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Skin-Microbiome Assembly in Preterm Infants during the First Three Weeks of Life and Impact of Topical Coconut Oil Application

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Abstract: The structure and function of infant skin is not fully developed until 34 weeks of gestation, and this immaturity is associated with risk of late-onset sepsis (LOS). Topical coconut oil improves preterm-infant skin integrity and may reduce LOS. However, data on early-life skin-microbiome succession and potential effects of emollient skin care in preterm infants are scarce. We therefore collected skin-microbiome samples from the ear, axilla, and groin on days 1, 7, 14, and 21 from preterm infants born <30 weeks of gestation as part of a randomized clinical trial of standard skin care vs. topical coconut oil. We found that within-sample microbiome diversity was highest on day 1 after birth, with a subsequent decline and emergence of *Staphylococcus* genus dominance from day 7. Moreover, microbiome assembly was less diverse in infants receiving coconut oil vs. standard skin care. Our study provides novel data on preterm-infant skin-microbiome composition and highlights the modifying potential of emollient skin care.

Keywords: coconut oil; preterm infants; skin microbiome

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1. Introduction

Human skin hosts a diverse, low-density microbiome of bacteria and fungi [1]. Emerging evidence suggests that commensal skin microbiota plays a fundamental role in regulating the physical integrity and repair of the skin barrier [2] and is crucial for developing the infant immune system [3,4]. The skin microbiota stimulates the development of skin immune cells and the production of antimicrobial peptides, short-chain fatty acids, and polyamines, which are critical for defense against infection [5]. The commensal skin microbiota produce metabolites that activate the aryl hydrocarbon receptor (AHR) in keratinocytes [2], which, in turn, accelerates epidermal differentiation and increases stratum corneum thickness. Moreover, commensal skin microbiota, such as *Staphylococcus epidermidis*, enhance skin barrier function by stimulating keratinocytes to produce ceramides, which are essential in reducing trans-epidermal water loss (TEWL) [6].

Skin colonisation begins immediately at, or even before, birth; however, the factors influencing the succession of the skin microbiota are not completely understood. For example, the effect of the mode of delivery on skin-microbiome composition remains controversial [7–10]. The skin of extremely-to-moderately preterm infants does not provide an adequate protective barrier against infection and water loss. The stratum corneum,

the external epidermal layer of the skin, begins to develop at 24 weeks of gestation. This layer has several cornified layers at 26 weeks and is fully developed by 34 weeks of gestation [11]. A mature stratum corneum provides a critical physical barrier, prevents invasion by microorganisms, and minimizes TEWL, preventing dehydration [11].

Infant skin at birth is initially rich in Firmicutes, followed by Actinobacteria, Proteobacteria, and *Bacteroides*. Preterm infants may have a higher skin abundance of Firmicutes, including *Staphylococcus*, *Corynebacterium*, and *Prevotella* bacteria, than full-term infants [8]. Since preterm infants are frequently exposed to antibiotic therapy and parenteral nutrition and are hospitalized for prolonged periods, they are susceptible to nosocomial infections, commonly with skin commensals [8]. Coagulase-negative staphylococci (CoNS), including *S. epidermidis* and *S. capitis*, colonize preterm infants and account for >75% of cases of late-onset sepsis (LOS) [12,13]. The vast majority of very preterm infants are exposed to empiric antibiotic therapy during their hospital stay, often repeatedly, and this may decrease cutaneous bacterial diversity [14].

Emollients used for neonatal skin care, both mineral-oil-derived and natural oils, aim to reduce TEWL and improve skin integrity [15], but their effects on skin barrier function and LOS in preterm infants are not fully understood. Skincare emollients may also have inadvertent deleterious effects on the barrier function of the skin of neonates, such as impairment of epidermal maturation by oleic acid in olive oil [16] or increase in sepsis risk in the smallest infants following the use of Aquaphor [17]. Coconut oil may be a more suitable emollient for neonatal skin care due to its high concentrations of lauric acid and its ester monolaurin, both with antimicrobial properties [18,19], and the absence of known adverse effects [20]. However, its effects on the neonatal skin microbiota have not been investigated.

Our previously described, randomized, controlled trial of topical coconut oil in very (born <30 weeks GA) preterm infants resulted in improved skin integrity, without adverse effects [19]. Here, we describe skin-microbial community assembly and the effect of the coconut oil intervention on the skin-microbial community in very preterm infants enrolled in this trial during the first 3 weeks of life.

2. Results

We collected skin swabs from 72 very preterm infants from the axilla, ear, and groin on days 1, 7, 14, and 21 after birth. Microbiome analysis was performed on samples with sufficient DNA content from 672 swab samples, from a total of 56 infants (28 infants each in intervention and control arms, respectively). The demographics of participants are described in Table 1. During the study intervention, there was one episode of LOS in the coconut oil intervention arm and three episodes in the control arm.

Table 1. Demographic characteristics of very preterm infants in control and intervention arms *.

Characteristics	Control Arm (n = 28)	Intervention Arm (n = 28)
Gestation (weeks)	28 (23.7–29.5)	27.6 (24.1–29.3)
Birth weight (g)	984 (424–1534)	1040 (590–1420)
Caesarean delivery	18	15
Male	13	19

* Data are expressed in medians.

For skin-microbiome analysis, a total of 52,972,312 reads were obtained from 672 swab samples. A total of 14,912 ASVs were assigned to reads after denoising with the DADA2 pipeline. The ASVs were assigned to 34 phyla, 81 classes, 209 orders, 354 families, 819 genera, and 540 species. We identified 40 ASVs as potential contaminants, using the Decontam pipeline [21] (Supplementary Table S1), and removed these ASVs from the feature table.

2.1. Succession of the Skin Microbiota of Preterm Infants in Both Control and Intervention Infants

There were substantial changes in the composition of the skin microbiome over the first three weeks of life (Figure 1). These were further visualized for age at sampling as longitudinal data using an alluvial plot for each body site (Supplementary Figure S1). At all sites, the *Staphylococcus* genus increased in relative abundance with age at sampling, most strikingly in the axilla. The groin skin microbiome was characterized by a profile dominated by the *Burkholderia* or *Staphylococcus* genus on day 1 but was dominated by *Staphylococcus* or *Bifidobacterium* at ages 7, 14, and 21 days. In differential abundance analysis, *Staphylococcus* showed higher relative abundance on day 7 (q-value = 2.43×10^{-9} and day 14 (q-value = 2.18×10^{-9}) compared to day 1 samples (Table 2). *Bifidobacterium* (q-value = 0.006) and *Cutibacterium* (q-value = 0.0003) also showed increased relative abundances on day 7 and day 14 compared to day 1. *Clostridium* (q-value = 9.46×10^{-5}) had higher relative abundance in the axilla vs. groin samples, while the opposite finding was observed for *Streptococcus* (q-value = 0.002).

Table 2. Bacterial genera with relative abundances that differed between body sites or with age at sampling. Differential abundance testing was performed with a mixed effects model in MaAsLin2, using Sample ID as a random effect and body site and age at sampling as fixed effects.

Taxon	Reference	Condition	Coefficient	q-Value
Corynebacterium	Axilla	Groin	−2.31	0.0001
Clostridium	Axilla	Groin	−1.84	9.46×10^{-5}
Streptococcus	Axilla	Groin	1.77	0.002
Staphylococcus	Day 1	Day 7	1.68	2.43×10^{-9}
Staphylococcus	Day 1	Day 14	1.71	2.18×10^{-9}
Bifidobacterium	Day 1	Day 7	1.88	0.006
Cutibacterium	Day 1	Day 14	−1.88	0.0003

The dissimilarity between microbial communities at different body sites and ages at sampling was assessed with non-metric multidimensional scaling (NMDS) using the Bray–Curtis dissimilarity index. Between-body-site distances were greater than within-site distances (PERMANOVA, $p = 0.001$) (Figure 2A). β-diversity also differed with age at sampling, with day 1 samples clearly separated from samples collected on later days (PERMANOVA, $p = 0.001$) (Figure 2B).

Using qPCR for *Bifidobacterium* spp., the proportion of swabs that were positive for the groin, ear, and axilla were 82.1%, 59.0%, and 95.4%, respectively. However, the axilla had the lowest *Bifidobacterium* bacterial load, the ear had an intermediate *Bifidobacterium* load, and the groin swabs had the highest load, with an increase from day 1 to day 7 ($p \leq 0.0001$) (Figure 3).

2.2. Factors Associated with Diversity of the Preterm-Infant Skin Microbiome

We tested the association between various participant-level factors and alpha diversity (within sample diversity) using linear mixed models. Bacterial richness (Chao1) in axilla samples was lower than that in groin samples ($p < 0.001$), while ear samples had higher richness than groin samples ($p < 0.001$) (Figure 4A). Using a different metric for α-diversity (Shannon–Weiner index, which also accounts for the evenness of taxa within samples) (Figure 4B), groin samples had higher diversity compared with the axilla or ear samples.

Bacterial richness was lower on day 7 ($p < 0.001$), day 14 ($p = 0.021$), and day 21 ($p < 0.001$) compared to day 1 (Figure 5A). Similar findings were observed using the Shannon diversity index (Figure 5B).

