Culture-independent detection of chlorhexidine resistance genes qacA/B and smr in bacterial DNA recovered from body sites treated with chlorhexidine-containing dressings

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Abstract
Purpose. Dressings containing chlorhexidine gluconate (CHG) are increasingly used in clinical environments for prevention of infection at central venous catheter insertion sites. Increased tolerance to this biocide in staphylococci is primarily associated with the presence of qacA/B and smr genes.

Methodology. We used a culture-independent method to assess the prevalence of these genes in 78 DNA specimens recovered from the skin of 43 patients at catheter insertion sites in the arm that were covered with CHG dressings.

Results. Of the 78 DNA specimens analysed, 52 (67 %) possessed qacA/B and 14 (18 %) possessed smr; all samples positive for smr were also positive for qacA/B. These prevalence rates were not statistically greater than those observed in a subsample of specimens taken from non-CHG treated contralateral arms and non-CHG-dressing exposed arms. A statistically greater proportion of specimens with greater than 72 h exposure to CHG dressings were qac-positive (P=0.04), suggesting that the patients were contaminated with bacteria or DNA containing qacA/B during their hospital stay. The presence of qac genes was not positively associated with the presence of DNA specific for Staphylococcus epidermidis and Staphylococcus aureus in these specimens.

Conclusion. Our results show that CHG genes are highly prevalent on hospital patients’ skin, even in the absence of viable bacteria.

INTRODUCTION
Chlorhexidine gluconate (CHG) is a water-soluble cationic bisbiguanide that is used as a front-line biocide for the decontamination of skin in clinical settings for infection prevention. CHG is present in hand disinfectant used by healthcare staff, for whole-body decolonization, and used as a general disinfectant at surgical and vascular access sites [1–3].

Increased tolerance to CHG has been reported for several major pathogens associated with catheter-related bloodstream infection (CRBSI) [4–10]. In Staphylococcus aureus and coagulase-negative staphylococci (CONS), acquisition of qac genes, including qacA/B and smr, is the major mechanism for increased CHG tolerance. These genes encode multidrug efflux pumps that export a variety of toxic molecules, including biocides, disinfectants and some antibiotics, from the bacterial cell, thereby increasing bacterial tolerance to these compounds [11].

Suwantarat et al. [12] reported that exposure to CHG through patient bathing with CHG-impregnated cloths increased the presence of CHG-tolerant bacteria on patient skin [12], suggesting that continued exposure to this biocide may be selecting for these organisms. Multiple dressing types containing CHG are increasingly used in clinical settings, and have been recommended in international...
infection prevention guidelines for use with peripherally inserted central catheters (PICCs) in high-risk patients [13, 14]. Unlike typical CHG preparations, CHG dressings are designed to release the biocide at the catheter insertion site over an extended duration [15], thereby preventing skin colonization over sustained time periods [16, 17]. A meta-analysis of nine randomized clinical trials has shown that the use of CHG dressings at catheter insertion sites greatly reduces both catheter colonization and associated CRBSI rates [18, 19] providing short-term benefits to patients. However, residual microbial colonization of the CHG-exposed skin surrounding the catheter insertion site remains and associated CRBSI is not completely eliminated [16].

The major aim of this study was to assess the prevalence of qacA/B and smr at PICC insertion sites covered with CHG dressings. To overcome the biocidal effect of CHG, a culture-independent DNA recovery method was developed.

**METHODS**

**Ethics**

The study was approved by the Human Research Ethics Committees from Queensland Health (HREC/11/QRCH/152 and HREC/13QRWB/454). All participants provided informed written consent prior to enrolment. All patient identifiers were removed from samples prior to laboratory transfer, with a unique study number assigned.

**Study design**

The study was primarily designed to assess the prevalence of qacA/B and smr in DNA recovered from the skin of patients at sites covered with CHG dressing. To determine if these rates differed from controls, DNA was also recovered from [1] contralateral arms not covered with CHG dressings (n=10) and [2] patients not exposed to CHG dressings (n=24) (Fig. S1, available in the online Supplementary Material).

