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Original Article

Relationship between chlorhexidine gluconate concentration and microbial colonization of patients' skin

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Abstract

Objective: To characterize the relationship between chlorhexidine gluconate (CHG) skin concentration and skin microbial colonization.

Design: Serial cross-sectional study.

Setting/participants: Adult patients in medical intensive care units (ICUs) from 7 hospitals; from 1 hospital, additional patients colonized with carbapenemase-producing Enterobacterales (CPE) from both ICU and non-ICU settings. All hospitals performed routine CHG bathing in the ICU.

Methods: Skin swab samples were collected from adjacent areas of the neck, axilla, and inguinal region for microbial culture and CHG skin concentration measurement using a semiquantitative colorimetric assay. We used linear mixed effects multilevel models to analyze the relationship between CHG concentration and microbial detection. We explored threshold effects using additional models.

Results: We collected samples from 736 of 759 (97%) eligible ICU patients and 68 patients colonized with CPE. On skin, gram-positive bacteria were cultured most frequently (93% of patients), followed by *Candida* species (26%) and gram-negative bacteria (20%). The adjusted odds of microbial recovery for every twofold increase in CHG skin concentration were 0.84 (95% CI, 0.80–0.87; $P < .001$) for gram-positive bacteria, 0.93 (95% CI, 0.89–0.98; $P = .008$) for *Candida* species, 0.96 (95% CI, 0.91–1.02; $P = .17$) for gram-negative bacteria, and 0.94 (95% CI, 0.84–1.06; $P = .33$) for CPE. A threshold CHG skin concentration for reduced microbial detection was not observed.

Conclusions: On a cross-sectional basis, higher CHG skin concentrations were associated with less detection of gram-positive bacteria and *Candida* species on the skin, but not gram-negative bacteria, including CPE. For infection prevention, targeting higher CHG skin concentrations may improve control of certain pathogens.

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Introduction

Daily chlorhexidine gluconate (CHG) bathing of intensive care unit (ICU) patients reduces skin microbial colonization and decreases the risk of bloodstream infections, multidrug-resistant organism acquisition, and blood culture contamination.[1–3] However, the association between CHG skin concentration and skin microbial bioburden is less understood. Some observational studies suggest that reaching threshold CHG skin concentrations may be needed for optimal inhibition of skin microbial growth,[4,5] but this relationship has not been consistently reproduced.[6] Understanding the association between CHG skin concentration and skin microbial reduction can inform strategies for improving CHG bathing, as bathing quality can be variable.[7–9]

In the context of a multicenter CHG bathing quality improvement study of adult ICU patients,[9] we performed a pre-planned analysis to characterize the relationship between CHG skin concentration and skin microbial detection. At 1 hospital, we additionally obtained samples from adult ICU and non-ICU patients colonized with carbapenemase-producing Enterobacterales (CPE). We hypothesized that patients with higher CHG skin concentrations would have less microbial detection on skin and

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sought to determine if a threshold effect existed in this relationship (ie, an 'adequate' CHG skin concentration beyond which skin microbial detection would be maximally reduced).

Methods

Study population

Multicenter cohort

Patients ≥18 years old who were admitted to the medical ICU were eligible for study participation at 7 academic hospitals with established daily CHG bathing (hereafter called "multicenter cohort"; see Supplement for participating sites). The median ICU bed capacity was 22 (range, 12–27 beds). Point prevalence surveys were conducted from January 2018 to February 2019.

CPE-colonized cohort

Due to the expected low prevalence of CPE-colonized patients in the multicenter cohort,[10] we obtained samples from an additional group of adult ICU and non-ICU patients who were confirmed to be colonized with CPE[11] by rectal or stool culture and were admitted from May 2018 to August 2019 (hereafter called "CPE cohort") at one of the participating hospitals (Rush University Medical Center). Patients in the CPE cohort were eligible for daily CHG bathing if admitted to the ICU or if they had a central venous catheter while cared for in non-ICU units. Five patients were analyzed in both the multicenter and CPE cohorts.

