

# The effects of daily chlorhexidine bathing on cutaneous bacterial isolates: a pilot study

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**Abstract:** Chlorhexidine gluconate (CHG) is a topical antiseptic used in a myriad of clinical settings. Recently, CHG baths have been shown to decrease multidrug-resistant organism acquisition and infections and catheter-associated bloodstream infections. The present study examined the effects of daily bathing with CHG on the recovery and antimicrobial susceptibility of cultivable cutaneous bacteria. The objectives of this study were to (1) explore the effects of clinical CHG bathing on cultivable cutaneous bacteria, (2) study the relationship between CHG minimum inhibitory concentration and antimicrobial susceptibility of coagulase-negative staphylococci, and (3) demonstrate the feasibility of the approach so a more definitive study may be performed. Significant decreases in bacterial colony counts and phenotypic diversity occurred with greater CHG exposure. The findings also suggest an inverse relationship between CHG minimum inhibitory concentration and antimicrobial susceptibility. Larger prospective studies are necessary to fully investigate the clinical impact of CHG usage.

**Keywords:** antiseptic, resistance, *Staphylococcus*, coagulase-negative

## Introduction

Chlorhexidine gluconate (CHG) is a topical antiseptic used in a myriad of clinical settings. Recently, CHG baths have been shown to decrease multidrug-resistant organism acquisition and infections and catheter-associated bloodstream infections.<sup>1,2</sup>

Limited in vitro data suggest that some organisms develop elevated minimum inhibitory concentrations (MICs) to CHG with continued exposure.<sup>3</sup> In *Staphylococcus aureus*, the *qacA* plasmid-borne resistance determinant is associated with efflux of CHG from bacteria<sup>4</sup> and with antimicrobial resistance.<sup>3,5–7</sup> Clinical data are needed to better understand the extent to which regular clinical CHG exposure is associated with elevated CHG MICs and reduced susceptibility to other antimicrobials. Furthermore, most prior studies focus on *qacA* activity in *S. aureus*, although coagulase-negative staphylococci (CoNS) commonly cause catheter-associated infections.

The objectives of this study were to (1) explore the effects of clinical CHG bathing on cultivable cutaneous bacteria, (2) study the relationship between CHG MIC and antimicrobial susceptibility of CoNS, and (3) demonstrate the feasibility of the approach so a more definitive study may be performed.

## Materials and methods

In the authors' hospital, Seattle Children's Hospital (Seattle, WA), patients with central lines are "bathed" daily by wiping their skin, from the neck down, with a 2% CHG-impregnated cloth (Sage Products, Inc, Cary, IL). Pediatric patients were eligible

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for study enrollment if they were aged 2 months or older, hospitalized between November 2010 and April 2011, and scheduled to receive daily CHG baths.

Samples were obtained by rubbing a saline-moistened sterile nylon-flocked swab (Copan Diagnostics Inc, Murrieta, CA) over a 9 cm<sup>2</sup> area of skin for 30 seconds. Patients were swabbed in the right axilla weekly until discharge, and the same researcher performed the swabbing each time. Swabs were vortexed in normal saline and aliquots were inoculated onto the following agars: sheep blood, chocolate, mannitol salt, MacConkey, and Sabouraud dextrose. Plates were incubated at 35°C and checked for growth by 48 hours; any growth was subcultured and identified as CoNS, *S. aureus*, *Streptococcus pneumoniae*, *Enterococcus* spp, viridans streptococci, group A streptococci, *Bacillus* spp, diphtheroids, yeast, or gram-negative (no further analysis of gram-negative organisms).<sup>8</sup> The total colony count of a swab was obtained from the blood agar plate.