**Study populations and samples**

CHG dressings (Biopatch; Johnson and Johnson) and matching skin swabs were collected from 43 patients (medical, surgical, oncology and haematological), aged >16, at the Royal Brisbane and Women’s Hospital (RBWH), a major tertiary teaching hospital in Queensland, Australia, from March 2014 to March 2015. All patients had PICCs in the middle of their upper arms, inserted into large deep veins.

Patient exclusion criteria were: (i) pre-existing bloodstream infection; (ii) burned or diseased skin at the catheter insertion site; (iii) existing skin tears or papery skin; (iv) being extremely diaphoretic; or (v) known allergy to CHG. The CHG dressings were replaced weekly while the catheter was in place, or earlier if clinically indicated (i.e. the dressing was not dry, clean and intact). Patient demographics, clinical symptoms of infection, antibiotic treatment, duration of CHG dressing use, number of previous CHG catheter dressings and hospital microbiology results, such as blood cultures, were all collected. Skin swabs were also collected from 24 consenting non-CHG-dressing-exposed patients (medical and surgical), aged >18, at the RBWH and Princess Alexandra Hospital, who had peripheral intravenous catheters (PIVCs) inserted into the peripheral veins of their arms from October 2013 to September 2014. The patient groups were enrolled in a ‘securing all intravenous devices effectively’ (SAVE) randomized control trial and their catheters were secured with non-CHG dressings [20]. Bacteria isolated from the PIVC insertion skin sites were used as a non-CHG-dressing-exposed patient control.

All samples were aseptically collected by experienced research nurses (ReNs). The dressings were removed from a patient’s skin by the ReNs weekly, and as needed to remain clean, dry and intact, wearing sterile gloves, and placed in a sterile container. Skin swabs were then taken from the CHG- and non-CHG-dressing sites surrounding the catheter insertion point using a sterile swab moistened with sterile 0.9% sodium chloride (Pfizer). A 2 cm² surface area at the site was swabbed for 10 s using back and forward motion and rolling of the swab tip. To determine if the duration of CHG dressing placement has any impact on the presence of CHG tolerance genes, a subset of patient (n=24) skin swab and CHG dressing samples were collected at multiple time points of dressing replacements – this was determined by patients’ needs for multiple dressing replacements and if ReNs were available to take samples. Skin swabs from non-CHG-exposed skin on the opposing arm were also collected from a subset of patients (n=10).

**Microbial culture**

The CHG dressing and skin swab samples were placed into separate tubes containing PBS, vortexed and centrifuged at 10,000 g for 5 min. The supernatant was removed and the pellet resuspended into 400 µl PBS. One hundred microlitres of the suspension was then plated onto horse blood agar (bioMérieux) and/or chocolate agar (Oxoid) plates, incubated at 37°C for 72 h and monitored daily for bacterial growth. The remaining 200 µl CHG dressing and skin swab samples were used for isolation of bacterial genomic DNA (gDNA). Any bacteria that did grow on agar plates were subsequently subcultured and identified using the Vitek MS (bioMérieux) system.

**DNA extractions**

Bacterial gDNA from CHG dressing and matched skin swab swabs was extracted using the QIAamp UCP Pathogen Mini kit (Qiagen) with minor modifications. Briefly, 100 µl of TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0) was added onto the CHG dressing and matched skin swab suspensions and then vortexed. The suspensions were then mixed with 500 µl ATL lysis buffer and 20 µl proteinase K, followed by incubation at 56°C for 60 min with frequent vortexing. The suspensions were transferred to a pathogen lysis tube for mechanical disruption. The remainder of the extraction procedure was performed as described in the manufacturer’s protocol. The ultraclean microbial DNA isolation kit (MO BIO Laboratories) was used for extraction of genomic DNA from individual bacteria isolated from non-CHG-
exposed control skin swabs as described in the manufacturer’s instructions.

