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: Chlorhexidine gluconate (CHG) containing dressings 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: Here 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 sample positive for smr were also positive for qacA/B. These prevalence rates were not statistically greater than that observed in a subsample of specimens taken from non-CHG treated contralateral arms and non-CHG dressing exposed patient's arms. A statistically greater proportion of specimens with greater than 72 hours exposure to CHG dressings were qac-positive (p= 0.04), suggesting 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 S. epidermidis and S. 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.

Title:

Culture independent detection of chlorhexidine resistance genes *qac*A/B and *smr* in bacterial DNA recovered from body sites treated with chlorhexidine containing dressings.

Running title: Prevalence of chlorhexidine resistance genes in association with chlorhexidine dressing's use

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Abstract:

Purpose: Chlorhexidine gluconate (CHG) containing dressings 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 *qac*A/B and *smr* genes.

Methodology: Here 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 *qac*A/B and 14 (18%) possessed *smr;* all sample positive for *smr* were also positive for *qac*A/B. These prevalence rates were not statistically greater than that observed in a subsample of specimens taken from non-CHG treated contralateral arms and non-CHG dressing exposed patient's arms. A statistically greater proportion of specimens with greater than 72 hours exposure to CHG dressings were *qac*-positive (p= 0.04), suggesting the patients were contaminated with bacteria or DNA containing *qac*A/B during their hospital stay. The presence of *qac* genes was not positively associated with the presence of DNA specific for *S. epidermidis* and *S. 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, whole body decolonisation, and 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 blood stream infection (CRBSI) (4-10). In *Staphylococcus aureus* and coagulase-negative staphylococci (CONS), acquisition of *qac* genes, including *qac*A/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 (2014) 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 increasing use in clinical settings, and have been recommended by international infection prevention guidelines for use with peripherally inserted central catheter (PICC) in high-risk patients (13, 14). Unlike topical CHG preparations, CHG dressings are designed to release the biocide at catheter insertion site over an extended duration (15), thereby preventing skin colonisation over sustained time periods (16, 17). A meta-analysis of nine randomised clinical trials has shown that the use of CHG dressing at catheter insertion sites greatly reduces both catheter colonisation and associated CRBSI rates (18, 19) providing short benefits to patients. However residual microbial

colonisation 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 *qac*A/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 for this purpose.

Materials and Methods:

Ethics: The study was approved by the Human Research Ethics Committees from Queensland Health (HREC/11/QRCH/152 and HREC/13QRBW/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 a *qac*A/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) (Figure supplementary 1).

Study populations and samples: CHG dressings (Biopatch^{TM}, Johnson & Johnson, New Jersey, USA) 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 their middle of upper arms inserted in 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) 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 dressings 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–September 2014. The patients groups were enrolled in a securing all intravenous devices effectively (SAVE) randomised control trial and secured with non-CHG dressings (20). Bacteria isolated from the PIVC insertion skin sites were used as non-CHG dressings exposed patient's control.

All samples were aseptically collected by experienced research nurses (ReN). The dressings were removed from patient's skin by the ReN 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 dressings sites surrounding the catheter insertion point using a sterile swab moistened with sterile 0.9% sodium chloride (Pfizer, New York, USA). A $2cm^2$ surface area at the site was swabbed for 10 seconds 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's (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 research nurses 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 x g for 5 minutes. The supernatant

was removed and pellet resuspended into 400µl PBS. One hundred microliters of the suspension was then plated onto Horse Blood agar (bioMerieux) and/or Chocolate agar (Oxoid) plates, incubated at 37ºC for 72 hours and monitored daily for bacterial growth. Remaining 200µl CHG dressing and skin swab samples were used for isolation of bacterial gDNA. Any bacteria that did grow on agar plates were subsequently subcultured and identified using the Vitek(R) MS (bioMerieux) system.