The project was evaluated independently by each institution's institutional review board and either deemed exempt or approved with a waiver of informed consent.

Point prevalence surveys and swab sample collection

For the multicenter study, we conducted 6 single-day point prevalence surveys at each hospital throughout the study period. For each survey, all patients in the ICU had unilateral skin swab samples collected from the anterior neck, axilla, and inguinal region. To measure CHG skin concentrations, we used sterile swabs moistened with sterile water (Bio-Swab, Arrowhead Forensics, Lenexa, KS) to swab a 5×5 cm^2 area from each body site. For bacterial and yeast cultures, an adjacent 5×5 cm^2 area from each body site was sampled using flocked swabs (FLOQSwabs, Copan, Murrieta, CA) and placed immediately into 1.2 mL Amies medium with neutralizers[12,13] but without ether sulfate.[14] Swab sample collection training sessions were held with research staff for uniform technique. For the CPE cohort, the neck, axilla, and inguinal skin sites were sampled, and an additional rectal or stool swab (BBL CultureSwab, Becton-Dickenson, Franklin Lakes, NJ) was collected to confirm CPE colonization.

We collected the following patient covariates at the time of survey: demographic information (age [≥ 90 years old recorded as 90 years], sex, body mass index), ICU and hospital length of stay, presence of invasive devices (mechanical ventilation via endotracheal tube or tracheostomy; central venous catheter), and receipt of CHG bath at any point during current hospitalization, prior to swab collection.

Laboratory methods

Swab samples were shipped in insulated containers on wet ice with continuous temperature monitoring and processed at a central laboratory (Rush University Medical Center) within 48 hours of collection. Skin swabs were tested for CHG concentration with a semiquantitative colorimetric assay, with a stepwise range of detection from 4.9 μg/mL to 20,000 μg/mL.[15] For culture, 100 μl volumes were inoculated onto 5% sheep's blood agar (Remel, Lenexa, KS) for total bacterial counts, Columbia CNA agar (Remel) to isolate gram-positive bacteria, MacConkey agar (Remel) to isolate gram-negative bacteria, CHROMagar™ Staph aureus (Becton-Dickenson, Franklin Lakes, NJ) to isolate *Staphylococcus aureus*, ChromID MRSA (bioMérieux, Durham, NC) to isolate methicillin-resistant *S. aureus*, CHROMagar™ Candida (Becton-Dickenson) to isolate *Candida* species, bile azide esculin agar (Remel) to isolate *Enterococcus* species, Spectra VRE agar (Remel) to isolate vancomycin-resistant enterococci, and mSuperCARBA (CHROMagar™, Paris, France) to isolate carbapenem-resistant Enterobacterales, *Pseudomonas* species, and *Acinetobacter* species. Plates were incubated in aerobic conditions at 35 ± 2°C for 16–24 hours for bacterial isolation, and CHROMagar™ Candida agar was incubated at 37°C for up to 7 days. Presumptive morphologic microbial identifications were confirmed using standard methods and matrix-assisted laser desorption ionization time-of-flight mass spectrometry (VITEK® MS bioMérieux). Antibiotic susceptibilities were confirmed using gram-negative and gram-positive panels (NM43, NC68, PC33, and PM29) on the MicroScan WalkAway System (Beckman Coulter, Indianapolis, IN). Organisms recovered on mSuperCARBA were tested for bla-KPC, bla-NDM, bla-OXA-48, bla-IMP, and bla-VIM carbapenemase genes by Xpert Carba-R (Cepheid, Sunnyvale, CA).

CHG minimum inhibitor concentration measurements

Isolates recovered from skin swab samples underwent broth microdilution testing to determine CHG minimal inhibitory concentrations (MICs) following modified Clinical and Laboratory Standards Institute (CLSI) guidelines,[16,17] starting with a 20% solution of chlorhexidine digluconate (Sigma-Aldrich, St Louis, MO). A representative sample of skin isolates was obtained by a mix of random and complete sampling to select isolates dependent on the number of isolates overall for a species, resistance type, or individual hospital level.

