Hygiene and Body Site Affect Your Skin Microbiome

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Introduction

Human skin is a habitat for millions of bacteria, fungi, viruses and microscopic communities referred to as “skin microbiome” [1]. The skin microbiome forms dynamic community relationships attributed to the diverse oily, moist and dry skin sites [2]. Studies recognize the skin microbiome plays a crucial role in homeostasis and disruption can lead to bacterial imbalance [3,4]. Skin of healthy individuals is dominated by members of the phylum *Actinobacteria, Firmicutes, Proteobacteria and Bacteroidetes* but the composition and diversity may vary between individuals due to intrinsic factors such as skin physiology and extrinsic factors such as hygiene routine [2,4]. Few studies have explored how personal care can inhibit survival of skin flora but it is still an understudied area [6-8]. This study used 16s rRNA genomic data across 5 skin regions of 15 participants to characterize the skin microbiome's spatial diversity and to test the “grandma hypothesis” [5]. The hypothesis tests whether less washed areas like behind ears (BE), between toes (BT) and belly button (BB) have different microbiomes than more frequently washed areas of forearms (FA) and calves (CA). Better understanding of the microbiome can inform hygiene guidelines and provide clarity to prevent dysbiosis.

Methods and Materials

Cohort: For this cross-sectional participants provided written consent then sampled as part of a Public Health Genomics Course at the Milken Institute of Public Health at the George Washington University (Washington, DC, USA)[5]. Participants self-reported information regarding skin conditions, cold or respiratory illness, antibiotic use and topical steroid at least a month prior. The study was approved by the GWU Committee on Human Subject Research, Institutional Review Board in 2024.

Sampling: 15 students participated in the study and provided self-swabbed regions consisting of oily, moist and sites in the five regions. Each region was sampled twice for 30 seconds and moistened with SCF-1 solution, placed in an Eppendorf tube containing the ZymoBIOMICS Lysis Solution then processed together directly after sampling [4,5].

16S amplicon sequencing: Total DNA was extracted using ZymoBIOMICS DNA Miniprep kit and followed metataxonomic protocol [4]. We targeted the V4 region of the bacterial 16S rRNA gene for primers to perform PCR amplification [4,5]. The PCR products were checked via gel electrophoresis then prepared for sequencing [4,5]. The GWU Genomics Core performed a single run of the Illumina MiSeq sequencing Platform.

Microbiome analyses: Processed MiSeq FASTQ sequence file reads were clustered into Amplicon Sequence Variants (ASVs) using the dada2 pipeline and assigned against the SILVA database. We uploaded ASV, taxonomic table, a phylogenetic tree and student metadata in MicrobiomeAnalyst [9,10] totaling 75 samples, 9443 initial sequence variants and 495 ASVs. Data filtering involved removal of singletons and samples FA-01, FA013, FA14, CA-14 with read count (<1000). We filtered the ASVs based on mean abundance, variance estimated by an

Interquartile range of 10% and strictly used Total Sum Scaling (TSS) for normalization. Final total of ASVs was 301. Overall community composition ranked by top 10 phyla and genera using relative abundance.The core taxa (phyla and genera) was analyzed across all samples at >80% prevalence and >0.01% relative abundance. The Shannon alpha-diversity index between skin regions and wash frequency for the ASV level was assessed using Kruskal-Wallis test. For beta diversity across the regions and wash frequency we used the PCoA ordination method on weighted UniFrac distance and the ANOSIM test at the ASV level. Taxon comparisons of phyla and genera between regions and wash frequency was determined after FDR correction for the ANOVA test at 0.05 adjusted p-value cut off.