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

Detection of *qac*A/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 1492R primers targeting conserved V1-V9 regions of 16sRNA. The presence of *qac*A/B and *smr* genes was determined using primers described by Noguchi *et al*., (21). All PCR reactions were carried out using 10µl GoTaq® Green Master Mix (Promega, USA), 1µl forward primer (10µM), 1µl reverse primer (10µM), 3 µl DNA (~20-50 ng) template in a final volume of 25µl. 16S PCR was performed with cycling conditions of 94ºC for 4 minutes for one cycle, 94ºC for 30 seconds, 58ºC for 1 minute and 72ºC for 2 minutes run up to 36 cycles and 72ºC for 7 minutes for the final extension. For *qac*A/B and *smr*, PCR was performed with cycling

conditions of 94ºC for 4 minutes for one cycle, 94ºC for 30 seconds, 56ºC for 30 seconds and 72ºC for 30 seconds run up to 30 cycles and 72ºC for 5 minutes for the final extension. DNA from reference strains (JCM16554, JCM16555, JCM16556) possessing *qac*A/B and *smr* were used as positive controls (Table 1). Genomic DNA from an *S. epidermidis* isolate previously shown to be negative for *qac*A/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 the quantitative real time PCR (*q*PCR) amplification of the *nuc*A and *tuf* genes (22, 23). The primers for *tuf* gene were optimised for real-time PCR. The specificity was confirmed with agarose gel electrophoresis. Of note, *nuc*A and *tuf* were for the specific detection of *S. aureus* and *S. epidermidis*, respectively (22, 23). For these assays, 10µl of Platinum SYBR Green qPCR SuperMix-UDG (Life Technologies, Australia), 1.25µl of both forward and reverse primers (10µM) (Table 1), 2µl of DNA template and sterile dH_2O were added to a final volume of 20µl. PCR cycling was performed by a three step temperature cycling procedure as follows: hold at 50ºC for 2 minutes, second hold at 95ºC for 2 min, 40 cycles of 95ºC for 15s, annealing temperature for 20s, 72ºC for 35s, melting between 72ºC and 95ºC rising by 1ºC. Amplification curve results were validated by analysing melting curves and the threshold melting temperature was set to ±0.5 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, Australia) and all data were generated with Rotor-Gene 6000 (version 7.1) software.

Statistical analysis: Statistical analysis was performed with GraphPad prism package (GraphPad Software Inc, San Diego, USA). The χ^2 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 dressings' exposed and non- exposed skin swabs. For a

comparison of CHG dressing/matched skin swab results by duration exposed to CHG dressing, the samples were divided (<72, 72-168 and >168 hours) by roughly 33% of the samples in each time-points. The χ^2 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.

Results:

Prevalence of *qac*A/B and *smr* and patient's clinical characteristics: To assess the presence of CHG tolerance genes at catheter insertions 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's skin swabs collected from catheter insertion sites covered with non-CHG dressings, demonstrating the effectiveness of CHG-dressings in killing bacteria at these sites (Supplementary Table 1). Additionally, when swabs were collected from the matched non-CHG exposed contralateral arm of ten patients with CHG-dressings in 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 *qac*A/B and *smr* genes at these sites. For this purpose, DNA was recovered independently from both the dressings (n=78) and matching skins swabs (n=66). Other 12 matching skin swabs had insufficient DNA recovered for analysis. Of the 43 patients, 34 (79%) were positive for *qac*A/B, 5 (12%) positive for *smr* and 4 (9%) positive for both *qac*A/B and *smr* (Table 2). Of the all DNA samples recovered directly from the 78 CHG dressings, 52 (67%) were positive for *qac*A/B, and 14 (18%) positive for *smr*, Thirteen of the 14 *smr*-positive DNA samples were positive for both *qac*A/B and *smr*. Of the 66 DNA samples directly recovered from skin swabs, 21 (32%) were positive for *qac*A/B and 6 (9%) positive for *smr*. All *smr* positive skin swabs were also positive for *qac*A/B. When the data from the CHG dressing and skin swab from matching patients was pooled, 52 (67%) of the DNA samples were positive for *qac*A/B, 14 (18%) positive for *smr*,

and 14 (18%) positive for both. In most cases, the samples positive for *qac*A/B was also contained *smr*.

