $qacG$, $H$ and $J$ primer sets, although control strains carrying $qacG$, $H$ and $J$ gave positive results. CA-MRSA isolates did not carry any of the $qac$ genes detected in these PCRs.

**Sequencing of PCR products**

Nucleotide sequencing of the PCR products revealed that all products formed by the $qacA/B$ primers were $qacA$. Comparison of the $qacA$ gene sequences with published $qacA$ sequence data revealed that two of the $qacA$-positive HA-MRSA isolates and two VISA isolates carried $qacA$ genes with a base substitution from C-T at position 1103. This alters the amino acid sequence of the protein: an isoleucine replaces a threonine at residue 368. This may have an impact on the function of the protein and more importantly the specificity of the pump. QacA is known to utilize a larger number of substrates and has a higher affinity for divalent cations than QacB. This is due to a difference in amino acid at codon 323 where $qacB$ encodes Ala and $qacA$ encodes Asp. Therefore, the proteins encoded by $qacA$ in these four clinical isolates could potentially have an altered specificity to other QacA pumps of isolates carrying ‘wild-type’ $qacA$. To test this, the MBC of each biocide for these four strains was compared with all other strains that carried $qacA$, using an unpaired two-tailed $t$-test. There was no significant difference in MBCs of $S. aureus$ isolate

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<th>$qacA/B$</th>
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<th>$qacG$</th>
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<td>CA-MRSA (25)</td>
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<td>MSSA (25)</td>
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Mediscrub. The MBCs of Trigene and MediHex-4 were higher for the four isolates that carried \textit{qacA} with the base substitution but this difference was not statistically significant.

\textbf{MBC and qac PCR}

Isolates were grouped according to the presence/absence of \textit{qac} genes (as defined by PCR) and results were analysed using the unpaired two-tailed \textit{t}-test. Isolates carrying \textit{qac} genes had significantly higher MBCs of Trigene (Figure 4) ($P < 0.0001$) and MediHex-4 (Figure 5) ($P < 0.0001$) than those without \textit{qac} genes. There was no significant difference in the MBC of Mediscrub for isolates carrying \textit{qac} genes and those without ($P = 0.08$).

\textbf{Increased tolerance to biocides following exposure to sublethal concentrations}

Following contact with low levels of biocide, isolates were gradually exposed to increasing concentrations of the agents. All isolates that carried \textit{qac} genes showed a significant increase in mean MBC after continued exposure to Trigene ($P < 0.05$). With MediHex-4, isolates that carried \textit{qac} genes appeared to show an increase in mean MBC following exposure to increased concentrations of the biocide; however, this increase was not statistically significant ($P = 0.08$). Following repeated exposure to increasing concentrations of Mediscrub, there was no statistically significant increase in MBC in isolates carrying \textit{qac} genes ($P = 0.1$). There was no significant difference in the initial and final MBC, following increased exposure to Trigene, MediHex-4 and Mediscrub, in \textit{qac}-negative isolates ($P = 0.1$, $P = 0.2$ and $P = 0.2$, respectively).

\textbf{Detection of induction of QacR-regulated genes by luciferase}

\textit{pQacLux} carries the \textit{qacR-qacA} intergenic region fused to the \textit{luxCDABE} operon encoding the luminescent protein luciferase. In the presence of a substrate of QacR, the \textit{qacR-qacA} intergenic region on \textit{pQacLux} will be transcribed along with the luciferase protein and light should be emitted. Biocides were added to RN4220/pQacLux cells and the level of light emission was measured. The maximum relative light units at each time point were divided by the OD for each of the biocides tested. The addition of subinhibitory concentrations of Trigene and MediHex-4 produced a significant increase in the level of light emitted from the cells in comparison with a biocide-free control ($P < 0.01$) (Figure 6). The addition of a subinhibitory concentration of Mediscrub to RN4220/pQacLux cells produced no significant increase in light emission from cells in comparison with the biocide-free control. This would suggest that Trigene and MediHex-4 induce the expression of \textit{qacA/B} and are likely substrates for these efflux pumps.

\textbf{Discussion}

The increasing prevalence of MRSA infections in hospitals worldwide has lead to greater public awareness of the threat of nosocomial infection and increased efforts to control this problem using suitable hygiene measures. Biocides are an essential part of infection control strategies employed in the hospital environment. In this study, the efficacy of three biocides commonly used in hospitals was tested for 38 clinical isolates of HA-MRSA, 25 CA-MRSA, 6 VISA and 25 MSSA isolates. All isolates had MBCs of Trigene, MediHex-4 and Mediscrub of 10–1000-fold lower than concentrations recommended for use by the manufacturers. This would suggest that, if these biocides are used in accordance with the manufacturers’ instructions, 100% of bacteria should be killed. In clinical practice a problem
may arise when biocides are used incorrectly, in dirty situations
where surfaces are not cleaned of organic matter prior to using a
biocide or ‘topping up’ biocides leading to the use of subinhibitory
concentrations. In the hospital environment bacteria grow in
biofilms on surfaces, which have been shown to afford the cells a
10–1000-fold higher tolerance of antimicrobials, and may be
a contributing factor to failure of disinfection.28

The potential for biocide-selected cross-resistance to clin-
cially important antibiotics is the subject of some discussion in
the literature.29,30 In this study, HA-MRSA isolates displayed a
significantly higher MBC of Trigene, MediHex-4 and Mediscrub
than MSSA isolates. This would suggest that there is a corre-
lation between antibiotic resistance and biocide tolerance.
Although a definitive link between antibiotic and biocide resis-
tance has not been established, it has been observed that the
MBC of QACs and chlorhexidine was significantly higher for
clinical isolates of MRSA than MSSA.31 Our study reinforces
this link in S. aureus and the issue of biocide–antibiotic
co-resistance should no longer be ignored.

