Volume 10:1-13

UJEMI+

The Undergraduate Journal of Experimental Microbiology & Immunology (+Peer Reviewed)

Individuals in Shared Dormitories who Rarely Wash their Sheets are Associated with Greater Sex-Specific Microbial Changes Compared to Individuals who Wash their Sheets Frequently

Lina Anwari, Abigail Cho, Dahyeon (Betty) Hong, Màiri MacAulay, Juliet Malkowski

Department of Microbiology and Immunology, University of British Columbia, Vancouver, British Columbia, Canada

SUMMARY The skin is the largest organ in the human body whose surface contains a diverse microbial community that contributes to human health by harboring and protecting against pathogens. The composition of the microbial community on the human hand is unique as it has frequent and direct interactions with the surrounding environment. Various factors impact the microbial species present on the dominant hand of an individual, with a primary intrinsic factor being sex and an extrinsic one being hygiene practice. While hygiene practices like hand washing have been shown to decrease overall microbial load, there is a current knowledge gap on how other hygiene practices, such as sheet washing, intersect with sex to impact the skin microbiome. This study therefore explored the impact of sex and the hygiene factor of sheet washing frequency on the microbial composition of hands among individuals residing in shared dormitories. Through microbial diversity and abundance analyses, our findings suggest that sex is a greater driver of hand microbial composition than sheet washing frequency, but that sheet washing frequency still has an effect, as less frequent sheet washing is associated with greater variations in hand microbial composition. Overall, the findings from our study contribute to the growing field of research on how hygiene habits influence the human microbiome in a sex-specific manner, providing a platform for further investigations on the effects of these intersecting factors on health outcomes.

INTRODUCTION

Hygiene practices have historically been associated with lowering microbial concentrations on the skin to reduce the transmission of diseases (1). One of the most common pathways pathogens are spread across populations is through touch, often done by the dominant hand (2). Investigation into the effect of hygiene practices on the hand microbiome can allow for the recommendation of more informed hygienic choices to prevent the spread of pathogenic diseases (3). Many studies have focused on hand washing as the primary hygiene practice to lower pathogenic transmission, leaving a current knowledge gap about the hygiene practice of sheet washing on the hand microbiome (1, 4).

Bed sheet washing has long been considered a beneficial hygiene practice to reduce the spread of disease, as inadequately washed sheets have been recognized to harbor pathogens (5). Humans come into direct contact with their bed sheets where their microbiota are then dispersed throughout the night (6). Infrequent sheet washing would lead to the accumulation of microbes and potential pathogens as many microorganisms can survive on similar surfaces for periods ranging up to several months (5). Due to the daily direct contact humans have

Published Online: September 2024

Citation: Anwari, Cho, Hong, MacAulay, Malkowski. 2024. Individuals in shared dormitories who rarely wash their sheets are associated with greater sex-specific microbial changes compared to individuals who wash their sheets frequently. UJEMI+ 10:1-13

Editor: Ronja Kothe, University of British Columbia

Address correspondence to:
https://jemi.microbiology.ubc.ca/

with bed sheets, the human hand microbiome has the potential to be influenced by the microbes present on bed sheets.

Another factor that contributes to the composition of the hand microbiome is sex (4). Genetic differences between male and female skin that participate in the formation of the microbial environment include skin thickness, number of hairs, sweat, sebaceous glands, and sex hormones (7). Sex has a substantial impact on determining hand microbial diversity, as women have been shown to consistently have a higher microbial diversity than men (4). Gender may also play a role in a social context, as a study has shown that worldwide women tend to adhere to hygiene practices more strictly than men (8).

While genetics is a major contributor to the composition of the microbial community on the skin, one of the most influential factors that are within human control is hygiene practices. Previous studies have shown that hygiene practices reduce the overall microbial load while sex creates distinct bacterial communities (4). Using data from Richardson *et. al,* our group investigated the hygiene practice of sheet washing in conjunction with sex to see if either factor influences the microbial community composition of the human skin (9). We hypothesized that both sheet washing frequency and sex would impact the microbial diversity of the hand microbiome.