CoNS isolates underwent antimicrobial susceptibility testing and were classified as susceptible or nonsusceptible using Clinical and Laboratory Standards Institute recommendations.<sup>9</sup> Antimicrobials tested were penicillin (10 µg), amoxicillin-clavulanate (20/10 µg), cefazolin (30 µg), cefoxitin (30 µg), ceftriaxone (30 µg), clindamycin (2 µg), erythromycin (15 µg), gentamicin (10 µg), trimethoprim-sulfamethoxazole (1.25/23.75 µg), ciprofloxacin (5 µg), rifampin (5 µg), linezolid (30 µg), and vancomycin (range 0.016–256 µg/mL). Vancomycin susceptibility was determined by Etest® (bioMérieux, Inc, Durham, NC); susceptibility to all other antibiotics was determined by disk diffusion. CHG MIC testing was conducted using a 96-well broth microdilution method.<sup>9</sup> Quality control organisms were included in each panel.

Polymerase chain reaction (PCR) amplification for *qacA/B* was performed with the GeneAmp® system (Applied Biosystems, Carlsbad, CA), using primers for sequences conserved between the two genes.<sup>10</sup> To distinguish *qacA* from *qacB* – which does not encode a CHG efflux pump – initial *qacA/B* PCR products were digested with the *AluI* restriction

enzyme; *qacA* and *qacB* fragments were detected by agarose gel electrophoresis.<sup>10</sup> Appropriate positive and negative control isolates were used in each step.

Every swab obtained was categorized into one of the following CHG exposure groups: none (no CHG exposure), moderate (1–14 daily CHG baths), and heavy (>14 daily CHG baths). Some patients contributed swabs to more than one exposure group; therefore, analyses were based on CHG exposure of the swabs, rather than that of the patients. To minimize the possibility of one patient's specimens heavily influencing the overall results, each patient was allowed to contribute only one swab per CHG exposure group for analyses of total colony and phenotype counts. If a patient had more than one swab in an exposure category, only the swab with the greatest CHG exposure was included. Similarly, each patient was allowed to contribute one CoNS isolate per CHG exposure category for analyses of CHG MIC. If a patient had more than one isolate in an exposure category, only the isolate with the highest CHG MIC was included. Lastly, for analyses of antimicrobial susceptibility versus CHG MIC, patients were allowed to contribute one CoNS isolate per CHG MIC category ( $\leq 1$  µg/mL or  $\geq 2$  µg/mL).

The authors compared bacterial colony counts per swab and number of bacterial phenotypes per swab across exposure groups using the Kruskal-Wallis test. The presence of antimicrobial susceptibility across exposure groups was evaluated using Fisher's exact test. Statistical analyses were performed using Stata (v 10.0; StataCorp LP, College Station, TX).

## Results

Twenty-four patients were enrolled in the study. An equal number of male and female participants were involved, and participants had a mean age of 5.7 years (range from 2 months to 19 years). Ultimately, four, fifteen, and ten swabs and three, eleven, and four individual CoNS isolates were included in the “none,” “moderate,” and “heavy” CHG exposure groups, respectively (Table 1). In the moderate and heavy exposure groups, the mean number of days receiving

**Table 1** Chlorhexidine gluconate (CHG) exposure versus measured outcomes

CHG exposure	Swabs (n)	Bacterial colony count per swab <sup>a</sup>	Bacterial phenotypes per swab (n) <sup>a</sup>	CoNS isolates (n)	CHG MIC <sub>50</sub> (µg/mL) <sup>a</sup>
None	4	5145 (15–12 870)	2 (1–3)	3	1 (1–2)
Moderate (1–14 CHG baths)	15	15 (0–30 750)	1 (0–3)	11	1 (0.5–2)
Heavy (>14 CHG baths)	10	0 (0–7500)	0 (0–1)	4	1.5 (1–4)

**Notes:** <sup>a</sup>Median values provided; numbers in parentheses reflect range values.

**Abbreviations:** CoNS, coagulase-negative staphylococci; MIC<sub>50</sub>, median minimum inhibitory concentration.

CHG baths was 7.5 (range 1–14) and 51.6 (range 17–139), respectively (data not shown).

Median bacterial colony count per swab decreased from 5145 (range 15–12, median 870) to 15 (range 0–30, median 750) to 0 (range 0–7500) for the “none,” “moderate,” and “heavy” CHG exposure groups, respectively ( $P=0.03$ ). The median number of phenotypically different bacteria per swab also decreased across exposure groups: 2 (range 1–3), 1 (range 0–3), and 0 (range 0–1), respectively ( $P=0.02$ ) (Table 1).