Statistical design and analysis

We performed linear mixed effects multilevel modeling to analyze the relationship between CHG skin concentration (log2-transformed) and microorganism recovery (yes/no as primary outcome and CFU/25cm^2 as secondary outcome), controlling for clustering of body sites within patients (random effect). Fixed effects in the model included hospital, body site, and CHG skin concentration. CHG concentrations below the limit of detection (< 4.9 μg/mL) were coded as 0 μg/mL for analysis. Descriptive statistics were also performed. SAS version 9.4 (Cary, North Carolina) was used for all analyses. A series of exploratory analyses considered the possibility of thresholds for microorganism detection by dichotomizing CHG concentration at each increment and adding the dichotomous variable to the model with the linear CHG skin concentration (log2-transformed). Ten thresholds were considered for each outcome.

Results

For the multicenter cohort, we obtained samples from 736 (97%) of 759 eligible patients from 7 hospitals, with a mean of 17.5 (SD = 5.2) patients per ICU per survey. A total of 2,176 skin sites were sampled. In the CPE cohort, 68 patients with CPE colonization based on rectal or stool cultures were identified, and 203 skin sites were sampled. Patient characteristics are shown in Table 1.

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Table 1. Patient demographics and clinical factors

Covariate	Multicenter cohort (N=736)	CPE cohort (N=68)
Age, years, mean (SD)	58.6 (16.3)	57.1 (16.6)
Male, n (%)	377 (51)	28 (41)
Body mass index, kg/m2, median (IQR)	27.3 (22.6, 32.6)	25.7 (22.2, 30.6)
Mechanical ventilation, n (%)	296 (40)	17 (25)
Tracheostomy, n (%)	117 (16)	26 (38)
Central venous catheter, n (%)	376 (51)	22 (33)
ICU day of swab specimen collection,[a] median (IQR)	4 (2, 8)	2 (1, 3)[b]
Hospital day of swab specimen collection,[a] median (IQR)	5 (3, 13)	2 (2, 4)
CHG bath received, n (%)	653 (90)	51 (75)
Hours since last CHG bath, median (IQR)	10 (5, 17)	11 (9, 29)
Median CHG skin concentration,[c] µg/mL (IQR)	39.1 (4.9, 312.5)	78.1 (<4.9, 312.5)

Note. CHG, chlorhexidine gluconate; CPE, carbapenemase-producing Enterobacterales; ICU, intensive care unit; IQR, interquartile range; SD, standard deviation.
[a]Days from admission to swab specimen collection.
[b]Includes 44 ICU patients at the time of sample collection.
[c]Includes 2,163 skin swabs for the multicenter cohort and 201 skin swabs for the CPE cohort.

Table 2. Prevalence of microorganisms by body site on skin of intensive care unit patients at 7 hospitals where chlorhexidine gluconate bathing was routine

Organism	Neck (N=732)	Axilla (N=730)	Inguinal (N=714)	Any site[a] (N=736)
Gram-positive bacteria, n (%)	614 (84)	463 (63)	457 (64)	682 (93)
Selected gram-positive species				
Staphylococcus aureus, n (%)	66 (9)	27 (4)	35 (5)	96 (13)
Methicillin-resistant *S. aureus,* n (%)	21 (3)	8 (1)	11 (2)	28 (4)
Enterococcus species[b], n (%)	73 (10)	41 (6)	127 (18)	177 (24)
Vancomycin-resistant enterococci[b], n (%)	24 (3)	12 (2)	45 (6)	61 (8)
Gram-negative bacteria, n (%)	62 (8)	46 (6)	89 (12)	150 (20)
Selected gram-negative species				
Acinetobacter species, n (%)	9 (1)	5 (0.7)	7 (1)	15 (2)
Klebsiella pneumoniae, n (%)	23 (3)	17 (2)	23 (3)	45 (6)
Pseudomonas aeruginosa, n (%)	16 (2)	12 (2)	21 (3)	44 (6)
Candida species, n (%)	78 (11)	61 (8)	120 (17)	188 (26)
Selected *Candida* species				
Candida auris, n (%)	1 (0.1)	2 (0.3)	0 (0)	3 (0.4)

