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Brief Report

The impact of chlorhexidine gluconate on the skin microbiota of children and adults: A pilot study

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We examined the effect of chlorhexidine gluconate (CHG) bathing on the skin microbiota of adult and pediatric patients. We observed no differences in pediatric patients; however, multiple genera of bacteria were observed to be significantly less abundant in the adults bathing with CHG. Further research is needed to determine the long-term impact of CHG use on the skin microbiota.

Bloodstream health care–associated infections (HAIs) are a significant cause of morbidity and mortality.[1] Daily bathing of patients with the broad-spectrum bactericidal antiseptic chlorhexidine gluconate (CHG) is effective in reducing bloodstream HAIs and is increasingly being used as part of routine care in hospitals.[2] However, little is known regarding the impact of daily CHG bathing on the skin microbiota.[3] Here we present a pilot study assessing the impact of CHG on the skin of children and adults in the nonintensive care unit setting.

METHODS

Pediatric and adult patients on both general medical and surgical units were bathed daily with 4% CHG foam as part of routine practice. We collected skin swabs (axillae and antecubital fossae) from adult and pediatric patients on admission prior to routine CHG usage (controls) and from patients bathed with CHG within 23 hours of sample collection between April and June 2017. Health care workers—primarily nursing staff—bathed pediatric patients, whereas adults bathed themselves whenever possible. All procedures were designated quality improvement by the University of Wisconsin-Madison Institutional Review Board.

Samples were collected using nylon flocked swabs (Copan Diagnostics, Murrieta, CA) dampened with sterile saline. Swabs were rubbed onto the skin applying firm pressure for 20 seconds. DNA extraction and 16S rRNA sequencing were carried out as previously described.[4] Sequences were binned into operational taxonomic units (OTUs) and taxonomy assigned using USEARCH/UPARSE (R.C. Edgar, CA).[5] All analyses were done in R v.3.4.0 (R Foundation for Statistical Computing, Vienna, Austria). Alpha diversity (inverse Simpson metric) and beta diversity (Bray-Curtis dissimilarity matrix plotted using nonmetric multidimensional scaling) were tested for significance using permutational multivariate analysis of variance. A P value of .05 was considered statistically significant. Tests for differential abundance were done using DESeq2. The Benjamini-Hochberg correction was used to reduce the false discovery rate. Additional sequencing methods are available upon request.

RESULTS

Specimens were collected from 24 patients (8 adult, 16 pediatric), with all patients providing both samples. Four adult and 6 pediatric patients had no known prior CHG exposure. Four adult and 10 pediatric patients were exposed to CHG.

Staphylococcus (Firmicutes), *Corynebacterium* (Actinobacteria), *Pseudomonas* (Proteobacteria), *Prevotella* (Bacteroidetes), and *Anaerococcus* (Firmicutes) were the top 5 genera in the pediatric axillae. There were no significantly different OTUs or significant differences