The median CHG MIC was 1 µg/mL for both low and moderate CHG exposure groups (range 1–2 and 0.5–2 µg/mL, respectively) and 1.5 µg/mL (range 1–4 µg/mL) for the heavy exposure group ( $P=0.35$ ) (Table 1).

All CoNS isolates were susceptible to vancomycin, linezolid, and rifampin. Except for penicillin, antimicrobial susceptibility was more frequent in isolates in the lower CHG MIC group (MIC ≤ 1 µg/mL) than in the higher CHG MIC group (MIC ≥ 2 µg/mL) (Table 2). Eighty percent of isolates in the higher MIC group were found to be resistant to three or more antimicrobials, compared with only 27% of isolates in the lower MIC group ( $P=0.11$ ).

Of 17 tested CoNS isolates, eleven (65%) carried *qacA/B*. Ten of these isolates carried *qacA* specifically; *AluI* digestion was unsuccessful for one PCR product. *qacA* was detected in 100% of isolates in the higher CHG MIC group and in 45% of isolates in the lower MIC group ( $P=0.09$ ); 50% of *qacA*-positive isolates had CHG MICs ≥ 2 µg/mL.

**Table 2** Comparison of antimicrobial susceptibility in isolates with a minimum inhibitory concentration of chlorhexidine gluconate (CHG MIC) ≤ 1 µg/mL and isolates with a CHG MIC ≥ 2 µg/mL

Antimicrobial agent	% susceptible to specified agent <sup>a</sup>		P-value <sup>b</sup>
	CHG MIC ≤ 1 µg/mL (n = 11)	CHG MIC ≥ 2 µg/mL (n = 5)	
Amoxicillin-clavulanate	100	80	0.31
Cefoxitin	72	20	0.11
Cefazolin	100	20	0.003
Ceftriaxone	72	40	0.30
Ciprofloxacin	100	20	0.003
Clindamycin	91	20	0.01
Erythromycin	45	20	0.59
Gentamicin	100	40	0.02
Penicillin	18	20	0.99
Trimethoprim-sulfamethoxazole	72	20	0.11

**Notes:** <sup>a</sup>All isolates were susceptible to vancomycin, linezolid, and rifampin; <sup>b</sup>from Fisher's exact test.

## Discussion

In a sample of pediatric patients with central lines and receiving daily CHG baths, greater CHG exposure was associated with lower colony counts of cultivable cutaneous bacteria and fewer different bacterial phenotypes. CoNS isolates with elevated CHG MICs were significantly less susceptible to several antimicrobials.

With increasing clinical use of CHG, there is concern that organisms may develop reduced susceptibility to the antiseptic and to other antimicrobials.<sup>3</sup> A slight increase was observed in the median CHG MIC of cutaneous CoNS for the highest exposure group, but the difference was not significant. Similarly, while *qacA* was detected more frequently in CoNS with higher CHG MICs, the difference was not significant. However, it was found that the frequency of antimicrobial nonsusceptibility was significantly higher among CoNS with higher CHG MICs, suggesting an association between CHG MIC and resistance determinant(s) in CoNS. Prior studies most clearly demonstrate plasmid-based linkages between *qacA/B* and beta-lactamase genes.<sup>5,6</sup> Another possible explanation is that antimicrobial resistance is, in part, mediated by the QacA efflux pump.

This study was limited by its observational design and small convenience sample. The small sample size limited the ability to detect significant differences in median CHG MIC and presence of *qacA* among the different exposure groups. Nevertheless, this study is unique in its emphasis on clinical CHG use and suggests important hypotheses for future confirmatory studies.

## Conclusion

The authors observed significant decreases in cultivable cutaneous bacterial colony counts and the number of phenotypes with increased CHG exposure. While the data did not demonstrate a clear association between CHG exposure and CHG MICs, the findings support an inverse relationship between CHG MIC and antimicrobial susceptibility in CoNS. Larger prospective studies are necessary to fully investigate the clinical impact of CHG usage.

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## Disclosure

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