[a]Organism detection on any body site.
[b]*E. faecalis* and *E. faecium.*

Microorganisms cultured

In the multicenter cohort, gram-positive bacteria were detected most frequently (93%), followed by *Candida* species (26%) and gram-negative bacteria (20%; see Table 2); there was variability in the distribution of pathogens by body site. In the CPE cohort, there were 78 CPE organisms identified from 68 rectal or stool swab samples; KPC-producing *K. pneumoniae* were identified most frequently (Supplementary Table S1). Of 68 patients confirmed to be CPE-colonized by rectal or stool culture, 26 (38%) had skin sites with detectable CPE. Of the 26 patients with CPE detected on both skin and rectal/stool cultures, 25 had concordant CPE species and resistance mechanisms detected. Three patients were co-colonized with carbapenemase-producing *K. pneumoniae* (2 KPC, 1 NDM) and another CPE (including KPC-producing *Citrobacter freundii*, KPC-producing *Escherichia coli*, and NDM-producing *E. coli*) based on rectal or stool culture; however, only carbapenemase-producing *K. pneumoniae* was cultured from the skin.

Relationship between CHG skin concentrations and microbial recovery

Median CHG skin concentrations are noted in Table 1. In the multicenter cohort, the adjusted odds of detecting gram-positive bacteria or *Candida* species on skin decreased linearly with increasing CHG skin concentrations. For every twofold increase in CHG skin concentration, the adjusted odds of microbial recovery decreased by 16% ($P < .001$) for gram-positive bacteria and 7% ($P = .008$) for *Candida* species (Figure 1, Table 3). We did not observe a significant association between CHG skin concentration and detection of gram-negative bacteria by culture (Figure 1, Table 3). In the CPE cohort, after adjusting for age and body site, we did not

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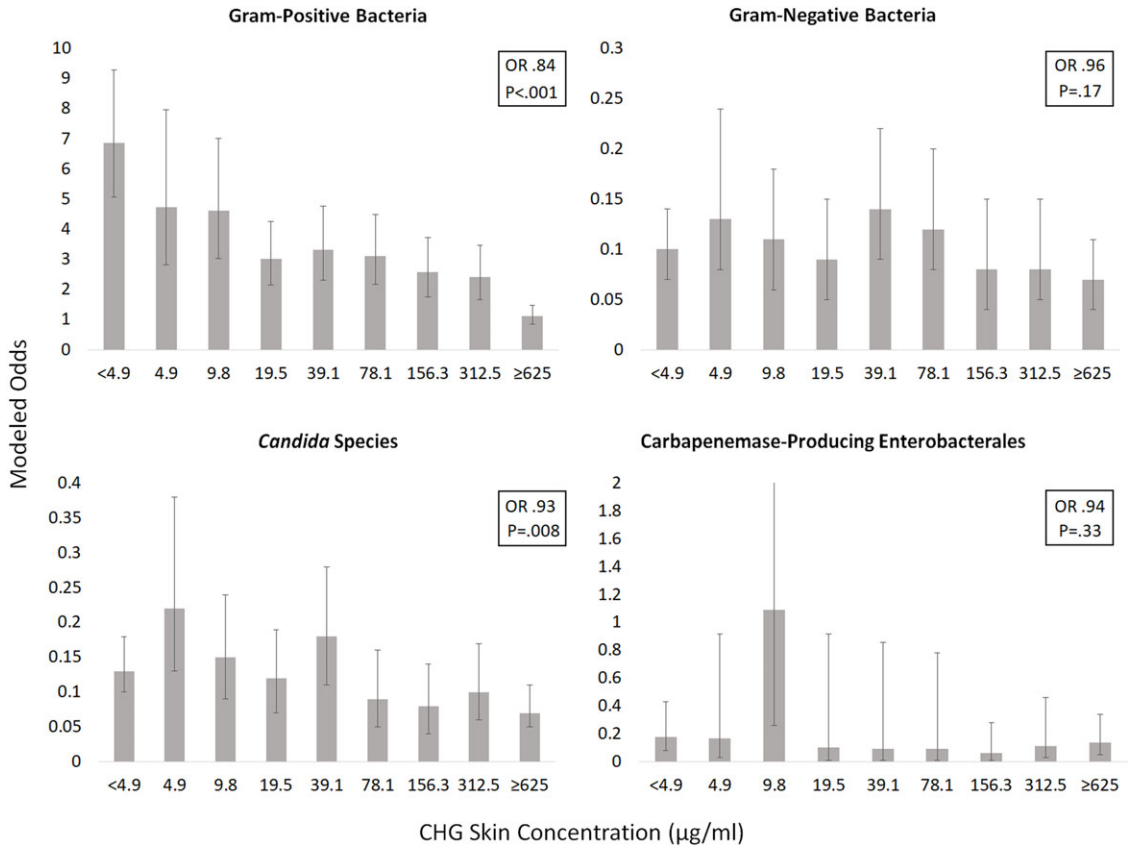