To examine the prevalence of *qac*A/B and *smr* in viable bacteria, we next assessed the prevalence of these genes in the viable bacteria recovered from the non-GCH dressing and non-CHG exposed control groups. Of the 24 skin swabs from non-CHG dressings (Table 2) 10 (42%) possessed *qac*A/B-positive, and 6 (25%) *smr* positive and 2 (8%) for both *qac*A/B and *smr* positive bacterial isolates. Acknowledging the differences of DNA samples isolated from bacterial cultures and non-cultured CHG dressing and skin swab, there was no significant difference (p=0.08, Fisher's test) in the detection of *qac*A/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, 7 of 10 (70%) possessed *qac*-positive staphylococci; 6 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 BSI or CRBSI, between patients who were positive (N=35) /negative (N=8) for *qac*A/B/*smr* (Table 3).

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 *qac*A/B and *smr* positive DNA samples recovered from 66 samples which collected at different time-points after the initial application of the CHG dressing. Our data showed that forty percent of DNA samples collected from the skin that had been exposed to CHG dressing for less than 72 hours (n=21) were *qac*-positive (Figure 1). For samples (n=22)

collected between 72-168 hours of CHG dressing exposure, 68% were *qac*-positive. Of samples (n=23) with greater than 168 hours exposure, 78% were *qac*-postive. A statistically significant difference in the prevalence rate was observed between the short exposure group ($\leq$72 hrs) and longer durations of exposure (p=0.04, Chi-square 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 *qac*A/B positive at all time-points. For the remaining 6 patients, DNA was negative for *qac*A/B at the first dressing change, but positive by the third or fourth dressing change. All patients who returned a positive *qac*A/B result remained positive for future samples. In one instance, *qac*A/B genes were detected in skin swabs collected only at the 4th dressing change, after 22 days of cumulative CHG dressing use. In another patient, the *smr* gene was detected from skin swabs only at the 4th dressing replacement after 14 days 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, *qac*A/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 presences 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 *qac*A/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 *qac*A/B, 12 (46%) possessed *S. epidermidis* DNA, 6 (23%) possessed *S. aureus* DNA, and 2 (8%)

246 possessed both *S. epidermidis* and *S. aureus*. Of the DNA samples positive for *smr* (n=14), 7
247 (50%) also possessed *tuf*, 7 (50%) possesses *nucA* and 3 (21%) possessed both *S. epidermidis*
248 and *S. aureus* DNA. Of the DNA samples that were negative for *smr*, 40 (63%) possessed *S.*
249 *epidermidis* DNA, 22 (34%) possessed *S. aureus* DNA, and 12 (19%) possessed both *S.*
250 *epidermidis* and *S. aureus*. No association between the presence of *qac*A/B and *smr* and DNA
251 from *S. aureus* or *S. epidermidis* was apparent (p=0.5; Fisher's test).

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 health-care settings when compared to bacteria recovered from non-health care environments (6-10). The use of CGH has also been implicated in the colonisation of patients with CHG tolerant bacteria. To our knowledge, this is the first report investigating potential associations between CHG dressings and the prevalence of GHG 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 also revealed high rates of *qac*-positive staphylococci, indicated that 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; a much larger samples size for both experimental and control groups is 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 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 health care workers to be colonised 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 heath care-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 were 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 the most studies focus 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*'s 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 method directly from CHG dressing and skin swab samples, as we were unable to recover any viable bacteria by standard culture methodologies, to examine the *qac*A/B and *smr* prevalence on the CHG dressing exposed skin of hospitalised patients. On the other hand, *qac*A/B and *smr* prevalence was determined in bacterial cultures

from non-CHG exposed control arms. The difference in the *qac*A/B and *smr* detection, one being directly from skin swab and dressing samples and the control non-CHG exposed arms swabs from bacterial cultures, may influence the analysis of the results.

The culture independent method has advantages over the 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 patient's skin for *qac* or other resistance determinants may become part of standard hospital practices.