Past research by students at the University of British Columbia used the same dataset from Richardson *et. al* to investigate the impact of roommates on an individual's microbiome and the abiotic environment of the shared space. Their findings showed significant differences in the microbiome of the abiotic samples between single and multiple occupancy dorms (10). To provide further information about the microbial communities of a shared space, our group aimed to expand on their findings focusing on hygiene practices and sex and their impact on biotic microbial composition.

Through our analyses of various diversity metrics, we found that both sex and sheet washing frequency influence hand microbiome diversity and composition, however, sex has a stronger effect. When sheets were washed less frequently, taxa bar plots revealed a greater variation in the abundance of phyla when comparing between sexes, and core microbiome analysis showed a greater diversity of unique microbial genera. DESeq2 results showed more unique upregulated genera in females than males across both sheet washing frequency groups. The two most upregulated genera found for females and males respectively were *Corynebacterium* and *Prevotella*. These two genera were common in the hand microbiome of both sexes, while the most abundant unique genera were *Qipengyuania* and *Anaerococcus* for females and *Aggregatibacter* and *Acinetobacter* for males in the high and low sheet washing frequency groups respectively. These findings contribute to current research being conducted on skin microbial community composition by investigating how hygiene habits influence the hand microbiome in a sex-specific manner. By expanding on current findings of hygiene practices and sex on the hand microbiome, hygienic practices can be recommended to reduce pathogenic transmission.

METHODS AND MATERIALS

Scripts.
https://github.com/mairi-macaulay/MICB475_Group13/tree/904fcbbf7b8b8d63e4068b5751eeeb8ebb70b3c9/Lab_Notebook

Dataset and metadata filtering. The dataset originated from a study conducted by Richardson *et al*. that examined the skin and environmental surfaces within a shared dormitory (9). The study was conducted with four time points over 3 months, during which samples were collected from 37 participants and their rooms in the dormitories at the University of Chicago. The selected metadata category used for this project was sex and weekly frequency of bed sheet washing. Before initiating the data processing, the sheet washing frequency was divided and added as an additional column in the metadata file using Microsoft Excel (v. 16.77.1). Samples were categorized into two groups based on the sheet washing frequency reported from histogram categorization prior to collection: 28 samples of "high" frequency, from individuals who washed sheets every 0 to 2 weeks, and 11 samples of "low" frequency, from individuals who washed their sheets more than every 6 weeks. Each sample was further categorized by sex, resulting in four groups: male high frequency (20

samples), female high frequency (8 samples), male low frequency (7 samples), female low frequency (4 samples).

Initial data processing in QIIME2. From the Quantitative Insights into Microbial Ecology Version 2 (QIIME2) server (11), we imported and demultiplexed the dorms dataset. The demultiplexed dataset was denoised to remove the low-quality reads, with a truncation length of 150 nucleotides (Figure S1). Then, Amplicon Sequence Variants (ASVs) were clustered using DADA2 (12). The V4 regions of the 16s ribosomal RNA gene from the SILVA database were extracted and were targeted with a 515F (5'-GTGCCAGCMGCCGCGGTAA-3')-806RB (5'-GGACTACHVGGGTWTCTAAT-3') primer pair (13). The denoised and clustered dataset was trained using the pre-trained classifier to assign the taxonomy of the reads. Mitochondria and chloroplast sequences were removed, and the metadata was filtered to keep only the skin (hand) samples. To address unequal sequencing depth and retain the majority of the samples and ASVs, the sampling depth was set to 6223 where 653,415 (38.06%) features were obtained in 105 (92.11%) samples (Figure S2). The ASVs are saturated and as a result, 9 samples were discarded at this rarefaction depth.

Formatting and filtering phyloseq object files for diversity analyses. Taxonomy, metadata, ASV tables, and phylogenetic tree from the preliminary QIIME processing steps were formatted and merged into a phyloseq object in R (v. 4.2.3) using packages phyloseq, ape, tidyverse, and vegan (14–17). For alpha diversity, beta diversity, and taxonomic bar plot analyses, the phyloseq object was filtered to remove non-bacterial sequences, samples with less than 100 reads, and samples where sheet washing frequency was not applicable. Phyloseq objects were rarefied to a sampling depth of 6223 to be consistent with preliminary QIIME processing rarefactions steps. This resulted in a reduction in the sample size of the four sex-specific sheet washing frequency groups: male high frequency (18 samples), female high frequency (6 samples), male low frequency (7 samples), female low frequency (3 samples). For DESeq2 and core microbiome analyses, the phyloseq object was not rarefied and additionally filtered to remove ASVs with less than 5 counts. Phyloseq objects for all analyses were also filtered for different sexes.