Figure 1. Relationship between chlorhexidine gluconate concentration and adjusted odds of microbial detection on the skin. Abbreviations: CHG, chlorhexidine gluconate; OR, odds ratio. *Note*: Odds of culture detection of microbial organisms on the skin at each CHG skin concentration were estimated using mixed effect models that included a random intercept for body sites clustered within the patient and fixed effects for hospital, body site, and CHG skin concentration. Bars represent 95% confidence intervals. OR represents the change in odds of microbial recovery for every twofold increase in CHG skin concentration, as presented in Table 3.

observe a significant association between CHG skin concentrations and recovery of CPE from skin. Through visual inspection (Figure 1) and modeling, we also did not observe a threshold CHG skin concentration for reduced detection of gram-positive bacteria, gram-negative bacteria, *Candida* species, and CPE. The relationship between CHG skin concentrations and skin detection of specific species of gram-positive and gram-negative bacteria is shown in Table 3.

In sensitivity analysis, we assessed the relationship between CHG skin concentration and skin microbial recovery on a continuous scale (colony forming units, or CFU/25cm^2); a stacked histogram depicting CHG skin concentration versus CFU of skin microbial recovery is presented in Figure 2. Adjusted analysis with continuous CFU/mL as a modeled outcome did not meaningfully change the results found in the primary analysis.

CHG minimum inhibitory concentrations

A subset of isolates (467) from patients in the multicenter cohort and 31 CPE isolates from the CPE cohort were selected for CHG MIC testing (Supplementary Table S2). Gram-positive bacteria tested such as *Staphylococcus aureus* demonstrated relatively low MIC values, compared with gram-negative bacteria and *Candida* species tested.

Discussion

Among hospitalized patients, in whom skin CHG concentrations and microbial cultures were obtained cross-sectionally, higher CHG skin concentrations were associated with less frequent skin detection of gram-positive bacteria and *Candida* species, but not with gram-negative bacteria, including CPE. For gram-positive bacteria and *Candida* species, the relationship was linear across all measured CHG skin concentrations, without a threshold effect observed.

We performed this study in the context of a CHG bathing quality improvement project in the ICU, which assessed the effectiveness of measurement and feedback of CHG skin concentrations to hospital unit leadership and bathing staff to improve the quality of CHG bathing.[9] A common question generated from feedback on CHG skin concentrations was whether there is an 'adequate' level of measured CHG skin concentration that would correlate with optimal microbial control. Based on limited data from prior studies, potential thresholds of 18.75 μg/mL for control of gram-positive bacteria and 128 μg/mL for control of CPE had been proposed.[4,5] Both prior studies utilized skin concentration measurement in a longitudinal fashion within patients (eg, serially before and after a CHG bath in the same patient). Our current study utilized a cross-sectional approach to CHG skin measurement that was independent of time from the last CHG bath received, representing a more pragmatic approach for unit-wide skin sampling by healthcare personnel. The findings of our current study and others[6] suggest that on a cross-sectional basis, there is not a threshold target for CHG skin concentration that correlates with optimal skin microbial control for some organisms.