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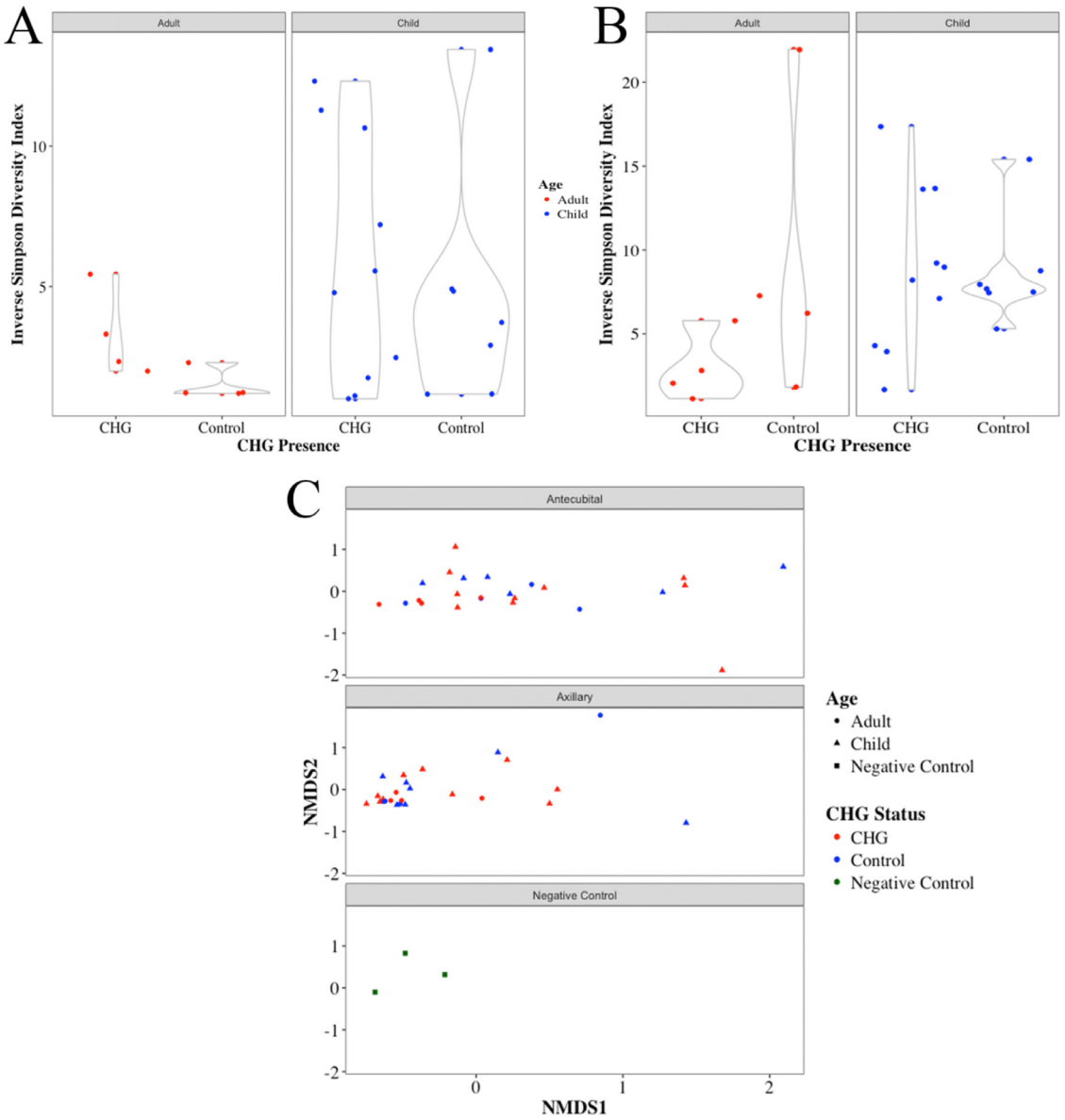

Fig 1. A, Violin plots of the alpha diversity as calculated by the inverse Simpson diversity index of all axillary samples. B, Violin plots of the alpha diversity as calculated by the inverse Simpson diversity index of all antecubital fossae samples. C, NMDS of all samples in both anatomic sites stratified by participant age and CHG status. Negative controls are laboratory controls used to ensure no laboratory contamination of samples occurred. *CHG*, chlorhexidine gluconate; *NMDS*, nonmetric multidimensional scaling.

in the alpha (difference in means, 4.53, $P = .574$) (Fig 1A) and beta diversity (r^2, 0.042, $P = .937$) (Fig 1C) between those exposed and the controls.

When considering the pediatric antecubital fossae, the most abundant genera were *Corynebacterium* (Actinobacteria), *Staphylococcus* (Firmicutes), *Streptococcus* (Firmicutes), *Micrococcus* (Actinobacteria), and *Acinetobacter* (Proteobacteria). Similar to the axillae, no significant differences were observed in any OTUs or alpha (difference in means, 1.56, $P = .917$) (Fig 1B) or beta diversity (r^2, 0.174, $P = .577$) (Fig 1C).

In the adult axillae, the most abundant genera were *Staphylococcus, Corynebacterium, Anaerococcus, Pseudomonas,* and *Prevotella*. Two OTUs—one belonging to the *Corynebacterium* genus (adjusted $P = .0414$) and the other to the *Anaerococcus* genus (adjusted $P = .0176$)—were more prevalent among the exposed. Similar to the pediatric axillary samples, there were no significant differences in alpha (difference in means, 1.78, $P = .0728$) (Fig 1A) or beta diversity between those exposed to CHG and the controls (r^2, 0.16, $P = .948$) (Fig 1C).

The most prevalent genera observed on the adult antecubital fossae were *Staphylococcus, Corynebacterium, Pseudomonas, Anaerococcus*, and *Streptococcus*. Fifteen OTUs belonging to 5 different phyla were significantly more abundant among the controls compared with those exposed to CHG (Table 1). Although there were significant differences in the composition of the microbiota between those exposed to CHG and the controls, there were no observed differences in alpha (difference in means, 1.78, $P = .206$) (Fig 1B) or beta diversity (r^2, 0.0455, $P = .218$) (Fig 1C).