Alpha and beta diversity analyses. Alpha and beta diversity analysis and subsequent statistical analyses were conducted in R (v. 4.2.3) using vegan, phyloseq and tidyverse packages (14, 16, 17). For alpha diversity, differences in Observed, Chao1, ACE, Shannon, Simpson, Inverse Simpson, and Fisher's metrics were analyzed between low and high sheet washing frequency groups categorized by sex to determine differences in hand microbial composition (18–23). Two-way ANOVA statistical analyses were performed on each alpha diversity metric (24). Significance was defined with a p-value cutoff of < 0.05. For beta diversity, differences in unweighted UniFrac, weighted UniFrac, Jaccard, and Bray-Curtis metrics were calculated between hand microbial compositions of differing sexes with varying sheet washing frequency habits (25–28). A pairwise permutational analysis of variance (PERMANOVA) was performed on the beta diversity metrics in R (29). Each metric was conducted with a p-value cutoff of <0.05 to define statistical significance.

Taxa bar plot analysis at the phylum level. Taxa bar plot analysis was conducted in R (v 4.2.3). The following packages were loaded: phyloseq, tidyverse, ggplot2, ape, and vegan (14–17, 30). To determine the distinct phyla and their abundance associated with the varying sheet washing frequencies and sex, the taxonomic data's relative abundance at the phylum level was calculated for groups categorized by sheet washing frequency (high, low) and sex (male, female). The phyla that represent a relative abundance greater than 1% were filtered for. Using ggplot2, the taxa bar plots at the phylum level were generated for analysis.

Taxa bar plot analysis at the genus level. The top 5 most abundant phyla were further analyzed at the genus level. The phyla of interest included Actinobacteriota, Firmicutes, Bacteroidota, Fusobacteriota, and Proteobacteria. Taxa bar plot analysis was conducted in R (v 4.2.3). The following packages were added: phyloseq, tidyverse, ggplot2, ape, and vegan (14–17, 31). To assess distinct genus and their abundance associated with the varying sheet

washing frequencies and sex, the taxonomic data's relative abundance at the genus level was calculated for groups categorized by sheet washing frequency (high, low) and sex (male, female). To generate five genus-level taxa bar plots, the data was filtered for each phylum of interest. Genera that had a relative abundance of less than 1% were removed. Taxa bar plots were generated for further analysis at the genus level.

Core microbiome analysis. To identify both shared and unique core microbiome genera associated with different sexes and sheet washing frequencies, a core microbiome analysis was conducted. By using the phyloseq (v. 4.2.3) and microbiome (v 1.22.0) R packages (14, 32), the non-rarefied phyloseq data was converted into relative abundance for both low and high sheet washing frequency groups for females and males. To determine the optimal prevalence and abundance threshold, a heatmap was generated to visualize the range of prevalence and abundance levels for individual bacteria at the genus levels (Figure S5, S6, S7, S8). This heatmap analysis utilized microbiome R packages (v 1.22.0) for taxonomic data analysis and RColorBrewer packages (v 1.1-3) for defining the colour palette in the figure. To visualize the result, ggVennDiagram package was used to generate a four-way Venn diagram, illustrating the core microbiome for our analysis (v 1.2.3) (33). The minimum prevalence and abundance parameters were set at 0.5 (50%) and 0.001 (0.1%), respectively.

DESeq2 analysis. To compare the differences in abundance between sexes and sheet washing frequency, a DESeq2 analysis was conducted in R (v 4.2.3) and used the phyloseq, ape, tidyverse, vegan, ggplot2, and DESeq2 packages (14–17, 31, 34). A non-rarefied phyloseq object was imported and edited to ensure the object contained no zeros. Two phyloseq objects were created by filtering for two sheet washing frequencies: high and low, with 28 and 11 samples, respectively. One phyloseq object was filtered for only high sheet washing frequency data and the other for low sheet washing frequency data. Two DESeq2 objects were then created from the phyloseq objects and analyses were run comparing differences in sex in the two sheet washing groups. Volcano plots were run and genera were filtered at a p-value cutoff of < 0.01, a $\log_2$ fold change >2, and a baseMean > 1. Genera were then pruned and a list of genus names from each group was created. Each genus was identified as being unique to either males or females per sheet washing frequency group. Using ggplot2, a bar plot was created at the genus level to identify the upregulated and downregulated ASVs present in both comparison groups as $\log_2$ fold change (31).