Whether CHG bathing effectively controls gram-negative bacterial skin colonization, transmission, and infection is uncertain. In a

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Table 3. Effect of chlorhexidine gluconate skin concentration on the odds of recovering selected microorganisms from the skin by culture[a]

Organism	Odds ratio (95% CI)	*P* value
Gram-positive bacteria	0.84 (0.80–0.87)	<.001
Selected gram-positive species		
Staphylococcus aureus[b]	0.85 (0.8–0.91)	<.001
Methicillin-resistant *S. aureus*	0.86 (0.75–0.98)	.02
Enterococcus species[b,c]	0.97 (0.93–1.02)	.19
Vancomycin-resistant enterococci[c]	0.97 (0.90–1.06)	.52
Gram-negative bacteria	0.96 (0.91–1.02)	.17
Selected gram-negative species		
Acinetobacter species	1.04 (0.88–1.21)	.66
Klebsiella pneumoniae	0.93 (0.84–1.02)	.13
Pseudomonas aeruginosa	1.02 (0.92–1.13)	.68
Carbapenemase-producing Enterobacterales species	0.94 (0.84–1.06)	.33
Candida species	0.93 (0.89–0.98)	.008

Note. CI, confidence interval; CHG, chlorhexidine gluconate; CPE, carbapenemase-producing Enterobacterales. Mixed effect models included a random intercept for body sites clustered within patient and fixed effects for hospital, body site, and CHG skin concentration. Hospitals without positive detection of the target microorganism were excluded from the analysis. Odds ratios represent the change in odds of microbial recovery for every twofold increase in CHG skin concentration.

[a]Results from the multicenter cohort of 7 hospital intensive care units, except CPE skin detection from patients with CPE colonization based on rectal or stool culture hospital-wide at a single center.

[b]Model with a random effect for multiple body sites within the patient did not converge; the random effect was removed for this outcome.

[c]*E. faecalis* and *E. faecium*.

longitudinal assessment of ICU patients, CHG bathing transiently reduced all pathogens, including gram-negative organisms on the skin, but rebound of microbial detection near baseline levels was observed at 4 hours post-bath for gram-negative organisms.[5] Routine CHG bathing in the ICU has not been consistently associated with reductions in gram-negative infections.[1,18,19] Nevertheless, CHG bathing has been shown to decrease KPC-producing *K. pneumoniae* skin colonization shortly after a bath.[4] Furthermore, CHG bathing has been utilized in a bundled intervention to interrupt the transmission of KPC-producing *K. pneumoniae* in the long-term acute care hospital setting, leading to decreases in KPC-producing *K. pneumoniae* and all-cause bacteremia.[20]

Our study has limitations. First, reductions in skin microbial colonization were used as a surrogate for reduced risk of pathogen transmission and infection. However, skin colonization contributes to the pathogenesis of infections such as central line-associated bloodstream infections (CLABSIs),[21] and reductions in skin microbial burden has been associated with reduced environmental and healthcare worker hand contamination.[3] Second, we may have been underpowered to detect correlations between CHG skin concentrations and less prevalent species or groups of organisms. Certain organism species may also demonstrate relationships with CHG skin concentrations that diverge from patterns observed at the genus level.[22] Third, we did not assess prior or current receipt of systemic antimicrobial agents. Additional research is needed on the impact of broad-spectrum antibiotics on the skin ecology of critically ill patients. Major strengths of our study included the multicenter prospective design over a geographically diverse group of healthcare facilities, standardized skin sampling techniques, and utilization of culture protocols that targeted multiple organisms of medical importance.