Detection of qacA/B and smr

Primers for all PCRs are listed in Table 1. Confirmation of bacterial DNA in extracted DNA samples was determined using 27F and 1492Primer targets conserved V1–V9 regions of 16S RNA. The presence of qacA/B and smr genes was determined using primers as described by Noguchi et al. [21]. All PCR reactions were carried out using 10 µl GoTaq Green Master Mix (Promega), 1 µl forward primer (10 µM), 1 µl reverse primer (10 µM), 3 µl DNA (~20–50 ng) template in a final volume of 25 µl. 16S PCR was performed with cycling conditions of 94°C for 4 min for one cycle, 94°C for 30 s, 58°C for 1 min and 72°C for 2 min run up to 36 cycles and 72°C for 7 min for the final extension. For qacA/B and smr, PCR was performed with cycling conditions of 94°C for 4 min for one cycle, 94°C for 30 s, 56°C for 30 s and 72°C for 30 s run up to 30 cycles and 72°C for 5 min for the final extension. DNA from reference strains (JCM 16554, JCM 16555, JCM 16556) possessing qacA/B and smr was used as positive controls (Table 1). Genomic DNA from a Staphylococcus epidermidis isolate previously shown to be negative for qacA/B and smr was used as the negative control.

Molecular identification of S. aureus and S. epidermidis DNA

Molecular detection of S. aureus and S. epidermidis in pooled dressing and swab samples was achieved through quantitative real-time PCR (qPCR) amplification of the nucA and tuf genes [22, 23]. The primers for tuf gene were optimized for real-time PCR. The specificity was confirmed with agarose gel electrophoresis. Of note, nucA and tuf were for the specific detection of S. aureus and S. epidermidis, respectively [22, 23]. For these assays, 10 µl of Platinum SYBR Green qPCR SuperMix-UDG (Life Technologies), 1.25 µl of both forward and reverse primers (10 µM) (Table 1), 2 µl of DNA template and sterile distilled H2O were added to a final volume of 20 µl. PCR cycling was performed by a three-step temperature cycling procedure as follows: hold at 50°C for 2 min, second hold at 95°C for 2 min, 40 cycles of 95°C for 15 s, annealing temperature for 20 s, 72°C for 35 s, melting between 72 and 95°C rising by 1°C. Amplification curve results were validated by analysing melting curves and the threshold melting temperature was set to ±0.5°C with respect to the reference sample. This step further reduces the number of non-specific products or false positives. All real-time PCR reactions were performed on the Rotor-Gene 6000 (Corbett) and all data were generated with Rotor-Gene 6000 (version 7.1) software.

Statistical analysis

Statistical analysis was performed with the GraphPad prism package (GraphPad Software). The χ² test and/or Fisher’s exact test was used to examine the relationship between clinical characteristics and CHG resistance, as well as the presence of qac genes in CHG-dressing-exposed and non-exposed skin swabs. For comparison of CHG dressing/matched skin swab results by duration exposed to CHG dressing, the samples were divided roughly into three for each time point (<72, 72–168 and >168 h). The χ² test and/or Fisher exact test were also used to determine the association of the presence of qac genes with bacterial species. P values less than 0.05 were considered statistically significant.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward/reverse (5’-3’)</th>
<th>Product size (bp)</th>
<th>Positive control</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>16S</td>
<td>AGAGTTTGATCTMGGGCTCAG</td>
<td>2465</td>
<td>S. epidermidis‡</td>
<td>[25]</td>
</tr>
<tr>
<td></td>
<td>CCGTTACCCTTGTTAGACTTG</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>qacA/B</td>
<td>GCGAGAAAAGTCAGAGTGCG</td>
<td>361</td>
<td>JCM 16555, JCM 16556</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CCAGTCCAATCATGCGCTG</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>smr</td>
<td>GCCATAAGTACTGAGTTATGGGA</td>
<td>195</td>
<td>JCM 16554‡</td>
<td>[21]</td>
</tr>
<tr>
<td></td>
<td>GACTACGGTTGTTAGACTAACCT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>nucA*</td>
<td>GCGATTGTATGGGTGATACGGTT</td>
<td>274</td>
<td>ATCC 25923</td>
<td>[23]</td>
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<td></td>
<td>AGCCAGCCTTGGAGGACACTAAGC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>tuf†</td>
<td>GCAGTTGAGGAGGATTCCT</td>
<td>412</td>
<td>ATCC 14990</td>
<td>[22]</td>
</tr>
<tr>
<td></td>
<td>CCATTGCTGATCCTGCAGTAA</td>
<td></td>
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</tbody>
</table>