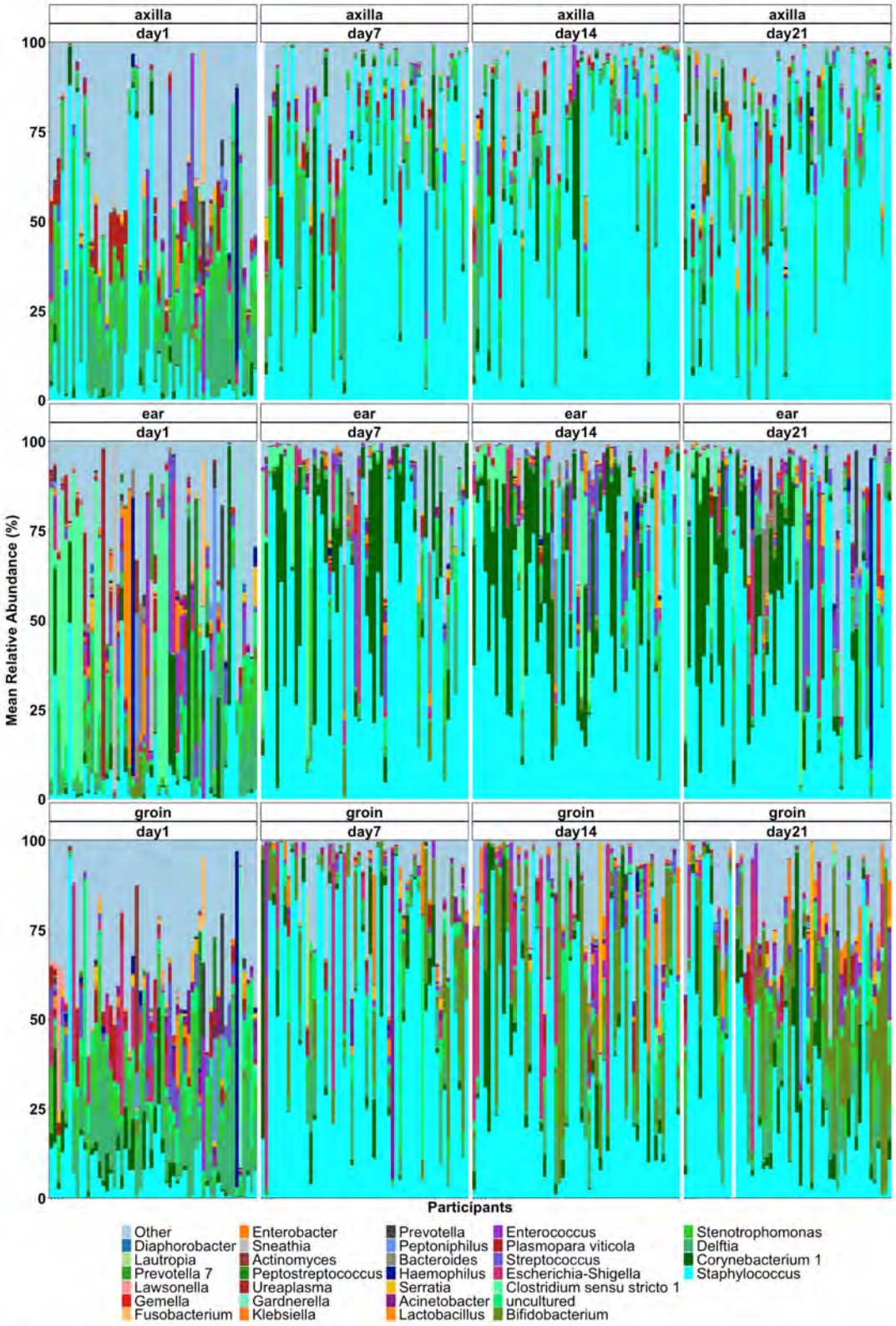

Figure 1. Relative abundance of bacterial taxa in individual participants. Compositional bar plot shows the mean relative abundance at the genus level for each individual participant at axilla, ear, and groin sites for samples collected on days 1, 7, 14, and 21. Genera that had a relative abundance of <10% are listed as "other".

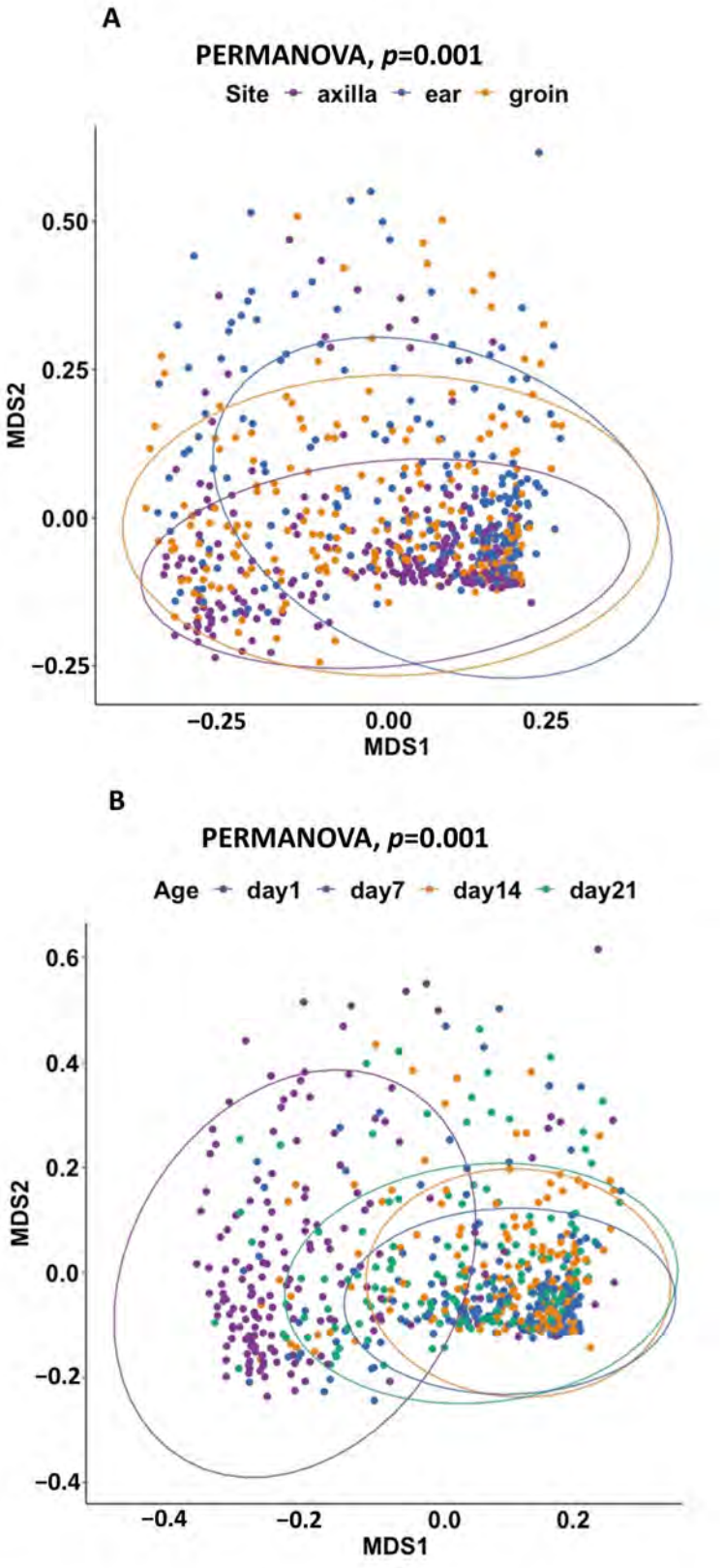

Figure 2. (**A**) Beta-diversity NMDS plot of different body sites. Non-metric-multidimensional-scaling (NMDS) plot at ASV level using Bray–Curtis dissimilarity index (PERMANOVA, *p*-value = 0.001). (**B**) Beta-diversity NMDS plot of age at sampling. Non-metric-multidimensional-scaling (NMDS) plot at ASV level using Bray–Curtis dissimilarity index (PERMANOVA, *p*-value = 0.001).