RESULTS

Diversity of hand microbial communities varies more significantly due to sex than sheet washing frequency. Alpha diversity analyses including Observed, Chao1, ACE, Shannon, Simpson, Inverse Simpson, and Fisher's diversity were conducted to determine differences in hand microbial composition on groups categorized by sheet washing frequency (low, high) and sex (female, male) of individuals (Figure 1, Figure S3). Based on two-way ANOVA statistical analyses, no alpha diversity comparisons were found to be significantly different. To determine whether the beta diversity of hand microbial communities differed between the variables, beta diversity metrics were run between groups categorized by sex (female, male) and sheet washing frequency (low, high). Regardless of sheet washing frequency, all beta diversity metrics (unweighted UniFrac, weighted UniFrac, Jaccard, Bray-Curtis) differed significantly between sexes (Table 1, Figure S4). The greatest difference between sex-specific sheet washing frequency groups was observed between the "female high" and "male high" groups (Table 1, Figure S4). The same-sex comparisons that differed only in terms of sheet washing frequency showed no significant differences in beta diversity metrics except for unweighted UniFrac for the "female high" vs "female low" comparison and Bray-Curtis for the "male high" vs "male low" comparison (Table 1, Figure S4). Therefore, there may be phylogenetically different microbial taxa present or absent between "female high" and "female low" group hand microbiomes, but the overall abundance of these differential taxa is small. Comparatively, there may be differences in the abundance of shared taxa between "male high" and "male low" group hand microbiomes, but no significant differences in the presence or absence of specific taxa. These results indicate that beta diversity of hand microbial communities varies more significantly due to sex than due to sheet

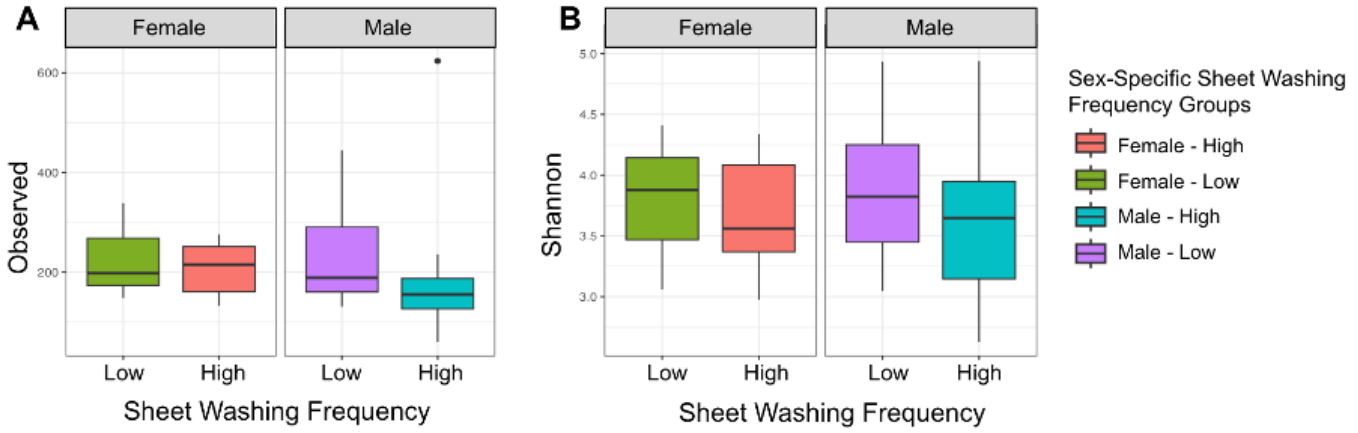

FIG. 1 Sex-specific sheet washing frequency groups do not significantly differ in alpha diversity. (A) Observed species diversity (richness) boxplot of groups categorized by sex and sheet washing frequency (p-values of 0.99 for Female Low/High and 0.64 for Male Low/High). (B) Shannon's diversity boxplot of groups categorized by sex and sheet washing frequency (p-values of 0.99 for Female Low/High and 0.65 for Male Low/High). Legend specifying groups categorized by sheet wash frequency and sex is shown on the right. Statistical analysis was performed using a two-way ANOVA, **p<0.05*.