Results

Across the overall skin microbiome, the most dominant phylum detected were *Firmicutes*, *Actinobacteria*, *Proteobacteria* and *Bacteroidetes* [Figure 1]. The most dominant genera were *Staphylococcus*, *Corynebacterium*, *Anaerococcus*, *Peptoniphilus* and *Porphyromonas* [Figure 1]. The core microbiome analysis revealed that only phylum Firmicutes and Actinobacteria meet our 80 %/0.01 % thresholds meaning they are universal skin residents. At the genus level, two core taxa include *Streptococcus* and *Corynebacterium*, suggesting they perform essential roles in skin health. Alpha-diversity varied by region and wash [Figure 2]. A Kruskal–Wallis test revealed alpha-diversity does differ by site and showed statistical significance ($p < 0.001$). The two less washed sites FA and CA exhibited the highest median Shannon indices (median FA = 3.1; CA = 3.0), both of which were greater than the least-diverse site, the belly button (median BB = 2.0). The difference by wash frequency was significant ($p < 0.01$) and the more washed group had higher median shannon index compared to the less washed group contrary to the grandma hypothesis. Beta diversity differed by region and wash frequency. In Figure 3, the two frequently washed FA and CA form a loose cluster while other less washed sites are in different quadrants suggesting strong spatial structure. Washing frequency explained 9% of the total compositional variance ($R^2 = 0.0905$) with $p = 0.001$ and FDR = 0.001 meaning the frequency accounts for community structure. The univariate analysis revealed that both washing frequency and region (FDR < 0.01) strongly influence which microbial lineages dominate your cohort's skin [Table 1]. More washed sites favor *Proteobacteria* and *Escherichia–Shigella*, while less-washed 'grandma' niches accumulate *Firmicutes* and *Staphylococci*.

Discussion

Our analysis confirms that both region and washing frequency shape the skin microbiome. The community composition across less washed versus more washed sites mirrors earlier work showing key taxa like *Firmicutes* and *Staphylococcus* dominate healthy skin. Contrary to the "grandma hypothesis," frequently washed sites exhibited higher α-diversity, consistent with Pérez-Losada et al [5], suggesting that routine cleansing may promote evenness. Wash frequency further partitions beta-diversity beyond site effects, in line with Grice et al [3]. Lastly, Proteobacteria enrich more washed sites, while Firmicutes and Staphylococcus predominate less-washed regions reflecting both differential removal by site-specific microenvironments. The findings refine our understanding of how hygiene and skin physiology interact to maintain distinct microbial communities.

Conclusion These findings validate the “grandma hypothesis,” demonstrating that hygiene routines and regional ecology jointly drive the assembly and maintenance of our resident microbial communities.