JCM, Japan Collection of Microorganisms.

*ntm, Thermostable nuclease gene.
†tuf, Elongation factor Tu; nucA specific for S. aureus and tuf is specific for S. epidermidis.
‡S. epidermidis (isolated from connectors in administration set).
§S. aureus; encoding multidrug efflux gene qacA.
∥S. aureus; encoding multidrug efflux gene qacB.
¶S. aureus; small multidrug resistance gene smr.
RESULTS

Prevalence of qacA/B and smr and patient clinical characteristics

To assess the presence of CHG tolerance genes at catheter insertion sites where dressings are impregnated with CHG, 78 CHG catheter dressings and matching skin swabs were collected from 43 consenting patients. Initial plating of these samples onto standard bacterial growth media failed to grow any viable bacteria. In contrast, 34 viable bacteria were recovered from 24 of 137 patient skin swabs collected from catheter insertion sites covered with non-CHG dressings, demonstrating the effectiveness of CHG dressings in killing bacteria at these sites (Table S1). Additionally, when swabs were collected from the matched non-CHG-exposed contralateral arm of 10 patients with CHG dressing on the other arm, 43 viable organisms were recovered from all of the samples. In all instances, the viable bacteria recovered from these sites were commonly CONS.

As no viable bacteria were recovered from the CHG-dressing-exposed group, we next used a culture-independent method to assess the presence of qacA/B and smr genes at these sites. For this purpose, DNA was recovered independently from both the dressings (n=78) and matching skin swabs (n=66). The other 12 matching skin swabs had insufficient DNA recovered for analysis. Of the 43 patients, 34 (79 %) were positive for qacA/B, five (12 %) positive for smr and four (9 %) positive for both qacA/B and smr (Table 2). Of all the DNA samples recovered directly from the 78 CHG dressings, 52 (67 %) were positive for qacA/B, and 14 (18 %) positive for smr. Thirteen of the 14 smr-positive DNA samples were positive for both qacA/B and smr. Of the 66 DNA samples directly recovered from skin swabs, 21 (32 %) were positive for qacA/B and six (9 %) positive for smr. All smr-positive skin swabs were also positive for qacA/B. When the data from the CHG dressing and skin swab from matching patients were pooled, 52 (67 %) of the DNA samples were positive for qacA/B, 14 (18 %) positive for smr, and 14 (18 %) positive for both. In most cases, the samples positive for qacA/B also contained smr.

To examine the prevalence of qacA/B and smr in viable bacteria, we next assessed the prevalence of these genes in the viable bacteria recovered from the non-CHG-dressing and non-CHG-exposed control groups. Of the 24 skin swabs from non-CHG dressings (Table 2), 10 (42 %) possessed qacA/B-positive, and six (25 %) smr-positive, and two (8 %) for both qacA/B-and smr-positive bacterial isolates. Acknowledging the differences between DNA samples isolated from bacterial cultures and non-cultured CHG dressing and skin swabs, there was no significant difference (P=0.08, Fisher’s test) in the detection of qacA/B or smr between arms exposed or not exposed to CHG dressings. Of the samples recovered from the contralateral arm of the CHG-exposed patients, seven of 10 (70 %) possessed qac-positive staphylococci; six of the 10 (60 %) samples contained smr-positive isolates.