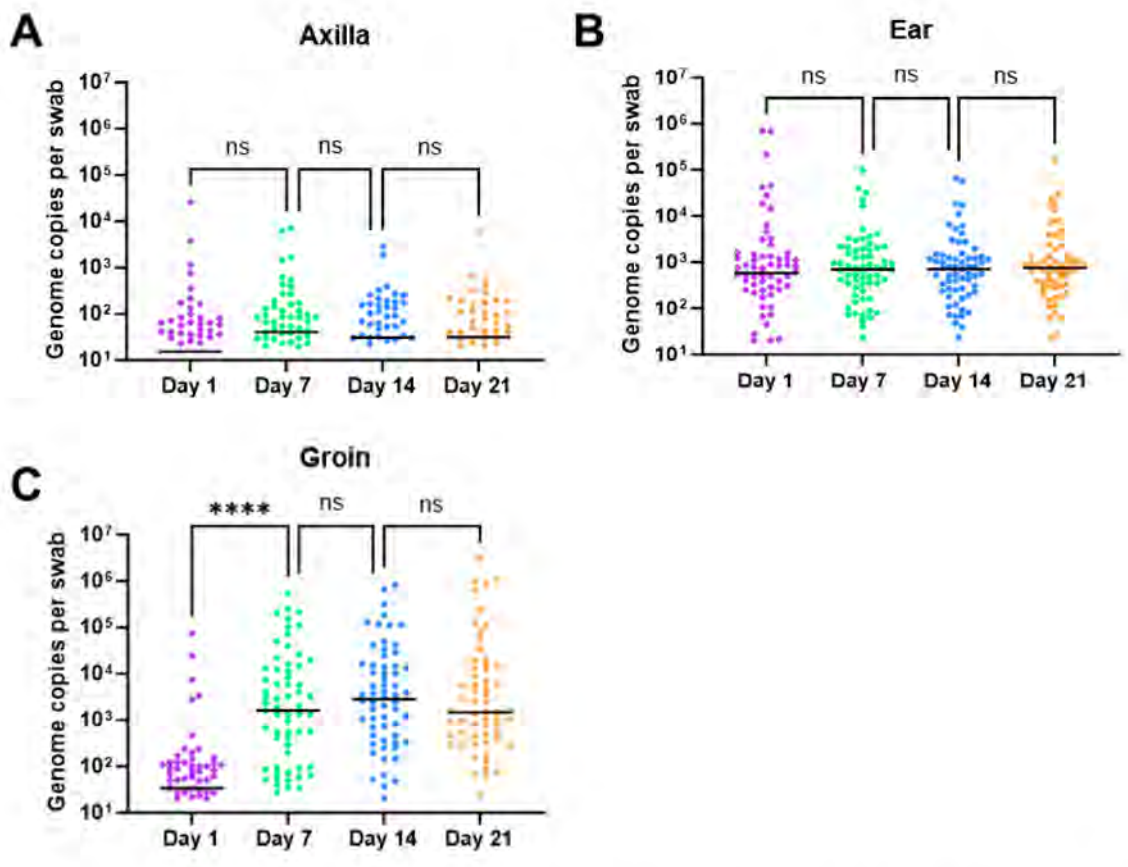

Figure 3. Quantitative PCR data for *Bifidobacterium* spp. at all time points for (**A**) axilla, (**B**) ear, and (**C**) groin. DNA in ng was measured using a standard curve and converted to total genomes per swab. Zero values are plotted as half the limit of detection (8 genome copies per swab). The median value for each time point is represented by a black bar. Each time point was compared with the other using a Kruskal–Wallis analysis of variance with Dunn's multiple comparisons test. Comparisons with significant differences are denoted in each figure (**** = $p < 0.0001$ and ns = $p > 0.05$). Each time point is represented by a different color (day 1 = purple, day 7 = green, day 14 = blue, and day 21 = orange).

We also analyzed the association between the total duration of antibiotic therapy, prior to each sampling time point, and alpha diversity. Lower Shannon diversity index was similar to prior antibiotic treatment ($p = 0.981$).

2.3. Effect of Topical Coconut Oil on the Skin Microbiome

We analyzed the effect of coconut oil emollient on within-sample diversity using a linear mixed model with participant ID as a random effect and with site and age as fixed effects. Compared to the control arm, there was a trend towards reduced microbiome richness in the intervention arm (beta = −9.97, 95% CI [−22.68, 2.74], $p = 0.124$). Using the Shannon–Weiner index of diversity, the intervention was negatively associated with diversity (beta = −0.16, 95% CI [−0.27, −0.04], $p = 0.006$) (Figure 6A,B). The overall composition of the skin microbiome, as measured by Bray–Curtis dissimilarity, differed between control and intervention arms on day 7 only for ear (PERMANOVA, $p = 0.01$) and groin (PERMANOVA, $p = 0.02$) samples (Figure 7). There were differences in individual taxa between the intervention and control arms using Maaslin2 analysis of the microbiome.

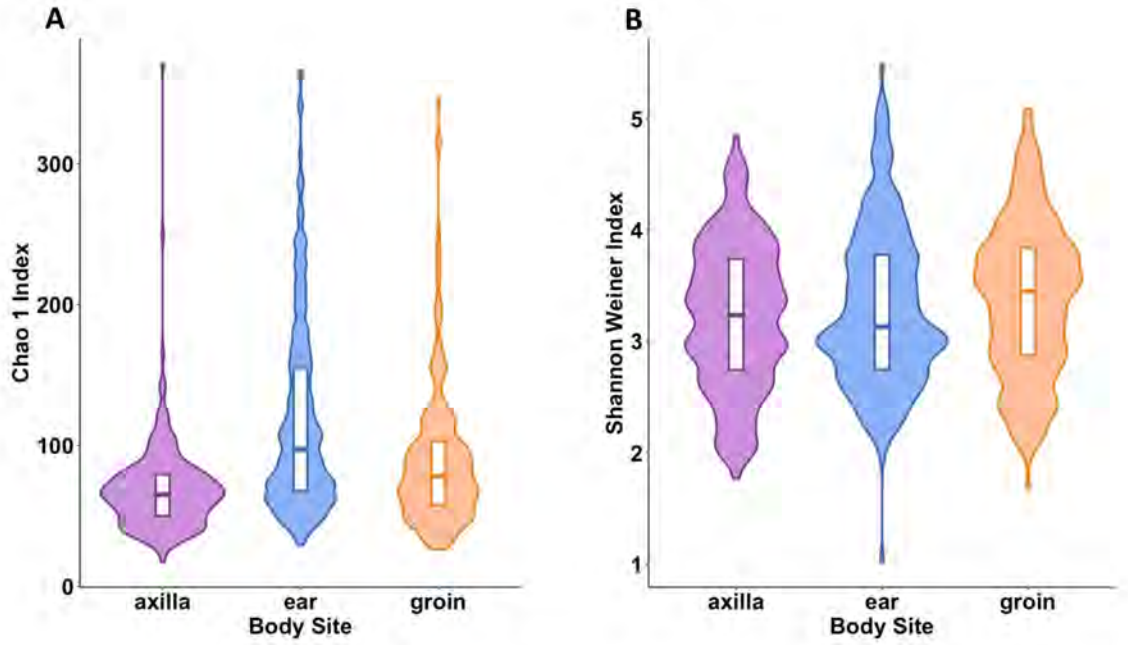

Figure 4. Alpha-diversity metrics for body site: axilla, ear, and groin. (**A**) Violin plot of Chao1 index (axilla: p-value $\leq$ 0.001 and $\beta = -28.07$; ear: p-value $\leq$ 0.001 and $\beta = 28.07$; both vs. groin). (**B**) Violin plot of Shannon–Weiner index (axilla: p-value = 0.006 and $\beta = 0.16$; ear: p-value = 0.11 and $\beta = -0.09$; both compared with groin). The box plot represents the interquartile range, and the middle line represents the median. The colored shape of the violin plot represents the density of measurements.

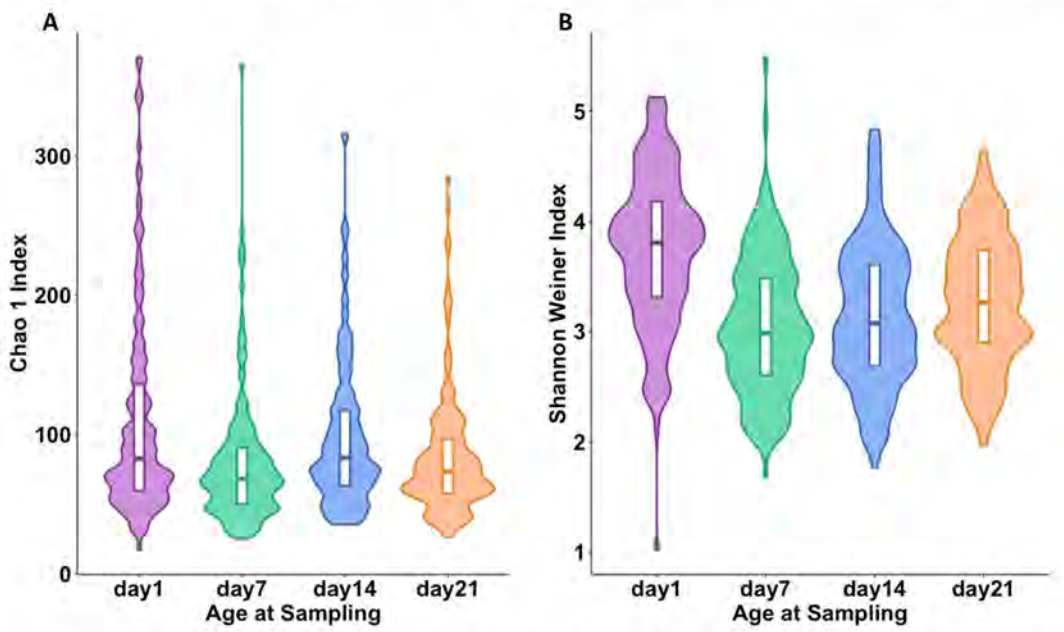

Figure 5. Alpha-diversity metrics for age at sampling: day 1, day 7, day 14, and day 21. (**A**) Violin plot of Chao1 index (day 7: p-value $\leq$ 0.001 and $\beta = -30.7$; day 14: p-value = 0.02 and $\beta = -11.95$; day 21: p-value $\leq$ 0.001 and $\beta = -26.59$; all compared to day 1). (**B**) Violin plot of Shannon–Wiener index (day 7: p-value $\leq$ 0.001 and $\beta = -30.75$; day 14, p-value = 0.02 and $\beta = -11.95$; day 21: p-value $\leq$ 0.001 and $\beta = -26.58$, all compared to day 1). The box plot represents the interquartile range, and the middle line represents the median. The colored shape of the violin plot represents the density of measurements. Each time point is represented by a different color (day 1 = purple, day 7 = green, day 14 = blue, and day 21 = orange).

Using qPCR, we analyzed the number of genome copies of CoNS and *S. aureus* in all samples. None of the axilla samples had any detectable *S. aureus* (Supplementary Table S2). Only two participants had detectable levels of *S. aureus* on day 7, both in the intervention arm and both from the groin. On day 14, two participants had *S. aureus* detected, one from a groin swab and the other from an ear swab. Both of these participants were in the control arm. Finally, on day 21, four participants had detectable levels of *S. aureus*, all in the control arm, from groin swabs (Supplementary Table S2). Except for one participant, who was positive for *S. aureus* on days 14 and 21, *S. aureus* detection was transient.