Conclusion

This study has revealed that *qac*A/B and *smr* are frequently recovered from skin sites where CHG-dressing are used, as well as body sites not exposed to CGH dressings. There is no evidence that CHG-dressing increases the frequency of bacteria harbouring CGH-tolerance genes at catheter insertion sites. However the high rates of recovery of CGH tolerance genes at body sites suggest the surveillance of CHG tolerance in hospitals may be warranted.

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Author Contributions Statement:

Wrote funding application: CR, DJM, RC, MAC. Conceived and designed the experiments: MAC DJM, CR. Performed the experiments: MAC, IR, HS, NG. Analysed the data: MAC, SB Contributed reagents/materials/analysis tools: DJM, CR, DLP, FH, MAC, Contributed to the writing of the manuscript: MAC, DJM, CR. Contributed to and approved final version of the manuscript: MAC, HS, UR, NG, RJC, NM, SB, FH, DLP, CMR and DJM.

Conflict of interest

CMR has undertaken consultancy work, and received unrestricted research/educational grants from makers of catheters and dressings including CHG dressings, but not the products tested in this study. All other authors have declared that no competing interests exist. No commercial entity had any involvement in the funding, design, undertaking or reporting of this study.

Competing Financial Interests

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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Figure legend:

Figure 1: Impact of duration of CHG dressing placement on the presence of CHG tolerance genes in CHG dressing/matched skin swab samples (N=66).

Table 1: Oligonucleotide sequences of primers used for detection of 16S, *qac*A/B, *smr*, *nucA* and *tuf* genes in bacterial DNA

Gene	Forward/reverse 5′-3′	Product size (bp)	Positive control	Reference
16S	AGAGTTTGATCMTGGCTCAG CGGTTACCTTGTTACGACTT	2465	*S. epidermidis*[a]	(25)
*qac*A/B	GCAGAAAGTGCAGAGTTCG CCAGTCCAATCATGCCTG	361	JCM 16555[b], JCM 16556[c]	(21)
smr	GCCATAAGTACTGAAGTTATTGGA GACTACGGTTGTTAAGACTAAACCT	195	JCM 16554[d]	(21)
nucA[e]	GCGATTGATGGTGATACGGTT AGCCAAGCCTTGACGAACTAA AGC	274	ATCC25923	(23)
tuf[f]	GCCAGTTGAGGACGTATTCT CCATTTCAGTACCTTCTGGTAA	412	ATCC14990	(22)

JCM; Japan Collection of Microorganisms; [a]*S. epidermidis* (isolated form connectors in administration set); [b] S. aureus; encoding multidrug efflux gene *qac*A; [c] S. aureus; encoding multidrug efflux gene *qacB*; [d] S. aureus; Small multidrug resistance gene *smr*; [e]*nucA* – Thermostable nuclease gene, [f]*tuf* – Elongation factor Tu; *nucA* specific for *S. aureus* and *tuf* is specific for *S. epidermidis*.

Table 2. CHG tolerance genes in bacterial DNA associated with CHG dressing use or contralateral arms.

Source of bacterial DNA samples		Number of samples/patients (%)			
		Presence of CHG tolerance genes			Absence of CHG tolerance genes
	DNA samples	*qac*A/B	*smr*	*qac*A/B and matched *smr*	
CHG dressing	78	52 (67)	14 (18)	13 (17)	25 (32)
CHG skin swab[a]	66	21 (32)	6 (9)	6 (9)	45 (68)
Pooled matched CHG dressing and skin swab[b]	78	52 (67)	14 (18)	14 (18)	26 (33)
	Patients				
CHG dressing and matched skin swab[c]	43	34 (79)	5 (12)	4 (9)	8 (19)
Non-CHG skin swabs from contra-lateral skin sites[d]	10	7 (70)	6 (60)	6 (60)	3 (30)
Non-CHG skin swabs from patients without CHG dressings [e]	24	10 (42)	6 (25)	2 (8)	10 (42)

[a] number of *qac*A/B and *smr* positive genes that detected in total number of skin swab DNA samples; [b]number of *qac*A/B and *smr* genes that detected in total number of CHG dressing and matched skin swab DNA samples combined; [c] number of *qac*A/B and *smr* genes that detected in bacteria isolated from patients who had matched CHG dressing and skin swab DNA samples; [d] number of *qac*A/B and *smr* genes that detected in bacteria that isolated from patients who had swabs taken from non-CHG exposed contra-lateral skin sites; [e] number of *qac*A/B and *smr* genes that detected in bacteria that isolated from patients who had swabs taken from non-CHG dressings exposed skin sites.