For bacterial DNA recovered from the CHG dressing/skin swab samples, there was no significant difference in gender (P=1.0), age (≥50 or <50, P=0.7), admitting diagnostic group (medical, haematology, oncology, surgical vascular, gastrointestinal or orthopaedic, P=0.8), and clinical diagnosis (yes/no, P=1.0) of bloodstream infection or CRBSI, between patients who were positive (n=35) or negative (n=8) for qacA/B/smrs (Table 3).

<table>
<thead>
<tr>
<th>Source of bacterial DNA samples</th>
<th>Number of samples/patients (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Presence of CHG tolerance genes</td>
</tr>
<tr>
<td></td>
<td>DNA samples</td>
</tr>
<tr>
<td>CHG dressing</td>
<td>78</td>
</tr>
<tr>
<td>CHG skin swab†</td>
<td>66</td>
</tr>
<tr>
<td>Pooled matched CHG dressing and skin swab†</td>
<td>78</td>
</tr>
<tr>
<td>Patients</td>
<td></td>
</tr>
<tr>
<td>CHG dressing and matched skin swab†</td>
<td>43</td>
</tr>
<tr>
<td>Non-CHG skin swabs from contralateral skin sites§</td>
<td>10</td>
</tr>
<tr>
<td>Non-CHG skin swabs from patients without CHG dressings</td>
<td></td>
</tr>
</tbody>
</table>

*Number of qacA/B and smr-positive genes that were detected in total number of skin swab DNA samples.
†Number of qacA/B and smr genes that were detected in total number of CHG dressing and matched skin swab DNA samples combined.
‡Number of qacA/B and smr genes that were detected in bacteria isolated from patients who had matched CHG dressing and skin swab DNA samples.
§Number of qacA/B and smr genes that were detected in bacteria isolated from patients who had swabs taken from non-CHG-exposed contralateral skin sites.
¶Number of qacA/B and smr genes that were detected in bacteria isolated from patients who had swabs taken from non-CHG-dressing-exposed skin sites.
Impact of duration of CHG dressing placement on the presence of CHG tolerance genes

To determine whether the length of exposure to CHG dressings was associated with an increased prevalence of CHG tolerance genes, we next compared the relative frequency of qacA/B- and smr-positive DNA samples recovered from 66 samples collected at different time points after the initial application of the CHG dressing. Our data showed that 40% of DNA samples collected from the skin that had been exposed to a CHG dressing for less than 72 h (n=21) were qac-positive (Fig. 1). For samples (n=22) collected between 72 and 168 h of CHG-dressing exposure, 68% were qac-positive. Of samples (n=23) with greater than 168 h exposure, 78% were qac-positive. A statistically significant difference in the prevalence rate was observed between the short exposure group (<72 h) and longer durations of exposure (P=0.04, χ² test).

For a subset of patients (n=24), skin swabs were from catheter insertion sites at multiple time points, coinciding with each CHG dressing replacement. For 18 of these patients, all samples collected were qacA/B-positive at all time points. For the remaining six patients, DNA was negative for qacA/B at the first dressing change, but positive by the third or fourth dressing change. All patients who returned a positive qacA/B result remained positive for future samples. In one instance, qacA/B genes were detected in skin swabs collected only at the fourth dressing change, after 22 d of cumulative CHG dressing use. In another patient, the smr gene was detected from skin swabs only at the fourth dressing replacement after 14 d of cumulative dressing use.