CoNS DNA was detected by qPCR in 66.8% of all samples. On day 1, CoNS DNA was detected in 40 participants (55.56%) from at least one site. By day 7, 70 participants (97.2%) had detectable levels of CoNS DNA from at least one site, with a concurrent increase in copy number. The CoNS load remained stable from day 7 onwards (Figure 8A).

For the axilla and ear sites, the median number of CoNS genomes detected was similar between the intervention and control arms at all time points (Figure 9A,B). From the groin swabs, the quantity of CoNS genomes was significantly higher in the intervention arm (420 genomes per swab) compared to the control arm (125 genomes per swab) ($p \leq 0.01$) on day 7 of sampling (Figure 9C). However, there was no significant difference between the intervention and control arms on day 14 and day 21 of sampling.

When all sites were combined, the number of CoNS genomes per swab increased from day 1 to day 7 for both the control and intervention arms ($p \leq 0.001$) (Figure 9A). On day 7, the number of CoNS genomes detected at all sites combined was higher in the intervention arm (median number of genomes = 395) compared to the control arm (median number of genomes = 115) ($p \leq 0.001$). A similar trend was observed on days 14 and 21 but was not statistically significant (Figure 9A). When all time points were combined and the total number of CoNS genomes was analyzed by site, the median number of detected CoNS genomes per swab was higher in the intervention arm at all sites compared to the control arm (axilla ($p \leq 0.05$) and groin ($p \leq 0.01$)) (Figure 9B).

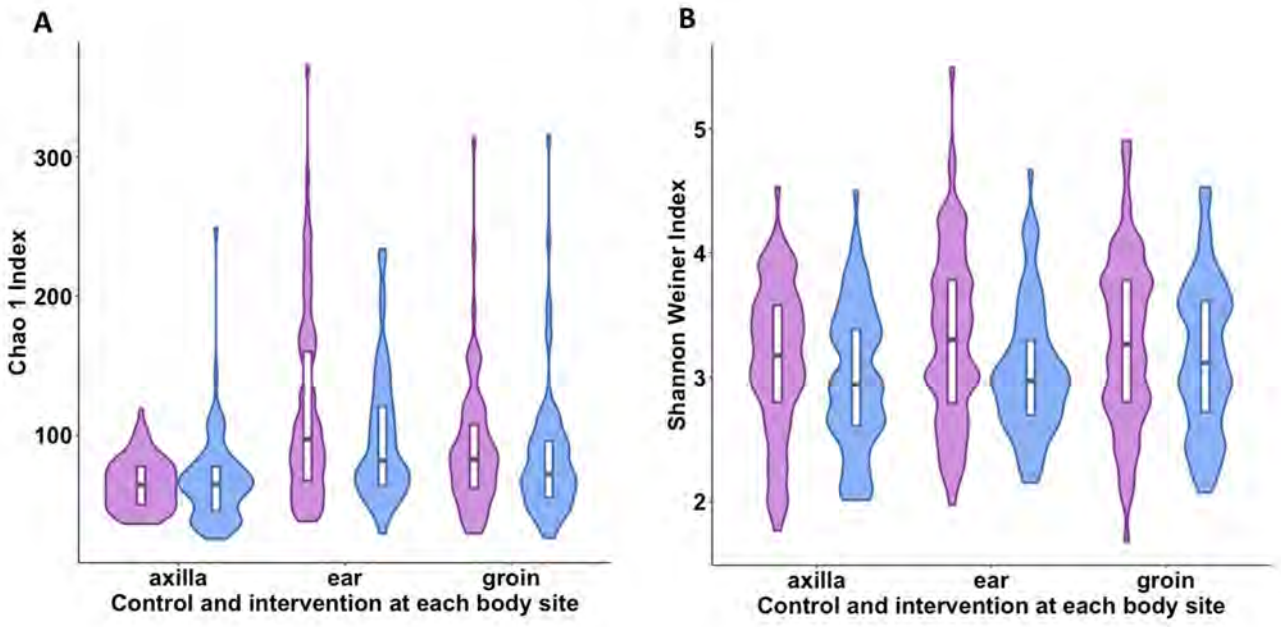

Figure 6. Alpha-diversity metrics for control and intervention arms at each body site. (**A**) Violin plot of Chao1 index (intervention: *p*-value = 0.12 and β = −9.97) and (**B**) violin plot of Shannon–Weiner index (intervention: *p*-value = 0.006 and β = −0.16) for control and intervention arms at axilla, ear, and groin body sites. The box plot represents the interquartile range, and the middle line represents the median. The colored shape of the violin plot represents the density of measurements.

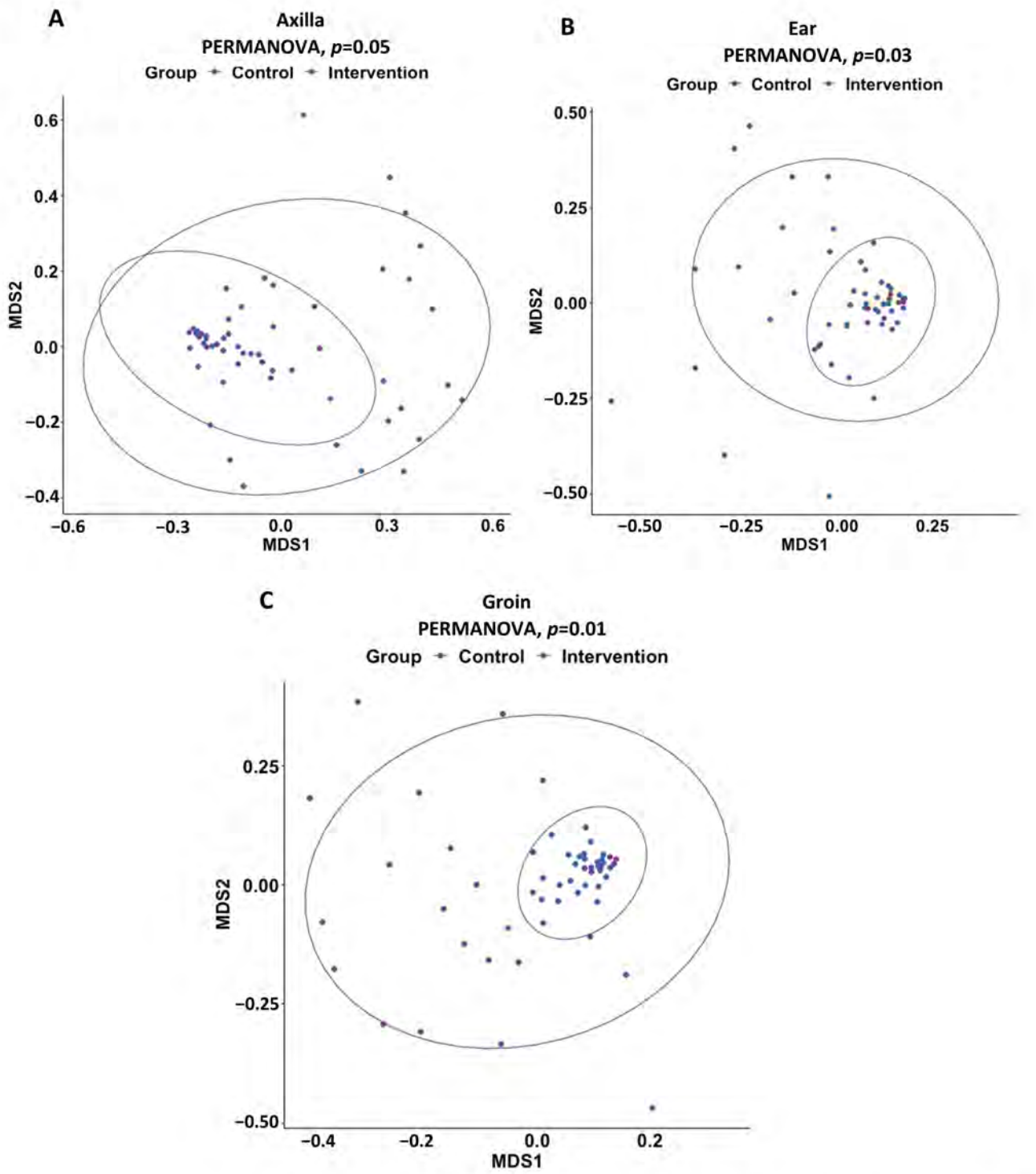

Figure 7. Beta-diversity NMDS plot for (**A**) axilla, (**B**), ear, and (**C**) groin in the intervention, and control arm on day 7. Beta diversity is shown in a non-metric-multidimensional-scaling (NMDS) plot using the Bray–Curtis dissimilarity index for each body site in control and intervention arms for day 7 samples.