436 Table 3. Clinical characteristics of 43 patients with or without CHG tolerance genes tolerance
437 genes bacterial DNA found in CHG dressings/matching skin swabs.

Characteristics	Number of patients/ samples (%) CHG tolerance genes positive (n=35)	CHG tolerance genes negative (n=8)	*p* value
Gender			
Male	20 (57)	4 (50)	1.0
Female	15 (43)	4 (50)	
Age (years)			
≥50	26 (74)	7 (88)	0.7
<50	9 (26)	1 (13)	
Admitting diagnosis			
Medical	7 (20)	2 (25)	0.8
Haematology	5 (14)	1 (13)	
Oncology	5 (14)	2 (25)	
Surgical Vascular	4 (11)	1 (13)	
Surgical Gastrointestinal	7 (20)	1 (13)	
Surgical Orthopaedic	6 (17)	1 (13)	
Clinical diagnosis			
BSI	2 (6)	0	1.0
CRBSI	1 (3)	0	

438 *p* value by chi-square test or Fisher's exact test.

439 Table 4. Bacterial species detected from pooled CHG dressing and skin DNA samples in 43
440 patients.

Presence of CHG tolerance genes	Number of samples (%)		
	S. epidermidis	*S. aureus*	*S. epidermidis* and *S. aureus*
*qac*A/B (n=52)	38 (73)	24 (46)	16 (31)
smr (n=14)	7 (50)	7 (50)	3 (21)
*qac*A/B + *smr* (n=13)	7 (54)	6 (46)	3 (23)
Absence of CHG tolerance genes			
*qac*A/B (n=26)	12 (46)	6 (23)	2 (8)
smr (n=64)	40 (63)	22 (34)	12 (19)