Screening of the 94 S. aureus isolates revealed that 10
HA-MRSA and 4 VISA isolates carried the qacA gene. Two
HA-MRSA isolates, one MSSA isolate and one VISA isolate
carried the qacC gene. One VISA isolate carried qacA and qacC
genes. HA-MRSA isolates that carried qacA or qacC had higher
MBCs of Trigene and MediHex-4 than isolates without the
genes. The isolates carrying qac genes also developed increased
tolerance to Trigene following exposure to increasing concen-
trations of this biocide. This suggests that carriage of qac genes
confers a selective advantage on these isolates in the presence of
Trigene and MediHex-4 and may contribute to their survival
after repeated exposure in the hospital environment to Trigene.
Only one MSSA isolate carried qacC. HA-MRSA isolates
had significantly higher MBCs of Trigene and MediHex-4
(\(P < 0.0001\) and \(P < 0.01\), respectively) than MSSA isolates
and this may be due to the higher frequency of carriage of qac
genes in these isolates.

VISA isolates had the highest MBC of Trigene and those car-
rying qac genes also displayed increased tolerance to this
biocide. Such VISA isolates have the potential to survive treat-
ment with diluted biocides and cause infection in the hospital
environment. Carriage of two qac genes (qacA and qacC) in
the case of one VISA isolate may afford added protection from a
wider range of substrates than carriage of a single efflux pump
gene. It is imperative that infection control practices restrict the
spread of VISA strains as there may be therapeutic failures in
clinical practice with vancomycin.

The CA-MRSA isolates included in this study did not carry
qac genes. This may be due to the reduced selective pressure
from biocides that these isolates experience in the community
setting compared with hospital strains.

None of the isolates carried the qacG, H or J genes. These
genes have been reported previously in S. aureus isolated from
food production areas and animals in Norway, where they have
been shown to increase tolerance to QACs.22–24 From the results
of this study, the qacG, H and J genes do not seem to pose a
problem among Scottish clinical isolates of S. aureus at the
moment. However, if carriage of these genes provides isolates
with a selective advantage for biocide tolerance, then their
appearance is a distinct possibility and must be monitored.

Mayer et al.14 examined the frequency of qac genes in 297
European MRSA isolates and found that 63% carried qacA/B
and 6.4% carried qacC. The frequency of qacA/B among
European isolates is considerably higher than the frequency
found in our study. This may be due to the larger number of iso-
lates screened in the European study, differences in epidemic
strains selected for each study or variation in the type of bio-
cides used regularly, applying different selective pressures on
MRSA isolates in each region.

Nucleotide sequencing revealed that all qacA/B-positive iso-
lates carried qacA. The multidrug efflux protein QacA is able to
utilize more than 30 cationic lipophilic antimicrobial compounds
that belong to 11 distinct chemical classes, whereas QacB has a
more limited substrate range.16 In the hospital environment, bac-
teria will encounter multiple classes of antimicrobial agents.
Therefore, it is beneficial for isolates to carry the gene that
encodes QacA which can extrude a larger number of toxic com-
pounds than QacB, increasing the chances of survival. Four of
the isolates carried qacAs that encoded a QacA with an amino
acid substitution. The amino acid substitution in the sequences
of QacA and QacB imposes a significant effect on the specificity
of the efflux pump. Therefore, the altered QacA found in isolates
in this study may be expected to alter efflux. Isolates carrying
the altered qacA gene displayed no significant difference in
MBC of Mediscrub but had higher MBCs of Trigene and
MediHex-4 than isolates with the ‘wild-type’ qacA. However,
the difference was not statistically significant.

The continued exposure of bacteria to residual levels of bio-
cides in the hospital environment is causing concern.12,13 This
study has shown that clinical isolates of S. aureus including
HA-MRSA, MSSA, CA-MRSA and VISA strains have MBCs of
the commonly used hospital biocides Trigene, MediHex-4 and
Mediscrub of 10–1000-fold less than the concentrations rec-
mended for use by the manufacturer. However, HA-MRSA
isolates had the ability to develop significantly increased toler-
ance to Trigene following repeated exposure to this agent. This
may suggest that repeated exposure of S. aureus to subinhibitory
concentrations of this biocide in the hospital environment could
enhance tolerance. HA-MRSA and VISA isolates frequently
carried qac efflux pump genes, which significantly increased
(\(P < 0.0001\)) the MBC of Trigene and MediHex-4 for these iso-
lates compared with isolates that did not carry qac genes.16
Trigene and MediHex-4 were found to induce the expression of
the genes encoding the QacA/B efflux pumps, which confirms
that these biocides are likely substrates. This suggests that in
the presence of these biocides, efflux-mediated increased tolerance
has the potential to develop. If biocides are used at concen-
trations recommended for use by the manufacturer in the hospi-
tal environment, then S. aureus isolates should be killed, as even
the increased tolerance displayed in isolates failed to develop
into complete resistance. However, the presence of qac genes in
the clinical S. aureus population and their ability to develop
increased tolerance highlights the importance of effective and
rigorous infection cleaning and infection control strategies and
the use of biocides at concentrations recommended by the
manufacturer.

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Transparency declarations

None to declare.

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