TABLE. 1 Sex impacts hand microbial community beta diversity metrics at low and high sheet washing frequency. Beta diversity metrics (Unweighted UniFrac, Weighted UniFrac, Jaccard, Bray-Curtis) were run on pairs of groups categorized by sex (Female, Male) and sheet washing frequency (Low, High). PERMANOVA statistical testing, **p<0.05; **p<0.01; ***p<0.001*.

Beta Diversity Metric	**PERMANOVA P-values**			
	Female High vs Female Low	Male High vs Male Low	Female High vs Male High	Female Low vs Male Low
Unweighted UniFrac	0.026*	0.088	0.008**	0.020*
Weighted UniFrac	0.082	0.054	0.019*	0.012*
Jaccard	0.211	0.053	0.018*	0.020*
Bray-Curtis	0.211	0.045*	0.014*	0.020*

washing frequency; however, sheet washing frequency in females can impact microbial presence and sheet washing frequency in males can impact microbial abundance.

A reduction in sheet washing frequency corresponds to an increased variation in the relative abundance of phyla observed between males and females. To determine if sheet washing frequency and sex affect the phyla present on the skin of those in shared dorms, we calculated the relative abundance of phyla present across the various sex-specific sheet washing groups (Figure 2). Through analysis of the plots generated, it is observed that the variation in the abundance of phyla between sexes increases as sheet washing frequency decreases (Figure 2A). With reference to this data, five additional taxa bar plots were generated, focusing specifically on the five phyla depicted in Figure 2A. In Figure 2B (Actinobacteriota) and Figure 2C (Firmicutes), an apparent trend emerges of higher frequency of sheet washing leading to reduced variation in the overall relative abundance percent of these phyla between males and females. Proteobacteria also demonstrates a similar trend; however, this difference is less pronounced in comparison to Actinobacteriota and Firmicutes (Figure 2E). In contrast, Figure 2D demonstrates that the relative abundance of Bacteroidota decreases in similarity between sexes as sheet washing frequency increases. The data also suggests that Fusobacteriota is only present on the skin samples of those with high sheet washing frequency and absent on those who do not wash their sheets as often (Figure 2F). Overall, there is a discernible trend which indicates that higher sheet washing frequency is associated with reduced variation in the relative abundance of phyla between males and females. Through analysis of the generated taxa bar plots, the primary genera constituting