441 n; number of samples

Figure1

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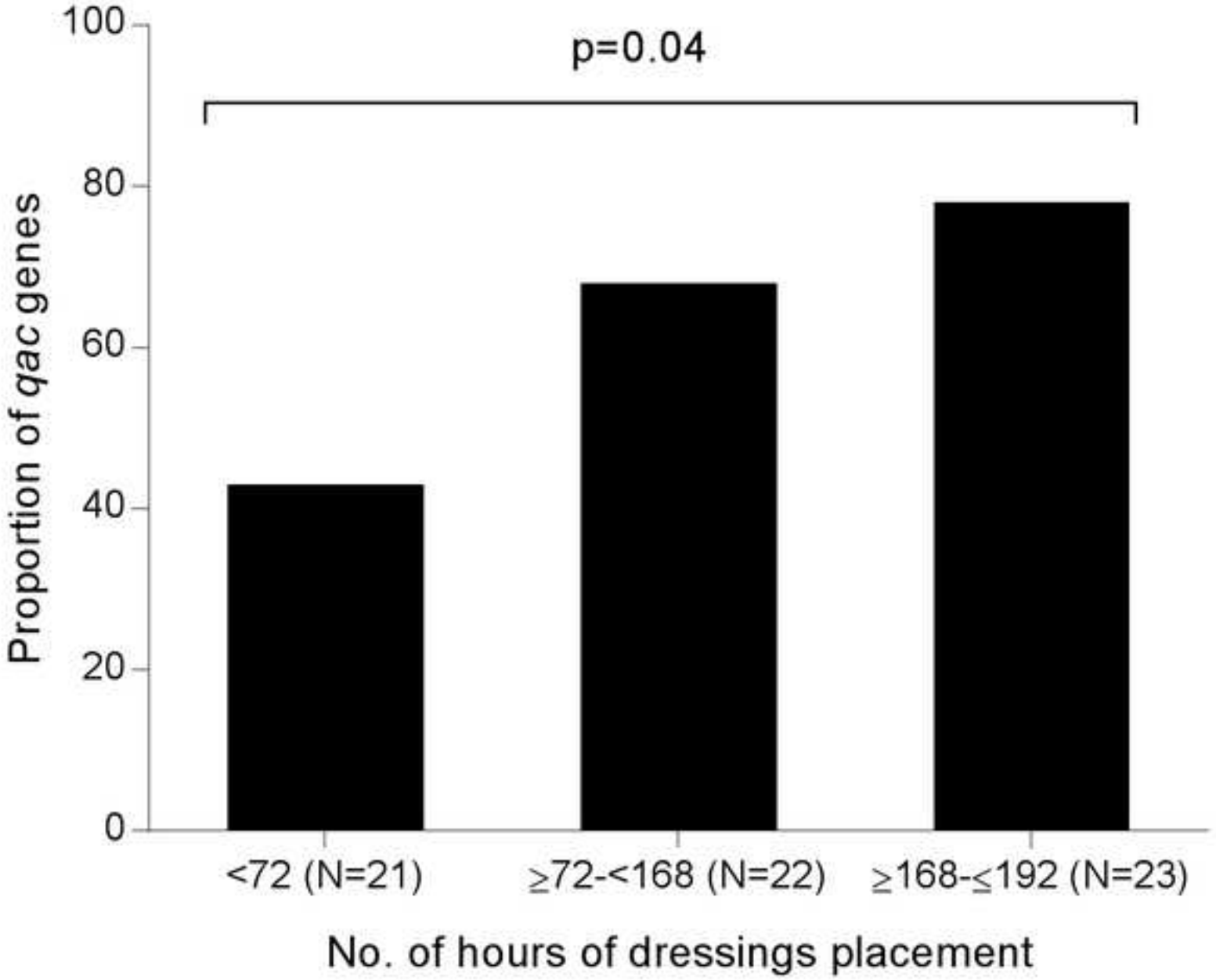
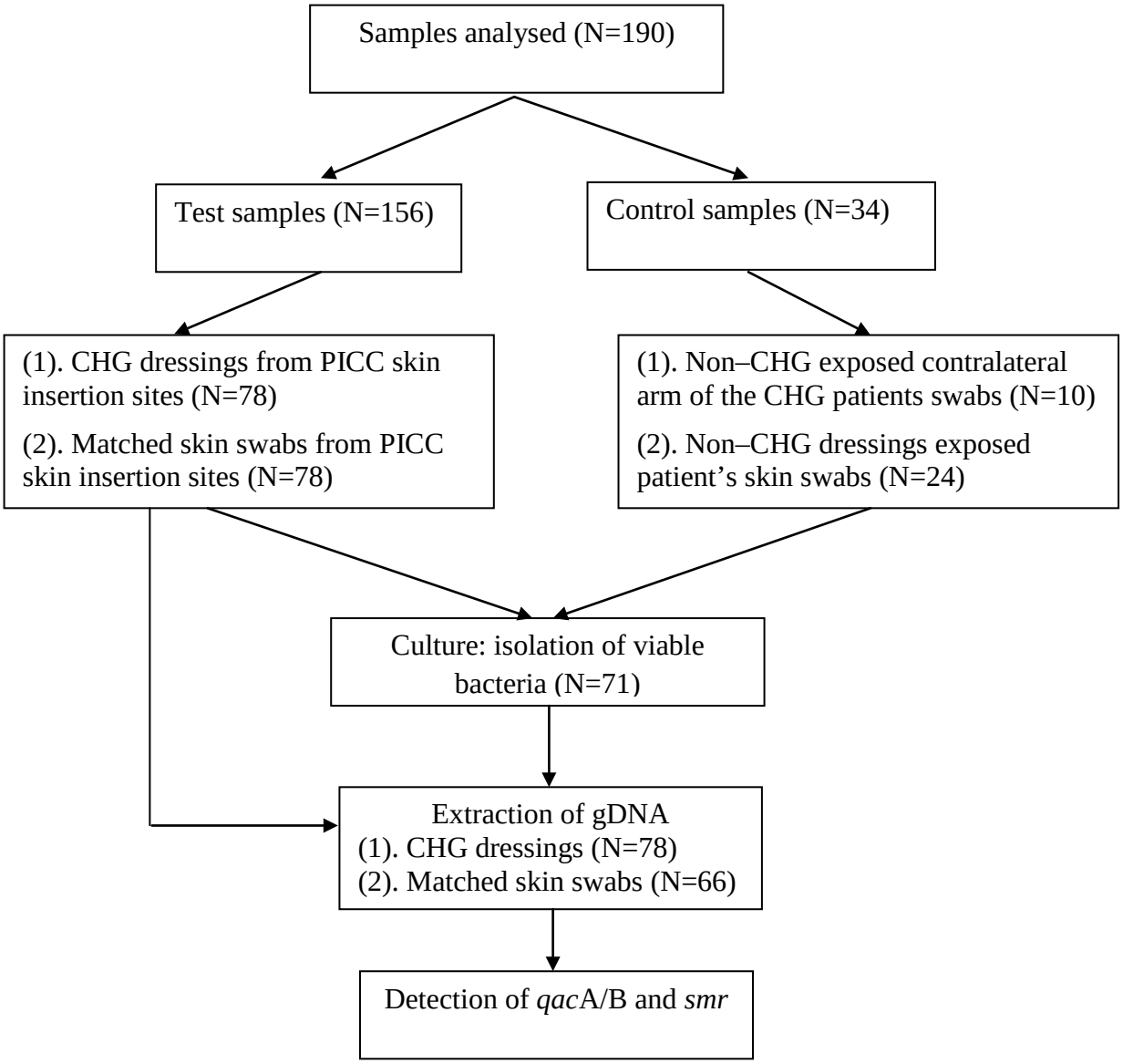