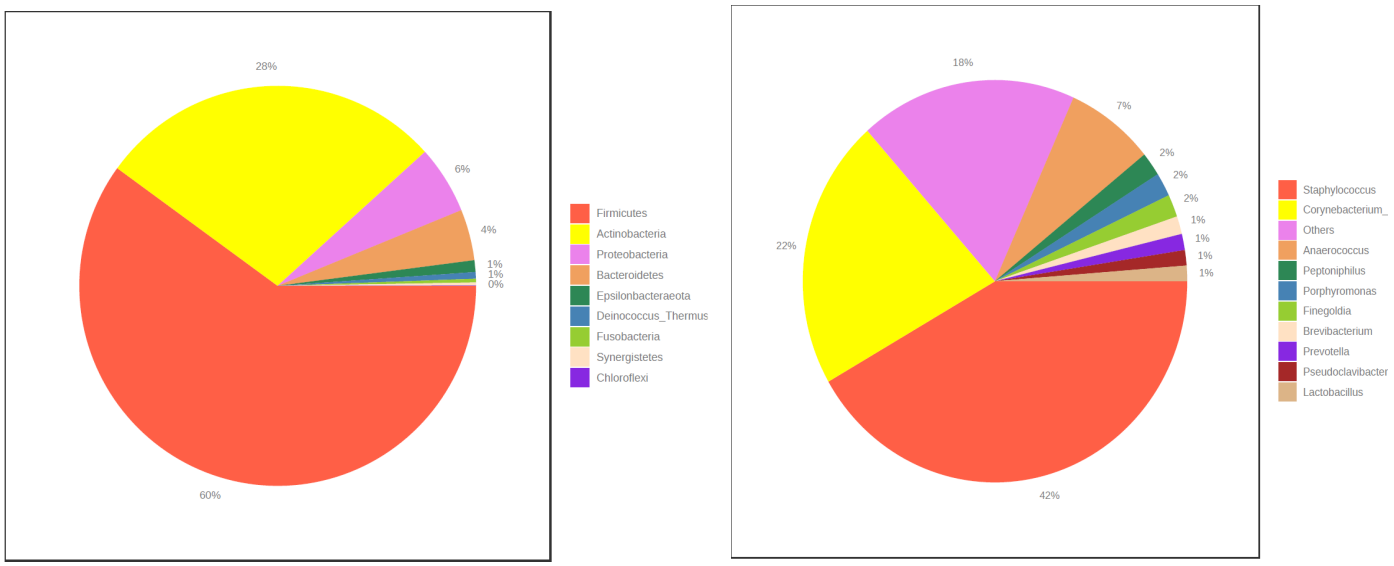

Figure 1.Relative abundance of top taxa across all skin samples at the Phylum (left) and Genus (right) level.

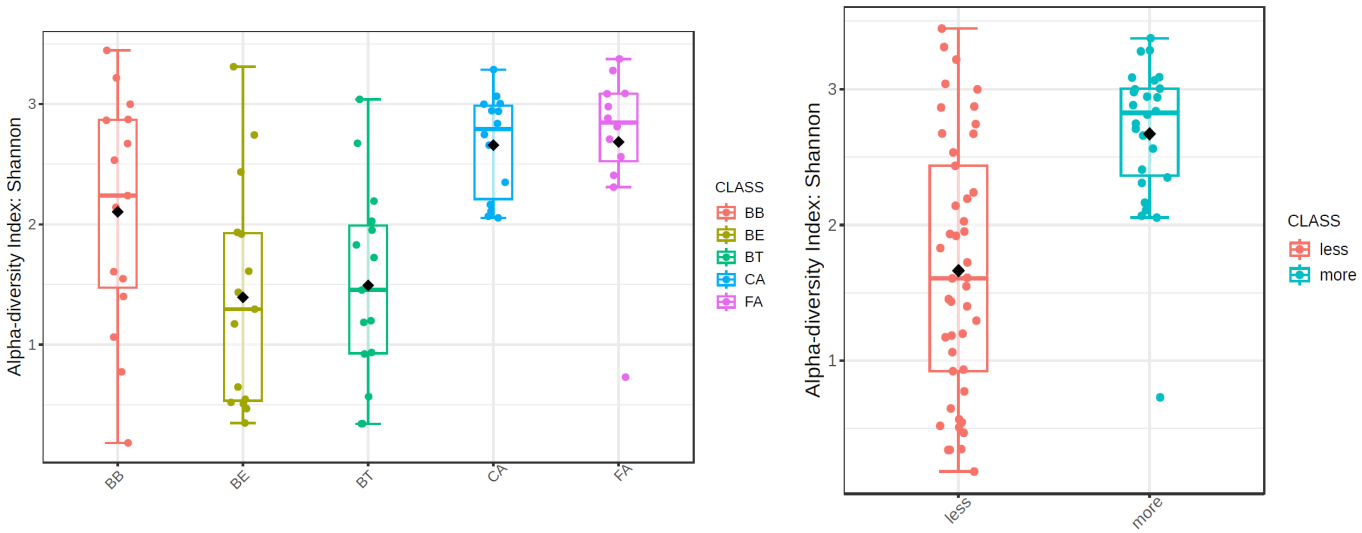

Figure 2. Abundance-based coverage estimator of alpha-diversity across skin regions alone and wash. Less = regions washed less: behind the ears (BE), between toes (BT), and belly button (BB); More = regions washed more often forearms: (FA) and calves (CA)