DISCUSSION

CHG reduces the overall bacterial burden in the hospital by reducing pathogens on patients' skin and health care workers' hands and in the environment,[6] thereby reducing HAIs.[7] We observed no impact

Table 1
Differentially abundant microbiota in the antecubital fossae of adult patients

OTU	Log 2-fold change	*P* value	Adjusted *P* value	Phylum	Genus
OTU 66	−23.597	3.11E-12	2.76E-10	Bacteroidetes	*Prevotella*
OTU 142	−22.874	1.39E-11	6.18E-10	Firmicutes	*Sporomusa*
OTU 318	−22.167	5.83E-11	1.73E-09	Firmicutes	*Caloramator*
OTU 18	−7.210	7.81E-06	.000174	Proteobacteria	*Haemophilus*
OTU 5	−7.530	4.76E-05	.000847	Firmicutes	*Lactobacillus*
OTU 53	−7.0741	.000202	.00300	Firmicutes	*Leuconostoc*
OTU 34	−9.398	.000239	.00304	Proteobacteria	*Mannheimia*
OTU 85	−5.700	.000485	.00540	Actinobacteria	*Rothia*
OTU 849	−6.777	.00138	.0137	Proteobacteria	*Acinetobacter*
OTU 71	−6.505	.00167	.0137	Fusobacteria	*Fusobacterium*
OTU 16	−5.393	.00170	.0137	Proteobacteria	*Neisseria*
OTU 3	−4.009	.00281	.0193	Firmicutes	*Streptococcus*
OTU 14	−5.905	.00275	.0192	Actinobacteria	*Corynebacterium*
OTU 65	−3.916	.00420	.0267	Proteobacteria	*Enterobacter*
OTU 68	−9.201	.00627	.0372	Bacteroidetes	*Porphyromonas*

NOTE. Negative log 2-fold change values represent OTUs that were significantly more abundant in the control group. *P* values were adjusted using the Benjamini-Hochberg correction. *OTU*, operational taxonomic unit.

of CHG on the pediatric skin microbiota and limited impact on adult patients, with significant changes in bacterial composition and relative abundance observed only on the antecubital fossae of adults. This is in contrast to other studies that have observed reductions in bacterial diversity and shifts in the skin microbiota following CHG use.[8]

Lower CHG concentrations result in higher bacterial loads and reduced efficacy duration postbath.[9] One possible explanation for the lack of difference in relative abundance in the pediatric population may be that CHG concentrations were not high enough to effectively decontaminate the skin. Although not reported here, CHG concentrations on the skin were measured via a colorimetric, semiqualitative indicator assay[9] 1 hour prior to, 1 hour post–, and 24 hours post–CHG bath and found to be lower in pediatric patients than adults. This could be due to several factors, including the bathing method used, duration of contact time, and thoroughness of bathing of pediatric patients. Observations of these baths were conducted as part of a larger study, and reduced contact time and procedure deviations were noted in the pediatric population.

In the adult population, we observed differences in CHG effectiveness by anatomical site, with more changes in relative abundance seen at the antecubital fossae. This has been reported by others[10] and may be due to variation in bathing practices, differing by body site and skin type.[9] One potential explanation for the decreased effectiveness at the axillae may be the larger number of hair follicles, sebaceous glands, and sweat glands, whose secretions may either recontaminate the axillae or reduce the duration of efficacy. Other exogenous factors, like deodorant use, may impact the effectiveness of CHG in the axillae but were not studied here.

Our study had several limitations, including small sample size in both patient populations, information collected over a short study duration, samples collected after only 1 bath, and the use of 16S rRNA sequencing, which does not allow for species-level identification. Because of the small sample size, there are potential differences between the groups we were not able to observe. Additionally, we did not collect patient-level information in this quality improvement study. Finally, this study was conducted in medical and surgical units, which may differ from intensive care unit settings. Despite these limitations, our study demonstrates how a microbiota assessment can help determine the efficacy of CHG bathing.

CONCLUSIONS

Daily bathing with CHG did not significantly impact the skin microbiota on either the axillae or antecubital fossae of children or the axillae of adults; however, significant reductions were observed on the antecubital fossae of adults. Bathing method and product (cloths vs foam) may play a crucial role in CHG efficacy. Longitudinal studies and studies using shotgun metagenomics are needed to understand the changes skin microbiota undergo during continued CHG exposure and provide insight into the clinical implications of CHG use.

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