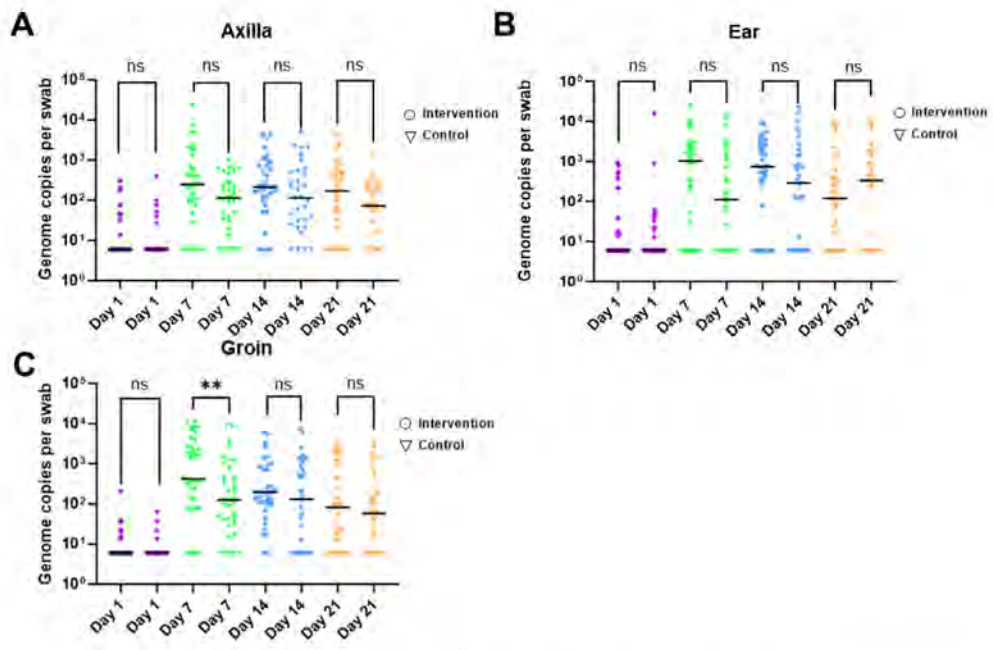

Figure 8. Quantitative PCR data for coagulase-negative staphylococci from participants at all time points for (**A**) axilla, (**B**) ear, and (**C**) groin. DNA in ng was measured using a standard curve and converted to total genomes per swab. Zero values are plotted as half the limit of detection (8 genome copies per swab). The median for each arm/time point is represented by a black bar. Each time point is represented by a different color (day 1 = purple, day 7 = green, day 14 = blue, and day 21 = orange). Each time point was compared between the treatment and control arms using a Mann–Whitney test. Comparisons with significant differences are denoted in each figure (** = $p < 0.01$, and ns = $p > 0.05$).

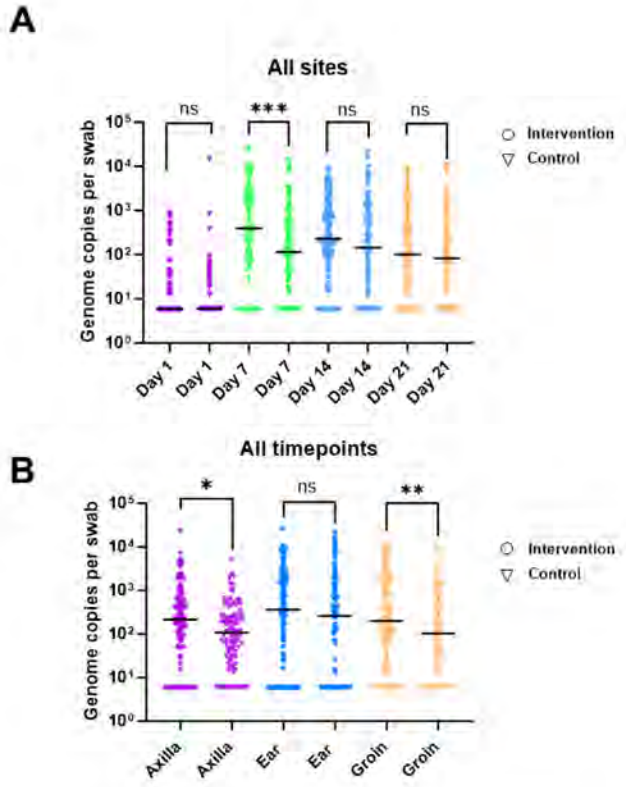

Figure 9. Quantitative PCR data for coagulase-negative staphylococci from participants with combined sites and time points. DNA in ng was measured using a standard curve and converted to

total genomes per swab. The median for each arm/time point is represented by a black bar (when a median value is 0, the bar is not shown). Each time point was compared between the intervention and control arms using a Mann–Whitney test. Comparisons with significant differences are denoted in each figure (* = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$, and ns = $p > 0.05$). (A) Total number of genome copies per swab is shown for all sites. Each time point is represented by a different color (day 1 = purple, day 7 = green, day 14 = blue, and day 21 = orange). (B) Total number of genome copies per swab is shown for all timepoints. Each site is represented by a different color (axilla = purple, ear = blue, and groin = orange).

3. Discussion

We investigated the effect of topical coconut oil emollient on the skin microbiome in preterm infants born <30 weeks of gestational age. Microbiome data were collected longitudinally from three body sites up to 21 days after birth. Overall, within-sample diversity was highest on day 1 after birth, with a subsequent decline and emergence of the *Staphylococcus* genus as the dominant taxon from day 7 onwards, consistent with previous studies [16]. qPCR data showed that this increase was largely due to commensal CoNS, rather than *S. aureus*. The coconut oil intervention was associated with lower bacterial diversity within samples and with an overall increase in the density of colonization with CoNS.

The reduction in diversity with age is likely due to a combination of host and environmental factors [16]. CoNS secrete products, such as lipoteichoic acid and proteases, that enhance skin barrier function and immunity [22], limiting colonization with *S. aureus* and other pathogenic microbes. Hence, the increased abundance of the *Staphylococcus* genus may play an important role in the development of the skin's immune function and compete with other microbes. Moreover, the age-related changes in the skin microbiome may also relate to the neonatal-intensive-care-unit (NICU) environment, which is common to all enrolled infants [22]. Previous studies indicate increasing similarity in NICU environmental and infant skin microbiome with duration of NICU admission over four weeks. Younge et al. [8] reported that *Escherichia*, *Staphylococcus*, and *Streptococcus* genera dominated the environment of preterm infants, taxa that were common on the skin of infants in our study.

Alpha diversity of the skin microbiome was significantly lower in the intervention arm than in the control arm, perhaps related to the higher absolute abundance of CoNS observed in children receiving coconut oil. In contrast, coconut oil application had no effect on the prevalence and absolute quantity of *S. aureus* on the skin of these infants. Indeed, *S. aureus* was detected infrequently and transiently in this study, with only seven infants having detectable levels of *S. aureus* from any site. A recent clinical trial on infants aged 3 to 6 months old found that the use of a glycerol-based emollient increased the alpha diversity of the skin microbiome compared to infants who did not use emollient [23]. Similar studies investigating emollients for the prevention and treatment of other diseases in infants have found that the skin-microbiome alpha diversity is significantly higher in the intervention group compared to the control group [24,25]. However, these differences between studies could be due to differences in the cohorts, as the physical and physiological properties of preterm-infant skin in our study are different from the skin of term infants [11]. Additionally, the antimicrobial effects of coconut oil may have impacted the skin microbiome of the infants in our study, resulting in reduced diversity [26].

We have previously reported that the implementation of topical coconut oil skin care in our NICU was associated with a lower frequency of LOS, without a change in the pattern of causative organisms [20]. The reduction in LOS due to coconut oil may be related to a key component of coconut oil, monolaurin. We have previously demonstrated that, in preterm infants, topical coconut oil administration resulted in higher plasma monolaurin levels compared to skin care without coconut oil [27], with potential direct antimicrobial and immunomodulatory effects. Monolaurin inhibits toxin production and biofilm formation in several bacteria, including *S. aureus* [28,29]. Previously, *S. aureus* has been reported to be inhibited by monolaurin in coconut oil [30].

Antibiotics decrease skin-microbiome alpha diversity in preterm infants [10]; however, in this study, systemic antibiotic treatment had little effect on the skin microbiome. This may be due to insufficient sample size and the almost universal administration of antibiotics in the first few days after birth (94.4%) and a substantial proportion (26.4%) of infants during the following weeks due to suspected sepsis. There have been conflicting reports of the impact of antibiotics on the skin microbiome, and the impact seems to be related to the timing and duration of treatment [8,10,14]. Salava et al. [14] showed that prophylactic benzylpenicillin and netilmicin administered intravenously in the first 5 days after birth significantly decreased the skin-microbial diversity, but no change in skin-microbial diversity was seen when vancomycin and netilmicin were administered intravenously to septic neonates. Another study [10] reported that bacterial richness and diversity were negatively correlated with exposure to intravenous antibiotics for more than 48 h.

Strengths of this study include its prospective integration into a randomized clinical trial comparing preterm infants' skin care with and without topical coconut oil, including serial sampling of three body sites. However, this study was underpowered to make conclusive findings about associations between the microbiome and antibiotic administration and clinical outcomes such as LOS. This study highlights the rapid succession of the skin microbiome, especially during the first week of life, and the potential modifying effects of topical coconut oil therapy.

4. Materials and Methods

4.1. Participant Recruitment and Sample Collection

This trial, conducted in the NICU at Edward Memorial Hospital, Perth, was approved by the institutional human research ethics committee (HREC2015191EW) and registered with the Australian Clinical Trial Registry (ACTRN12616000042448). Infants born <30 weeks' gestational age without major congenital malformations or congenital skin conditions were enrolled. Virgin coconut oil (Nature Pacific, Varsity Lakes, QLD, Australia) was provided in individually sealed 5 mL sachets for each application. Coconut oil (5 mL/kg) was applied by trained nursing staff to neonates in the intervention arm every 12 h for 21 days, commencing within 24 h of birth. The control arm was given standard neonatal care as per NICU guidelines without topical coconut oil [19].

Skin swabs (eNAT FLOQ Swabs®, Copan Diagnostics, CA, USA) were collected from the ear lobe, axilla, and groin at 1, 7, 14, and 21 days after birth. Swabs were stored in eNAT tubes, containing a nucleic acid preservation solution, at −80 °C until further processing. Clinical data were extracted from routine electronic databases.