Supplementary Figure 1. Study design flow chart



Supplementary Table 1: Bacterial species detected from 24 patient's skin swabs collected at catheter insertion sites covered with non-CHG dressings.

Patients no.	Isolates no.	Organism	*qac*A/B	*smr*
1	1A	*Pseudomonas aeruginosa*	Positive	Negative
	1B	*Staphylococcus epidermidis*	Negative	Negative
2	2A	*Bacillus cereus*	Negative	Negative
	2B	*B. Cereus*	Negative	Negative
3	3	*Micrococcus luteus*	Negative	Negative
4	4	*S. hominis*	Positive	Negative
5	5	*S. epidermidis*	Positive	Negative
6	6A	*S. capitis*	Negative	Negative
	6B	*S. aureus*	Negative	Negative
7	7	*S. epidermidis*	Positive	Negative
8	8	*S. epidermidis*	Negative	Negative
9	9A	*S. capitis*	Negative	Positive
	9B	*S. epidermidis*	Negative	Positive
	9C	*S. capitis*	Negative	Positive
10	10	*S. capitis*	Negative	Negative
11	11	*S. epidermidis*	Positive	Negative
12	12	*S. epidermidis*	Negative	Negative
13	13	*S. hominis*	Negative	Positive
14	14	*S. epidermidis*	Negative	Negative
15	15	*Micrococcus luteus*	Positive	Positive
16	16	*Kytococcus sedenticus*	Positive	Negative
17	17A	*S. epidermidis*	Positive	Negative
	17B	*S. carnosus*	Negative	Negative
18	18	*Micrococcus luteus*	Negative	Negative
19	19A	*Micrococcus luteus*	Negative	Negative
	19B	*S. haemolyticus*	Positive	Negative
20	20	*Micrococcus luteus*	Negative	Negative
21	21A	*S. hominis*	Negative	Negative
	21B	*S. epidermidis*	Positive	Positive
22	22	*Micrococcus luteus*	Negative	Negative
23	23	*S. epidermidis*	Negative	Positive
24	24a	*S. epidermidis*	Negative	Positive
	24B	*S. capitis*	Negative	Positive