Molecular identification of species-specific bacterial DNA

A limitation of culture-independent methods is the inability to determine which bacterial species the CHG genes originate from. In the context of this study, qacA/B and smr are found in multiple staphylococcal species. To determine whether the presence of these genes is associated with the presence of DNA from two major staphylococci pathogens associated with CRBSI (i.e. S. epidermidis and S. aureus), qPCR using primers specific for these species was performed on the pooled DNA samples. Based on the presence of nucA, 33 (43%) of the samples were positive for S. aureus. Fifty-two of the samples were tuf-positive, an indicator of S. epidermidis. Of the qacA/B-positive samples, 38 (73%) were positive for S. epidermidis DNA, and 24 (46%) positive for S. aureus DNA. Sixteen (31%) possessed DNA from both S. epidermidis and S. aureus (Table 4). Of the DNA samples that were negative for qacA/B, 12 (46%) possessed S. epidermidis DNA, six (23%) possessed S. aureus DNA, and two (8%) possessed both S. epidermidis and S. aureus. Of the DNA samples positive for smr (n=14), seven (50%) also possessed tuf, seven (50%) possessed nucA and three (21%) possessed both S. epidermidis and S. aureus DNA. Of the DNA samples that were negative for smr, 40 (63%) possessed S. epidermidis DNA, 22 (34%) possessed S. aureus DNA, and 12 (19%) possessed both S. epidermidis and S. aureus. No association between the presence of qacA/B and smr and DNA from S. aureus or S. epidermidis was apparent (P=0.5; Fisher’s exact test).

Table 3. Clinical characteristics of patients swabs were recovered from in this study

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Number of patients (n) /samples (%)</th>
<th>CHG tolerance genes positive (n=35)</th>
<th>CHG tolerance genes negative (n=8)</th>
<th>P value*</th>
</tr>
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<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>20 (57)</td>
<td>20 (57)</td>
<td>4 (50)</td>
<td>1.0</td>
</tr>
<tr>
<td>Female</td>
<td>15 (43)</td>
<td>15 (43)</td>
<td>4 (50)</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>≥50</td>
<td>26 (74)</td>
<td>26 (74)</td>
<td>7 (88)</td>
<td>0.7</td>
</tr>
<tr>
<td>&lt;50</td>
<td>9 (26)</td>
<td>9 (26)</td>
<td>1 (13)</td>
<td></td>
</tr>
<tr>
<td>Admitting diagnosis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medical</td>
<td>7 (20)</td>
<td>7 (20)</td>
<td>2 (25)</td>
<td>0.8</td>
</tr>
<tr>
<td>Haematology</td>
<td>5 (14)</td>
<td>5 (14)</td>
<td>1 (13)</td>
<td></td>
</tr>
<tr>
<td>Oncology</td>
<td>5 (14)</td>
<td>5 (14)</td>
<td>2 (25)</td>
<td></td>
</tr>
<tr>
<td>Surgical vascular</td>
<td>4 (11)</td>
<td>4 (11)</td>
<td>1 (13)</td>
<td></td>
</tr>
<tr>
<td>Surgical gastrointestinal</td>
<td>7 (20)</td>
<td>7 (20)</td>
<td>1 (13)</td>
<td></td>
</tr>
<tr>
<td>Surgical orthopaedic</td>
<td>6 (17)</td>
<td>6 (17)</td>
<td>1 (13)</td>
<td></td>
</tr>
<tr>
<td>Clinical diagnosis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BSI</td>
<td>2 (6)</td>
<td>2 (6)</td>
<td>0</td>
<td>1.0</td>
</tr>
<tr>
<td>CRBSI</td>
<td>1 (3)</td>
<td>1 (3)</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

*P value by χ² test or Fisher’s exact test.
As no viable bacteria were present at these sites, we developed a culture-independent DNA recovery method to investigate the presence of qac genes and for bacterial species identification.