In conclusion, we found that within the range of CHG skin concentrations detected among hospitalized patients undergoing routine CHG bathing, there was an association between higher CHG skin concentration and less frequent detection of gram-positive bacteria and *Candida* species on the skin, without an observed threshold effect. We did not find such a relationship for gram-negative bacteria. For infection prevention, CHG bathing

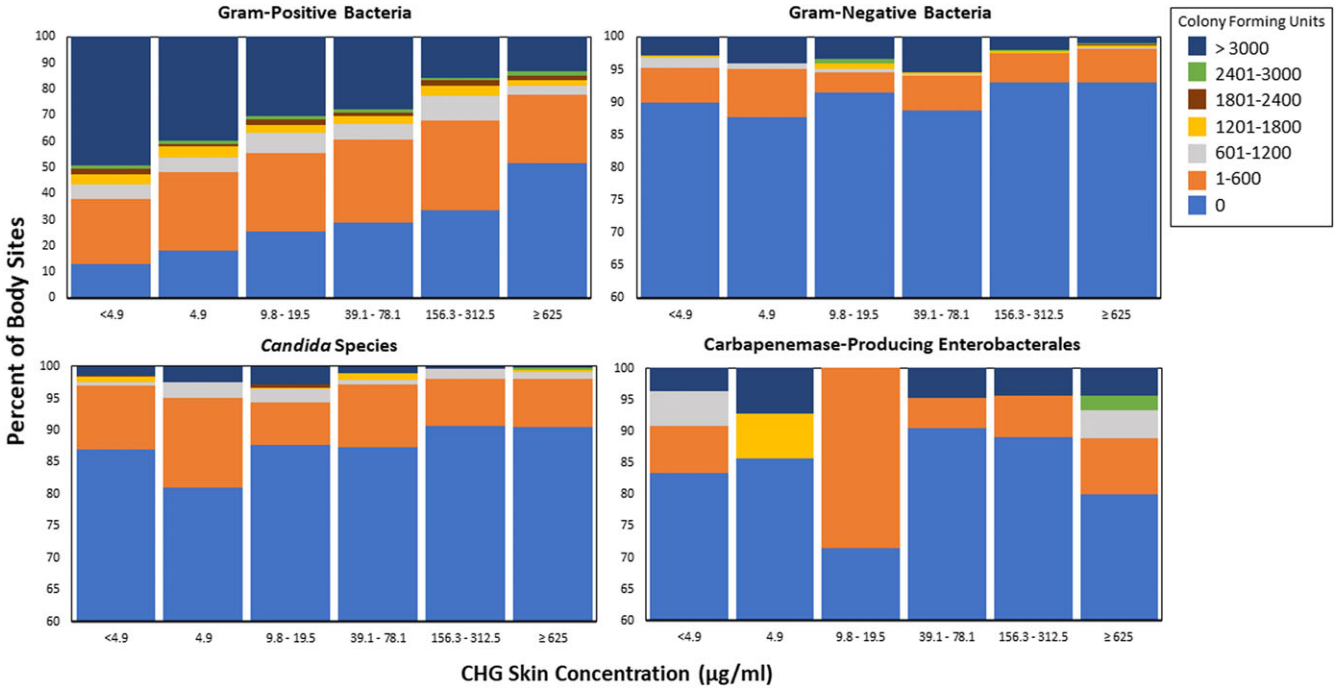

Figure 2. Unadjusted relationship between chlorhexidine gluconate concentration and microbial colony forming units on skin. Abbreviations: CHG, chlorhexidine gluconate. *Note*: Different *y*-axis scales on panels. The skin area swabbed is 25 cm^2.

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strategies that achieve higher CHG skin concentrations may improve control of certain pathogens.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/ice.2024.81.

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Competing interests. M.K.H. has been a co-investigator on several research studies for which Sage Products (now part of Stryker Corporation), Mölnlycke, and Medline provided CHG products at no charge to hospitals and skilled nursing facilities participating in the research. Neither M.K.H. nor her employer (Rush University Medical Center) received chlorhexidine products. C.R. reports royalties from UpToDate, Inc. and consulting fees from Pfizer and Cytovale for topics unrelated to this study. D.W. was a consultant for Mölnlycke Health Care AB after the completion of the study. M.Y.L. has received research support in the form of a CHG product from Sage Products (now part of Stryker Corporation).

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