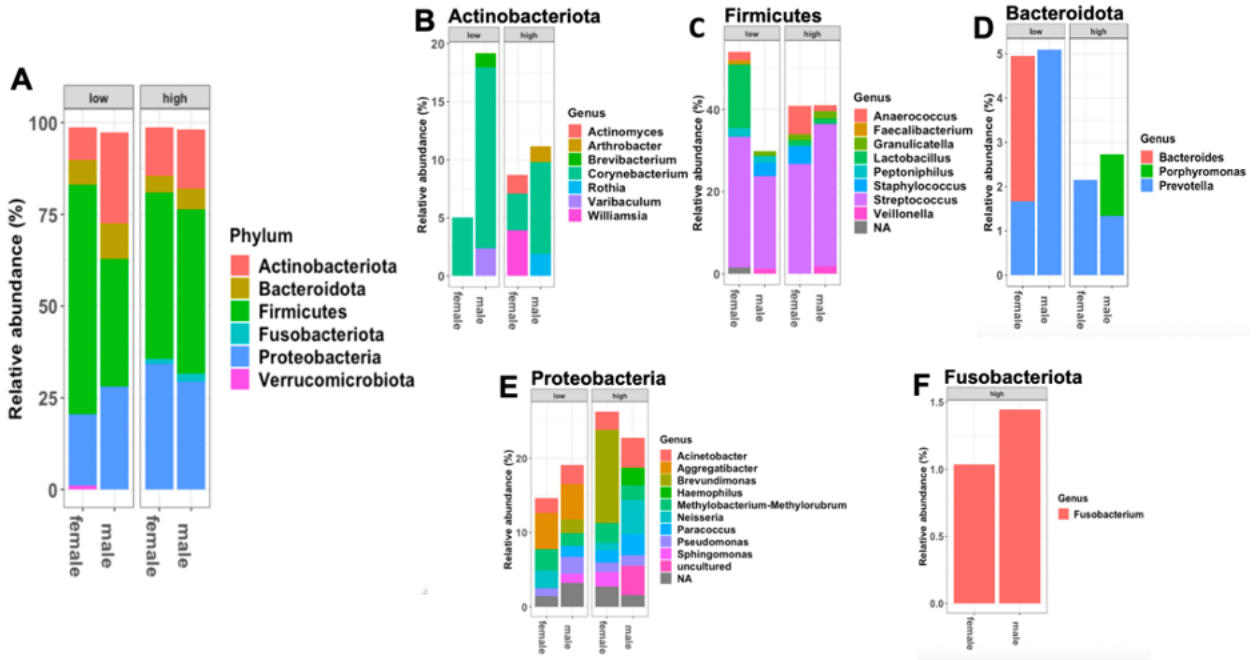

FIG. 2 Low sheet washing frequency results in higher variation in the relative abundance of phyla observed between males and females. (A) The phyla present in the various sex-specific sheet washing groups are represented by differing colours in the bars. The phyla of interest, which include (B) Actinobacteriota, (C) Firmicutes, (D) Bacteroidota, (E) Proteobacteria, and (F) Fusobacteriota, are further analyzed through taxa bar plots which visualize variation at the genus level of the various sex-specific sheet washing groups. All figures have legends on the right which represent the various genera (B-F) and phyla (A) present in the taxa bar graphs.

each phylum can be identified. This includes *Corynebacterium* for Actinobacteriota, *Streptococcus* for Firmicutes, *Prevotella* for Bacteriodiota, and *Fusobacterium* for Fusobacteriota. Proteobacteria have a variety of genera; therefore, it does not have one dominating or prevalent genus.

Both sexes exhibit a greater diversity of unique microbial genera when bed sheets are washed less frequently. A core microbiome analysis was conducted to explore the relationship between sex and bed sheet washing frequency, both in relation to each other and within each variable. The Four-way Venn diagram revealed 15 core microbial genera, constituting 20% of the overall core microbiome, which remained unaffected by sex or the frequency of bed sheet washing (Figure 3). Our findings revealed greater microbial diversity among females who infrequently wash their sheets in comparison to females who frequently wash their sheets (Figure 3). This observed pattern is consistent for males as well, where lower sheet washing frequently is associated with higher genera diversity (Figure 3). These results suggest a divergence in microbial genera between the sexes, particularly when the sheets are washed less frequently.

DESeq2 analysis shows fewer shared differentially abundant genera than those unique to one group with more upregulated genera in female groups across both sheet washing frequencies. A DESeq2 analysis was run to compare differences in ASV abundance between sexes in conjunction with sheet washing. Volcano plots revealed twenty-five significant values in the high sheet washing group and twenty-two significant values in the low group (Figure 4A-D). In the high sheet washing frequency group, results showed that more unique genera are abundant in the female group in comparison to the reference male group as seen in the number of genera present on the female side in Figure 4B. The shared genera between the two groups consisted of five genera including *Corynebacterium*, *Prevotella*, *Paracoccus*, *Staphylococcus,* and *Kocuria,* listed in the same order as Figure 4B. In the high sheet washing frequency group for females, sixteen genera were found to be significantly upregulated, thirteen of which were unique to the female group (Figure 4B). Some of the most unique upregulated genera included *Qipengyuania*, *Williamsia*,