4.2. DNA Extraction, PCR, and Amplicon Sequencing

DNA was extracted from the eNAT tubes using the Qiagen Blood Mini Kit with several modifications. Briefly, 1 mL of sample was aliquoted in sterile 2 mL tubes containing 1.0 mm and 0.1 mm sized silicon beads. The tube was centrifuged at 10,000× *g* for 10 min, and 750 µL of supernatant was discarded. A total of 200 µL of AL buffer was added to the sample pellet and resuspended. To this, 20 µL of Proteinase K was added. Sample tubes were placed in Fastprep® (24 Classic Instrument)(MP Biomedicals™, Irvine, USA) for 1 min at a frequency of 4 m/s, after which the samples were immediately incubated for 10 min at 60 °C. Sample tubes were then centrifuged at 8000× *g* for 30 s. A total of 200 µL of chilled 99% ethanol was added, and the tubes were gently mixed by tapping onto the vortex three times at medium speed. A total of 700 µL of supernatant was discarded, and the remaining volume was then used as per the manufacturer's protocol. DNA was eluted in 20 µL (pre-warmed at 60 °C) of nuclease-free water. The quality of the DNA was analyzed using the NanoDrop 1000 Spectrophotometer, and 4 µL of DNA was used to run 1% (*w*/*v*) agarose gel. DNA was quantified using the Qubit HS dsDNA kit (Invitrogen, Melbourne, Australia). All extracted DNA was stored at −20 °C until further processing.

The 16S rRNA gene V4 region was amplified using 515F and 806R primers with 50 ng of DNA in final volume of 50 µL. Several negative controls were amplified using

water as a template. The amplification and amplicon size were confirmed by running samples on 1% (*w*/*v*) agarose gel. The concentration was determined using the Qubit HS dsDNA kit (Invitrogen, Australia). The barcoded amplicons were subsequently pooled at a concentration of 2 ng/µL. The pool was purified using AMPure XP (Beckman Coulter, Australia), and the quality and size of the pool were checked by visualizing on a 1% (*w*/*v*) agarose gel. The composite mixture was sequenced on the Illumina Miseq platform at the UNSW Ramaciotti sequencing facility, Australia.

4.3. Bioinformatics and Statistical Analysis

Demultiplexed sequences were quality filtered using QIIME (Quantitative Insights into Microbial Ecology) version 2.2020.2 [31]. Briefly, adapters were removed from paired-end reads using Cutadapt [31]. Demultiplexed sequences were denoised (cutoff 240 bp), filtered, and trimmed with the DADA2 plugin [32]. The sequences were truncated at the base and chimeras were removed. A feature table containing amplicon sequence variants (ASVs) was generated. Finally, the ASVs were classified using the q2-feature-classifier with the SILVA v.138 database self-trained for the V4 region of the 16S rRNA gene.

To remove contaminants that were seen in sequenced controls, we used an in silico approach using the Decontam package in R software (v 4.3.2) [21]. Decontam implements a statistical classification procedure that identifies contaminants that appear at higher frequencies in low-concentration samples and negative controls. All ASVs identified as contaminants were removed from the feature table. The resulting clean feature table was used for all downstream analyses. A de novo phylogenetic tree was generated using $\log_{10}$ read counts and the phylogeny align-to-tree-mafft-fasttree plugin for downstream analysis.

All univariate statistical analysis was carried out in the R software using the Vegan package version 2.5.7 [33] and Microbiome package [34]. Alpha (α)-diversity at the ASV level was calculated using the richness, evenness, and Shannon–Weiner diversity index. To compare α-diversity measures between arms, we applied linear mixed models. Random effect models were used to assess longitudinal data, using sample ID as a random effect, to account for multiple samples per participant. We used linear mixed effects models to assess whether coconut oil treatment influenced the skin-microbiome diversity. We fitted a LMM (estimated using REML and nloptwrap optimizer). The normality of the data was confirmed using the Shapiro–Wilk test. For indices that were not normally distributed, such as Chao1 index, we applied a generalized linear model. Beta diversity (β) of microbial communities was calculated with nonmetric multidimensional scaling (NMDS) using Bray–Curtis dissimilarity [35] at the ASV level, and statistical significance was assessed with pairwise ANOSIM. All plots were produced using 'ggplot2' [36]. Longitudinal dynamics were visualized using an alluvial plot created with an in-house script for the matplotlib python package. The alluvial plots visualizations were made with the seaborn python package. Differential abundance analysis was carried out in the MaAsLin 2 package [37] in R software v 4.3.2 using random and fixed effect functions with sample ID as a random effect. The *p*-values derived from MaAsLin2 were adjusted using the Benjamin-Hochberg false discovery rate, and q-Values and coefficients were determined. Finally, we created the composition plots using the Fantaxtic v2.0.1 package in R software. In the composition plots, the relative abundance of each taxon is shown at the genus level. Genera with a relative abundance of <10% were listed as "other".

4.4. Quantification and Detection of *Staphylococcus* spp.

Staphylococcus aureus and coagulase-negative staphylococci (CoNS) were detected and quantified from extracted DNA using real-time PCR targeting the *spa* (encoding Staphylococcal protein A) and *tuf* (encoding elongation factor Tu) genes respectively. The primers and probe for *spa* were specific for *S. aureus* only, while the primers and probe for *tuf* were specific to all *Staphylococcus* species (Supplementary Table S3). Real-time PCR for the detection of *mecA* (encoding methicillin resistance) and *pvl* (encoding the virulence factor Panton-Valentine leukocidin) was also used (Supplementary Table S3). Reactions

were performed in quadraplex, with a final reaction mixture of 1X Taqman FAST Advanced Master Mix (Life Technologies), 0.3 µM of each primer, 0.15 µM of each probe (except for *spa* which had a final concentration of 0.2 µM), 5 µL of template DNA, and nuclease-free water (Integrated DNA Technologies) to a final volume of 20 µL. PCR cycling conditions consisted of an initial denaturation of 95 °C for 2 min, followed by 40 cycles of 95 °C for 30 s and 60 °C for 30 s (data acquiring). All reactions were performed on the QuantStudio™ 6, and data were analysed using QuantStudio Real-Time PCR Software v1.3 (Life Technologies). The quantity of DNA in ng for CoNS and *S. aureus* was determined using a standard curve and an internal positive control. This was converted to total genomes per swab and plotted on a scatter plot. The total amount of CoNS genomes was calculated by subtracting the total number of *S. aureus* genomes if *S. aureus* was detected in that sample. Within each body site, the treatment and control arms were compared using a Mann–Whitney test. Comparison was also made between age of sampling (days 1, 7, 14, and 21).

4.5. Quantification and Detection of *Bifidobacterium* spp.

As per the NICU standard protocol at KEMH, all infants received oral supplementation with *Bifidobacterium breve* M16V, independent of skin care allocation. *Bifidobacterium* spp. were detected and quantified from extracted DNA using genus-specific real-time PCR targeting the 16S rRNA gene sequence [38]. Reactions were performed as a single reaction, with a final reaction mixture of 1X Taqman FAST Advanced Master Mix (Life Technologies), 0.3 µM of each primer, 0.15 µM of each probe 5 µL of template DNA, and nuclease-free water (Integrated DNA Technologies) to a final volume of 20 µL. PCR cycling conditions and analysis was as for *Staphylococcal* spp. All sampling ages were compared using a Kruskal–Wallis analysis of variance with Dunn's multiple comparisons test.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/ijms242316626/s1. (References [39,40] are contained in supplementary material).

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Sepsis Prevention

Treatment of Dermal Infections with Topical Coconut Oil

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Treatment of Dermal Infections With Topical Coconut Oil

A review of efficacy and safety of Cocos nucifera L. in treating skin infections

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Abstract

Coconut oil, and many other portions of the plant *Cocos nucifera L*, have been hypothesized to have antimicrobial and antifungal activity. Medium-chain fatty acid constituents of coconut oil including lauric acid, capric acid, and others provide antimicrobial effect by disrupting bacterial, fungal, and viral cell membranes, leading to cell death. This review summarizes in vivo and in vitro studies of topical anti-infective properties of coconut oil and the medium-chain fatty acids contained within, and describes the proposed use of coconut products for dermal infections.

Introduction

Referred to as the “tree of life” because of its many uses, coconut, *Cocos nucifera L.*, is a fruit tree found in warm, humid climates with well-drained soil. Different cultures around the globe have cultivated the coconut tree and utilized the various parts of the coconut fruit [water, meat (from which coconut oil is isolated), and husks] for a plethora of uses from biofuel to food. Coconut oil has traditionally been used as a medicinal agent for cancer, diabetes, diarrhea, dry skin, and psoriasis and is used as an antibacterial, antifungal, and antiviral agent for the treatment of dermal infections.[1–3] Evaluation of *Cocos nucifera L.* as an anti-infective agent is very important due to the increased prevalence of antibiotic-resistant infectious microorganisms, and the dearth of novel antibiotics in the pipeline.[4,5]

Medicinal properties of *C. nucifera* are attributed to 3 medium-chain fatty acids found in coconut fat: lauric acid, the most abundant fatty acid, capric acid, and caprylic acid.[3] Lauric acid is a medium-chain fatty acid that, when esterified with glycerol, results in the monoglyceride monolaurin.[6] Monolaurin has been suggested as the most potent antimicrobial agent among those found in *C. nucifera*.[7]

The anti-infective mechanism of fatty acids such as those found in *C. nucifera* is poorly understood. One hypothesis is that fatty acids interfere with the bacterial cell structure and acids interfere with cellular energy production, causing disruption of the electron transport chain and oxidative phosphorylation.[3,6] Fatty acids may also inhibit enzyme activity, impair nutrient uptake, generate cellular degradation products, or cause direct lysis of infectious cells.[3,6]

This literature review summarizes *in vivo* and *in vitro* studies of virgin coconut oil (VCO), lauric acid, capric acid, monolaurin, and other fatty acids as microbicides against bacteria, fungus, and viruses that cause dermal infections.