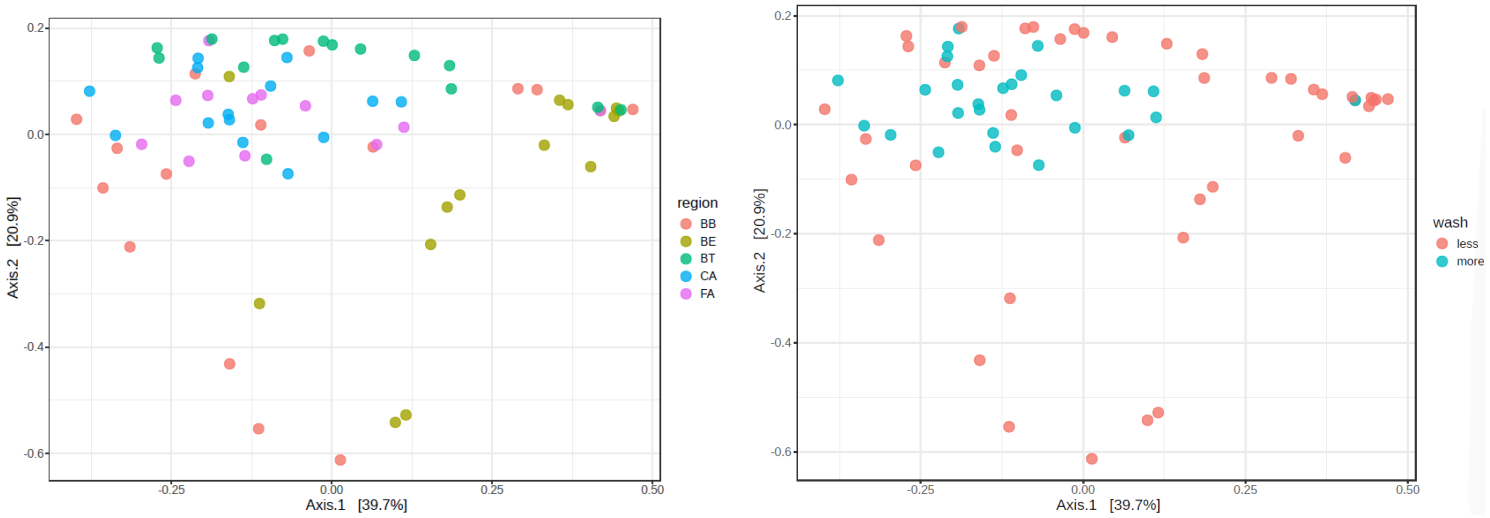

Figure 3: PCoA analysis of skin regions using weighted UniFrac index and the ANOSIM test across skin regions and wash. Less = regions washed less: behind the ears (BE), between toes (BT), and belly button (BB); More = regions washed more often forearms: (FA) and calves (CA)

Test	Level	Taxon	p-value	FDR
Wash frequency	Phylum	Proteobacteria	<0.001	2.30×10^{-7}
Wash frequency	Phylum	Firmicutes	<0.001	7.71×10^{-4}
Body region	Phylum	Proteobacteria	<0.001	5.24×10^{-8}
Body region	Phylum	Firmicutes	<0.001	1.95×10^{-5}
Body region	Phylum	Actinobacteria	0.0011	3.23×10^{-3}
Wash frequency	Genus	Escherichia–Shigella	<0.001	4.73×10^{-3}
Wash frequency	Genus	Staphylococcus	<0.001	1.28×10^{-2}
Wash frequency	Genus	Bacillus	0.0020	4.60×10^{-2}
Wash frequency	Genus	Streptococcus	0.0020	4.60×10^{-2}
Wash frequency	Genus	Anaerococcus	0.0024	4.60×10^{-2}
Wash frequency	Genus	Noviherbaspirillum	0.0026	4.60×10^{-2}
Body region	Genus	Streptococcus	<0.001	1.07×10^{-4}
Body region	Genus	Corynebacterium_1	<0.001	1.21×10^{-3}
Body region	Genus	Escherichia–Shigella	<0.001	1.84×10^{-3}
Body region	Genus	Staphylococcus	<0.001	1.84×10^{-3}
Body region	Genus	Lawsonella	<0.001	1.74×10^{-2}
Body region	Genus	Anaerococcus	0.0014	2.44×10^{-2}
Body region	Genus	Bacillus	0.0020	3.18×10^{-2}
Body region	Genus	Micrococcus	0.0037	4.95×10^{-2}
Body region	Genus	Noviherbaspirillum	0.0044	4.96×10^{-2}
Body region	Genus	Allorhizobium-Neorhizobium-Parahizobiu	0.0045	4.96×10^{-2}
Body region	Genus	Paenibacillus	0.0051	4.96×10^{-2}

Table 1: Differentially abundant taxa (phyla and genera) across body regions and wash frequency

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