The large percentage of qac-positive DNA samples recovered from CHG-dressing-exposed catheter insertion sites was a surprising result. However, subsequent analyses of viable bacteria from skin sites covered with non-CHG dressings, or no dressing at all, revealed high rates of qac-positive staphylococci, indicating that the use of CHG dressings themselves was not associated with an increase in the prevalence of these genes at these sites. Rather, the prevalence of these genes is intrinsically high in the general hospital patient population sampled here. However, the current study was not adequately powered to address whether no statistical differences exist in the prevalence of CHG tolerance genes between CHG-dressing and non-CHG-exposure groups; much larger sample sizes for both experimental and control groups are needed to quantify the impact of CHG dressings on the development of CHG tolerance.

In an apparently contrasting finding, our results also showed that duration of exposure to CHG dressings was positively associated (P=0.04) with an increase in recovery of CHG tolerance genes in a DNA sample. However, this trend may reflect the prolonged stay of these patients in the hospital environment rather than CHG exposure. CHG is commonly used as a biocide in hospital environments, providing positive-selection pressure for qac-positive staphylococci at a hospital-wide level, as opposed to local (i.e. CHG dressing). In this regard, previous studies have shown healthcare workers to be colonized with qac-positive CONS or S. aureus at greater rates than the general population [10, 24]. The regular handling and replacement of the catheter and CHG dressing may therefore facilitate the transmission of qac-positive strains from healthcare workers to the catheter site. The observation that individual patients who were qac-negative became qac-positive throughout the study, but no patients transitioned from a qac-positive to qac-negative status, supports this theory.

The fact that DNA encoding qac genes was consistently recoverable at such a high rate in this study may have clinical implications regarding the development of qac tolerance and other antimicrobial resistance. Whereas most studies focusing on the transfer of antibiotic tolerance genes focus on conjugative bacteria to bacteria transfer, our results suggest that even after bacteria have been killed, uptake of naked DNA through transformation may be an alternative means by which bacteria may acquire these determinants in clinical environments. In this scenario, the killing of bacteria, such as through the use of biocide, would not be sufficient to prevent the transfer of these genes to another bacterial isolate. As qac and antibiotic tolerance genes are normally plasmid encoded [11], recombination after transformation would be unnecessary for maintenance of these genes after uptake by the recipient organism.

This study used DNA extraction directly from CHG dressing and skin swab samples, as we were unable to recover any viable bacteria by standard culture methodologies, to examine the qacA/B and smr prevalence on the CHG-dressing-exposed skin of hospitalized patients. On the other hand, qacA/B and smr prevalence was determined in bacterial cultures from non-CHG-exposed control arms. The difference in the qacA/B and smr detection, one being directly from skin swab and dressing samples and the control non-CHG-exposed arm swabs from bacterial cultures, may influence analysis of the results.

The culture-independent method has advantages over culture-based methods in that DNA from non-viable cells is detectable. A disadvantage of this approach is that mobile genetic elements with broad host range, such as those encoding qac and antibiotic resistance genes, cannot be linked directly to specific bacterial species. However, the fact that DNA encoding qac genes was consistently recoverable at high rates demonstrates direct molecular techniques are highly sensitive, and could be applied to the detection of other clinically relevant genes that may provide data on species and/or antibiotic resistance determinants. Indeed, culture-independent methods are increasingly being used for diagnostic applications. As such tests become more economically viable, screening a patient’s skin for qac or other resistance determinants may become part of standard hospital practice.

### DISCUSSION
Chlorhexidine is ubiquitous in healthcare settings. It is present in surgical hand-scrub, general disinfectants, and increasingly present in wound dressings. As a consequence, multiple studies have reported increases in prevalence of CHG tolerance genes in bacteria recovered from healthcare settings when compared to bacteria recovered from non-healthcare environments [6–10]. The use of CGH has also been implicated in the colonization of patients with CHG-tolerant bacteria. To our knowledge, this is the first report investigating potential associations between CHG dressings and the prevalence of CHG tolerance genes at catheter insertion sites. As no viable bacteria were present at these sites, we developed a culture-independent DNA recovery method to investigate the presence of qac genes and for bacterial species identification.