Literature Review

A MEDLINE, International Pharmaceutical Abstracts, Natural Standard, and Natural Medicine database search was conducted for clinical trials published in English using the key terms coconut oil, *Cocos nucifera*, *Cocos nucifera L*., lauric acid, monolaurin, dermal infection, skin infection, antibiotic, and antimicrobial. *In vitro* and *in vivo* trials published in English that evaluated the anti-infective efficacy and safety of coconut oil and its components were selected and evaluated.

Clinical Evidence

Studies have evaluated the antimicrobial activity of *Cocos nucifera L.* husk fiber, coconut oil, and lauric acid and monolaurin extracts. Table 1 summarizes *in vitro* and *in vivo* studies.

A double-blind, randomized controlled trial compared virgin coconut oil (VCO) to virgin olive oil (VOO) for efficacy in removing colonized *Staphylococcus aureus* in 26 patients aged 18 to 40 years with atopic dermatitis (AD). This study included patients with new and old AD with low to high moderate scores on the SCORing Atopic Dermatitis (SCORAD) severity index (O-SSI, an objective scoring system that

accounts for spread and intensity of lesions, as well as subjective symptoms such as pruritus and insomnia; scores range from 0 to 40). Patients were excluded if they had grossly infected lesions requiring antibiotics, any dermatologic diagnosis other than AD, hypersensitivity to VCO or VOO, or an immunocompromised state (including diabetes), or if they were on topical steroids or topical/oral antibiotics within the past 2 weeks. Before initiation and after 4 weeks of treatment, cotton swabs of well-defined lesions were obtained and analyzed for presence of *S. aureus*. Both groups applied 5 mL of either VCO or VOO on the affected area twice daily and were instructed not to put any other emollients, creams, or oil-based products on the lesions.[8]

Patients were on average 31.5 years old, approximately 50% were female, and duration of AD was 16.5 years; there was no difference in baseline O-SSI scores. At baseline, 20 patients in the VCO group and 12 in the VOO group were colonized with *S. aureus*. Of the patients who were initially colonized, after 4 weeks of treatment, 1 patient (5%) treated with VCO remained colonized with *S. aureus compared* to 6 patients who were treated with VOO (50%) (RR=0.10, 95% CI: 0.01–0.73, *P*=0.028). Post-intervention O-SSI scores in the VCO group were significantly lower than the VOO group (mean difference -4.1, *P*=0.004). No adverse effects to VOO or VCO were reported.[8]

A mixed *in vitro* and *in vivo* study examined the antibacterial activity of lauric acid against *Propionibacterium acnes* and other skin flora. *P. acnes* is the main causative organism of acne vulgaris, a disease that affects between 50% and 95% of adolescents at some point in their lives and 40 million people in the United States.[9] Current treatments, such as benzoyl peroxide (BPO), have undesirable side effects including burning, drying, irritation, and erythema.[10] *S. aureus*, *Staphylococcus epidermidis*, and *P. acnes* were co-cultured with either BPO or lauric acid. Following incubation of agar plates containing *P. acnes* and either BPO or lauric acid, the minimum inhibitory concentration (MIC) against each organism for BPO were 15.6, >100, and 62.5 mcg/mL, respectively, compared to 0.9, 3.9, and 3.9 mcg/mL, respectively, for lauric acid. EC50 were 30, not determined, and 60 mcg/mL for BPO, respectively, versus 6, 4, and 2 mcg/mL for lauric acid, respectively. Lauric acid was bactericidal to *P. acnes* at concentrations over 60 mcg/mL. In the *in vivo* portion, BALB/C mice ears were injected intradermally with 1 X 107 colony forming units (CFU) of *P. acnes*. After 24 hours, significant swelling was observed in the *P. acnes* injected ear. Inflamed ears were then treated with intradermal injections and epicutaneous applications of lauric acid. After 1 day ear

inflammation thickness was significantly reduced ($P<0.05$), as were *P. acnes* CFU ($P<0.0005$). TUNEL assays (Terminal deoxynucleotidyl transferase dUTP nick end labeling, a method that identifies DNA fragmentation that results from abnormal apoptosis or cellular DNA damage) reveal that lauric acid was not toxic to keratinocytes.[11]

Eight *in vitro* studies assessed the antimicrobial activity of lauric acid and monolaurin on a wide variety of microorganisms, and all are reviewed in chronological order below.[6,10,12–17] The first *in vitro* study evaluated bactericidal properties of 30 different fatty acids including lauric, capric, and caprylic acids and their derivatives against gram-negative organisms (*Proteus vulgaris*, *P. mirabilis*, *P. rettgeri*, *Escherichia coli*, *Serratia marcescens*, *Pseudomonas aeruginosa*, and *Salmonella typhimurium*), gram-positive organisms (*S. aureus*, *S. epidermidis*, beta-hemolytic streptococci, group D streptococcus, *Bacillus subtilis*, *Sarcina lutea*, *Micrococcus sp.*, *Nocardia asteroids*, *Corynebacterium sp.*, and pneumococcus) and *Candida albicans*. The MIC was determined for each organism after an 18-hour incubation time on an agar plate. Lauric acid and capric acid were active against all gram-positive and gram-negative bacteria and *C. albicans*. Compared to glyceride derivatives, the free acid form of lauric acid had the highest bacteriostatic activity. MIC ranges for lauric acid were 0.062–2.249 micromoles/mL, for capric acid were 1.45 and 5.8 micromoles/mL, and for caprylic acid were not inhibitory at concentrations tested.[12]

Next, an *in vitro* study evaluated fungicidal activity of *C. nucifera* fatty acids capric acid, lauric acid, and a variety of monoglycerides against *Candida albicans*. *C. albicans* was incubated with a suspension of each fatty acid. After a short inactivation time of 10 and 30 minutes and 2 and 5 hours, CFU of yeast were measured. The study determined that capric acid had the fastest killing of *C. albicans* with no colonies present after 10 minutes versus 3.5 $\log_{10}$ CFUs in the lauric acid sample. Higher concentrations of both lauric and capric acids were more effective at killing than lower concentrations. Following longer incubation times of 30 minutes and above, lauric acid had the most reliable killing compared to other agents with no detectable growth at 30 minutes, 2 hours or 5 hours.[13]

Another study evaluated the activity of 15 antimicrobial medications (including, but not limited to, ampicillin-sulbactam, vancomycin, oxacillin, levofloxacin) and 6 saturated fatty acids (lauric acid, stearic acid, octanoic acid, myristic acid, palmitic

acid) against methicillin sensitive and 4 strains of methicillin resistant *S. aureus* (MSSA and MRSA, respectively). MICs for antibacterial drugs were determined following microbroth dilution. Time-kill curves were also determined, as were MICs in the presence of human plasma. Lauric acid inhibited growth of all strains of S. aureus, and had a lower MIC compared to other saturated fatty acids (400 mcg/mL for all strains versus 800–1,600 mcg/mL for other fatty acids). MICs were increased to 800 mcg/mL in the presence of 10% human plasma. All antimicrobial agents displayed much lower MICs than the fatty acids (on the order of ≤0.5-2 mcg/mL for most agents against MSSA, and ≤0.5–>16 mcg/mL for MRSA with arbekacin as the agent with the lowest MIC). Lauric acid showed a bacteriostatic effect at concentrations at and above the MIC, and was bactericidal at 6 hrs at concentrations 2 and 4 times the MIC.[14]

Ogbolu et al (2007) performed an *in vitro* study that focused on the antifungal properties of coconut oil compared to fluconazole, a first-line option for a variety of *Candida* yeasts. Fifty-two isolates obtained from vaginal, endocervical, urine, ear swab/discharge, and wounds were studied for their susceptibilities to VCO and fluconazole using an agar-well diffusion technique. Progressively dilute solutions of VCO or fluconazole were placed on an agar medium with yeasts. After 24-hours, the sensitivity patterns were measured, and zone of inhibition diameter ≤27 mm were considered resistant. All *Candida* species were sensitive to 100% coconut oil vs 92% in fluconazole. It is important to note that some species such as *C. krusei* and *C. tropicalis* are known to be less susceptible to fluconazole, and this medicine is not recommended for these infections. *C. albicans* had the highest susceptibility to coconut oil, and the percent of *Candida* species sensitive to VCO was greater than the percent of species sensitive to fluconazole at most concentrations.[15]

An *in vitro* study of skin samples from infected atopic dermatitis and impetigo lesions from 100 newborn to 18-year-old patients evaluated sensitivity to monolaurin. Dermatoses were infected with gram-positive organisms and gram-negative organisms including *S. aureus*, coagulase negative *Staphylococcus*, *Streptococcus pyogenes*, *E. coli*, *Serratia marcescens*, *Klebsiella rhinosclermatis,* and others. Skin scrapings were incubated for 24 hours then added to a blood agar plate with monolaurin, penicillin, oxacillin, erythromycin, mupirocin, fusidic acid, or vancomycin. All *S. aureus*, coagulase-negative *Staphylococcus*, *Streptococcus spp*, *Enterobacter*, *Enterococcus*, and *E. vulneris* species were 100% sensitive to monolaurin; 100% sensitivity was not observed in any of the antibiotics. *K.*

rhinosclermatis were less sensitive to monolaurin, but still showed 92.31% sensitivity to monolaurin compared to 0–7.69% sensitivity to other antibiotics ($P<0.05$ for each antibiotic).[6]

Another *in vitro* study by Yang et al compared the effectiveness of lauric acid, palmitic acid, and oleic acid against *P. acnes* on Brucella broth agar plates after 3 days incubation. Lauric acid began to kill *P. acnes* at concentrations above 50 mcg/mL and completely killed *P. acnes* at 80 mcg/mL. Growth was still noted at concentrations as high as 100 mcg/mL for both palmitic and oleic acids. To improve water solubility and potentially improve delivery of medicinal agents, lauric acid was loaded into liposomes. It was found that lauric acid–loaded liposomes could fuse with *P. acnes* bacterial membranes and were effective at delivering lauric acid. Complete killing of *P. acnes* by liposomal spheres was noted at concentrations above 51 mcg/mL.[10]

Coconut oil in water cream emulsions varying in concentrations from 5–40% were tested for *in vitro* antimicrobial activity against *C. albicans*, *Aspergillus niger*, *S. aureus*, and *Ps. aeruginosa*. Each cream was inoculated with a standardized culture of bacteria or yeast, and survival was measured at 6, 24, and 48 hrs as well as at 7, 14, and 28 days. Creams were compounded as preservative-free or with preservatives of lemon grass oil, parabens, or cetrimide. No growth of *S. aureus* was observed after 6 hrs for any of the creams, no growth of *Ps. aeruginosa* after 48 hrs, and none after 7 days for *Candida* or *A. niger*. The results of this study indicate that coconut oil could be formulated into a cream and maintain its antimicrobial activity on both fungus and bacteria.[16]

Lastly, Fischer et al (2012) examined the efficacy of different sphingoid bases and fatty acids against 4 gram-positive and 7 gram-negative bacteria typically found in the oral and epithelial microbiome, including *Fuscobacterium nucleatum*, *S. aureus*, *Streptococcus sanguinis*, *S. marcescens*, *Streptococcus mitis*, *E. coli*, *Ps. aeruginosa*, *Corynebacterium bovis*, *C. striatum*, and *C. jeikeium*. Bacterial cultures were added to dilute lipid suspensions in microtiter plates, and after incubation, the MIC and MBC were measured and evaluated. While all sphingoid bases were antimicrobial for gram-positive organisms (MIC range 0.3–13 mcg/mL), lauric acid was the only fatty acid that displayed antibacterial activity against *C. bovis*, *C. striatum*, and *C. jeikeium*. Lauric acid did not have any activity against *E. coli*, *Ps. aeruginosa*, or *S. marcescens* (MBC >500 mcg/mL) in this study.[17] This study had different results

toward gram-negative bacteria than previous studies, and this may be due to difference in study methodology (direct inoculation of bacteria followed by incubation with fatty acids on an agar plate versus addition of bacteria to a dilute fatty acid suspension followed by incubation on microtiter plates).

Adverse Effects

When applied topically, coconut oil has a very low risk of allergic reaction or adverse effects. However IgE binding proteins are present, and allergic reactions have been described in a small number of patients, as has localized pruritus.[1,18–20] Lightening of skin tone may occur.[21] Avoid topical use of coconut oil if a known allergy or hypersensitivity exists to coconut, coconut oil, or any member of the *Arecaceae* family. Systemic absorption is low for coconut oil; however, when administered orally, hypotension and hyperlipidemia have been noted, as have reductions in serum lipids.[22,23] Lauric acid is known to be excreted in breast milk and may induce allergies in infants.[20] There are no known drug or food interactions with coconut oil when applied topically; however, antihypertensive and antihyperlipidemic medications may be affected if administered orally.[1,8,24] Topical coconut oil has been studied in children, the elderly, and pregnant and lactating women; adverse effects are similarly rare in all groups.[1]

Conclusions

Cultures across the globe have used the *Cocos nucifera L.* plant for many generations. Constituents of coconut oil, predominantly lauric acid, have *in vitro* and *in vivo* evidence for killing a wide variety of gram-positive and gram-negative bacteria and *Candida* species. Though lauric acid has a lower MIC compared to other fatty acids, it does not achieve the same bacteriostatic or bactericidal potential as commercially available antibiotics. Coconut oil can be prepared in emulsions and liposomes and retain anti-infective properties. Given the low side effect burden, it may be a reasonable option for patients with mild to moderate dermal infections, especially acne vulgaris caused by *P. acnes*, polymicrobial atopic dermatis, impetigo, or wound infections. Additional randomized controlled trials are needed to solidify the place in therapy of *C. nucifera* as a treatment of dermal infections.

Table 1: Summary of in vitro and in vivo studies of the antimicrobial properties of coconut oil

Reference	Study Design	Study Overview	Study Results
Verallo-Rowell, 2008	DB, RCT	VCO was compared to VOO in removing colonized S. aureus from 26 patients aged 18 to 40 with mild to moderate high scores on SCORAD O-SSI atopic dermatitis. Patients were administered 5 mL of either VCO or VOO twice daily.	Of patients who were originally colonized with S. aureus, only 1 patient treated with VCO remained colonized after 4 weeks vs 6 patients treated with VOO (RR=0.10, 95% CI: 0.01–0.73, P=0.028). Post-intervention O-SSI scores in the VCO group were significantly lower than the VOO (mean difference -4.1, P=0.004). No ADEs to VOO or VCO were reported.

Nakatsuii, 2009	Mixed: In vivo and in vitro	In vitro portion: S. aureus, S. epidermidis, and P. acnes were co-cultured with either BPO or lauric acid on agar plates. In vivo portion: BALB/C mouse ear were injected intradermally with to P. acnes. Inflamed ears were then treated intradermally and epicutaneous with lauric acid.	MIC for S. aureus, S. epidermidis, and P. acnes against each organism for BPO were 15.6, >100, and 62.5 mcg/mL, respectively, versus 0.9, 3.9, and 3.9 mcg/mL, respectively, for LA. After 1 day ear inflammation thickness was significantly reduced ($P<0.05$), as were P. acnes CFU ($P<0.0005$), and TUNEL assays reveal that lauric acid was not toxic to keratinocytes.
Kabara, 1972	In vitro	The bactericidal properties of 30 FAs including LA and CA and their derivatives were studied against gram-negative and gram-positive bacteria and C. albicans. MICs were determined after an 18-hour incubation on an agar plate.	LA and CA were active against all gram-positive and gram-negative organisms and Candida. MIC ranges for lauric acid were 0.062–2.249 micromoles/mL, and were 1.45 and 5.8 micromoles/mL for capric acid.

Bergusson, 2001	In vitro	The susceptibility of C. albicans to CA and LA were evaluated particularly following 10 min, 30 min, 2 hr, and 5 hr incubation periods.	CA was found to have to fastest killing of C. albicans, and LA had the most reliable killing at times greater than 30 min.
Kitahara, 2004	In vitro	The MIC of saturated FAs, including LA, was determined against 6 strains of MRSA and MSSA using a microbroth dilution and compared to a variety of antibiotics.	Of the saturated FAs examined, LA was the most effective against strains of S. aureus with a MIC of 400 μg/mL vs 800–1,600 μg/mL for other FAs. LA was not as effective compared to antibiotic agents that had MICs as low as 0.5 μg/mL.
Ogbolu, 2007	In vitro	52 isolates of Candida species obtained from vaginal, oral, wound, and ears were studied for their susceptibilities to VCO and fluconazole by using the agar-well diffusion technique.	100% of Candida species were susceptible to VCO, including Candida species known to have inherent resistance to fluconazole. C. albicans had the highest susceptibility to VCO and VCO consistently killed more species than fluconazole.

Carpo, 2007	In vitro	Skins samples for 100 pediatric patients, newborn to 18 years old, with infected impetigo or actopic dermatitis dermatoses were tested for sensitivity against monolaurin, and a variety of antibiotics. Infections were polymicrobial.	All the present gram-positive organisms were 100% susceptible to monolaurin, as were Enterobacter spp., E. vulneris, and Enterococcus spp. K. rhinosclermatis was less sensitive to monolaurin, at 92.31%, but antibiotic sensitivities ranged from 0–7.69% ($P<0.05$ for each antibiotic)
Yang, 2009	In vitro	LA, palmitic acid, and oleic acid were incubated with P. acnes then diluted and grown on agar plates for 3 days. After 3 days, CFUs of P. acnes were quantified. LA was also formulated into liposomes in an effort to increase delivery of LA, and then evaluated for antimicrobial activity.	LA completely killed P. acnes at 80 mcg/mL, and this was not mimicked by palmitic and oleic acids. LA loaded liposomes fused with P. acnes, were effective at delivering lauric acid, and completely killed at concentrations above 51 mcg/mL

Oyi, 2010	In vitro	VCO in water emulsion creams with either no preservative or preservative were evaluated for antimicrobial activity against C. albicans, A. niger, S. aureus, and Ps. aeruginosa.	Regardless of preservative status VCO in water emulsions killed S. aureus was by 6 hrs, Ps. aeruginosa by 48 hrs, Candida or A. niger by 7 days.
Fischer, 2012	In vitro	The MIC and the MBC of sphingoid bases, including LA, were evaluated from antimicrobial assays measured the susceptibility of 4 gram-negative and 7 gram-positive bacteria.	All FAs were antimicrobial for gram-positive organisms (MIC range 0.3–13 mcg/mL), and LA was the only FAs that was antibacterial to C. bovis, C. striatum, and C. jeikeium. Lauric acid did not have any activity against E. coli, Ps. aeruginosa, or S. marcescens (MBC >500 mcg/mL) in this study

Abbreviations: CA: capric acid, CFU: colony forming units, DB: double blind, FA: fatty acid, LA: lauric acid, MBC: minimum bactericidal concentration, MIC: minimum inhibitory concentration, MRSA: methicillin resistant Staphylococcus aureus, MSSA: methicillin sensitive Staphylococcus aureus, RCT: Randomized controlled trial, spp: species, VCO: virgin coconut oil, VOO: virgin olive oil

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