The large percentage of qac-positive DNA samples recovered from CHG-dressing-exposed catheter insertion sites was a surprising result. However, subsequent analyses of viable bacteria from skin sites covered with non-CHG dressings, or no dressing at all, revealed high rates of qac-positive staphylococci, indicating that the use of CHG dressings themselves was not associated with an increase in the prevalence of these genes at these sites. Rather, the prevalence of these genes is intrinsically high in the general hospital patient population sampled here. However, the current study was not adequately powered to address whether no statistical differences exist in the prevalence of CHG tolerance genes between CHG-dressing and non-CHG-exposure groups; much larger sample sizes for both experimental and control groups are needed to quantify the impact of CHG dressings on the development of CHG tolerance.

In an apparently contrasting finding, our results also showed that duration of exposure to CHG dressings was positively associated (P=0.04) with an increase in recovery of CHG tolerance genes in a DNA sample. However, this trend may reflect the prolonged stay of these patients in the hospital environment rather than CHG exposure. CHG is commonly used as a biocide in hospital environments, providing positive-selection pressure for qac-positive staphylococci at a hospital-wide level, as opposed to local (i.e. CHG dressing). In this regard, previous studies have shown healthcare workers to be colonized with qac-positive CONS or S. aureus at greater rates than the general population [10, 24]. The regular handling and replacement of the catheter and CHG dressing may therefore facilitate the transmission of qac-positive strains from healthcare workers to the catheter site. The observation that individual patients who were qac-negative became qac-positive throughout the study, but no patients transitioned from a qac-positive to qac-negative status, supports this theory.

The fact that DNA encoding qac genes was consistently recoverable at such a high rate in this study may have clinical implications regarding the development of qac tolerance and other antimicrobial resistance. Whereas most studies focusing on the transfer of antibiotic tolerance genes focus on conjugative bacteria to bacteria transfer, our results suggest that even after bacteria have been killed, uptake of naked DNA through transformation may be an alternative means by which bacteria may acquire these determinants in clinical environments. In this scenario, the killing of bacteria, such as through the use of biocide, would not be sufficient to prevent the transfer of these genes to another bacterial isolate. As qac and antibiotic tolerance genes are normally plasmid encoded [11], recombination after transformation would be unnecessary for maintenance of these genes after uptake by the recipient organism.

This study used DNA extraction directly from CHG dressing and skin swab samples, as we were unable to recover any viable bacteria by standard culture methodologies, to examine the qacA/B and smr prevalence on the CHG-dressing-exposed skin of hospitalized patients. On the other hand, qacA/B and smr prevalence was determined in bacterial cultures from non-CHG-exposed control arms. The difference in the qacA/B and smr detection, one being directly from skin swab and dressing samples and the control non-CHG-exposed arm swabs from bacterial cultures, may influence analysis of the results.

The culture-independent method has advantages over culture-based methods in that DNA from non-viable cells is detectable. A disadvantage of this approach is that mobile genetic elements with broad host range, such as those encoding qac and antibiotic resistance genes, cannot be linked directly to specific bacterial species. However, the fact that DNA encoding qac genes was consistently recoverable at high rates demonstrates direct molecular techniques are highly sensitive, and could be applied to the detection of other clinically relevant genes that may provide data on species and/or antibiotic resistance determinants. Indeed, culture-independent methods are increasingly being used for diagnostic applications. As such tests become more economically viable, screening a patient’s skin for qac or other resistance determinants may become part of standard hospital practice.
Conclusion
This study has revealed that qacA/B and smr are frequently recovered from skin sites where CHG dressings are used, as well as body sites not exposed to CHG dressings. There is no evidence that CHG dressings increase the frequency of bacteria harbouring CHG tolerance genes at catheter insertion sites. However, the high rates of recovery of CHG tolerance genes at body sites suggest that surveillance of CHG tolerance in hospitals may be warranted.

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Conflicts of interest
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References