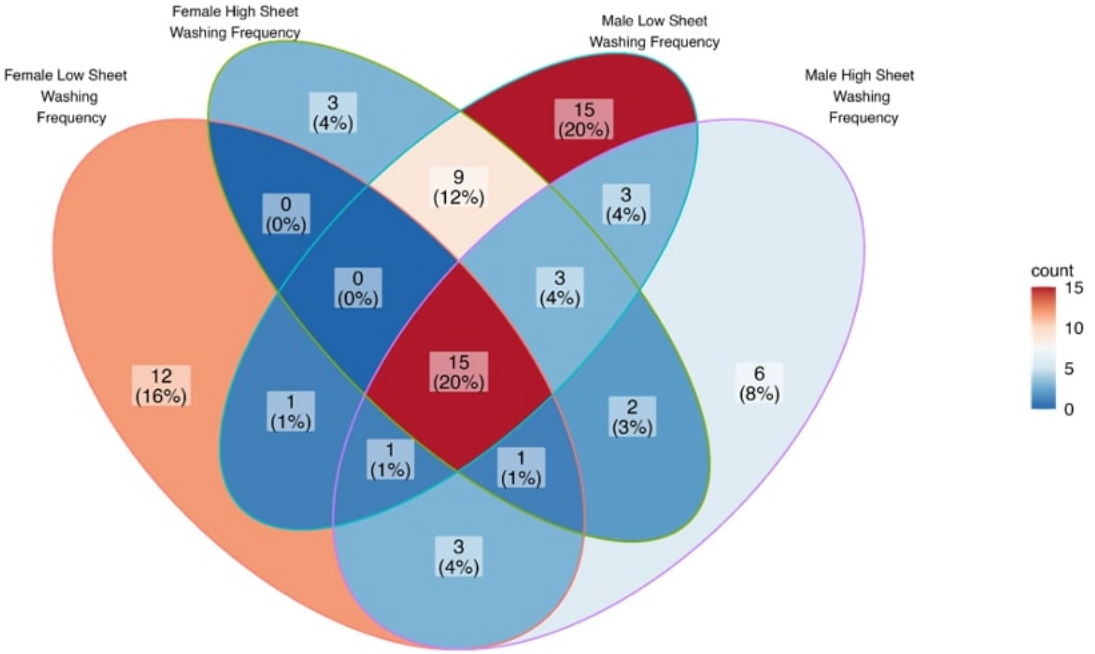

FIG. 3 For both sexes, individuals with low sheet washing frequency have more unique core microbiomes than individuals with high washing frequency. Four-way Venn diagram illustrating the percentage and number of overlapping and unique microbial genera present in females with low sheet washing frequency, females with high sheet washing frequency, males with low sheet washing frequency, and males with high sheet washing frequency. The diagram has a minimum prevalence threshold of 0.5 (50%) and an abundance threshold of 0.001 (0.1%). The numbers in each circle represent the genera above these thresholds. The colour corresponds to the number of counts of genera, with a darker red indicating a higher count and a darker blue indicating a lower count.

Mycobacterium, *Brevundiomonas*, and *Nocardioides* (Figure 4B). For the male high sheet washing frequency group, the three unique genera were *Aggregatibacter*, *Fusobacterium*, and *Rothia* (Figure 4B). The unique genera upregulated in the high sheet washing group are different from the low sheet washing group, as seen by the labeled genera on the y-axis between Figures 4B and 4D. In the low sheet washing frequency group, there were eleven significantly upregulated genera for females and six for males (Figure 4D). The unique species for the female low sheet washing frequency group included *Anaerococcus*, *Blautia*, *Subdoligranulum*, *Lactobacillus*, and *Bacteroides* while the male low sheet washing group consisted of the genera *Acinetobacter*, *Pseudomonas*, and *Brachybacterium* (Figure 4D).

DISCUSSION

The primary motivation of this study was to explore the impact of sex and the hygiene factor of sheet washing frequency on the microbial composition of hands among individuals residing in shared dormitories.

Our first analysis aimed to explore the broad differences in microbial diversity within and between sex-specific sheet washing frequency groups through alpha and beta diversity analyses. Alpha diversity analyses did not yield significant results, suggesting that richness, abundance, and evenness levels within individual hand samples were similar between sexes and sheet washing frequency groups (Figure 1). This is consistent with a previous study that revealed that Chao1, Shannon's diversity, and phylogenetic distance did not differ significantly when only looking at sex (35). Beta diversity analyses revealed significance, notably between sexes, indicating that sex may influence microbial community diversity (Table 1, Figure S4). This finding supports a previous study that observed significant differences in beta diversity metrics of facial skin samples between sexes (36). There was also significance in some beta diversity metrics between sheet washing frequency groups, specifically unweighted UniFrac and Bray-Curtis (Table 1, Figure S4). While there is minimal literature that explores sheet washing, other hygiene practices like hand washing

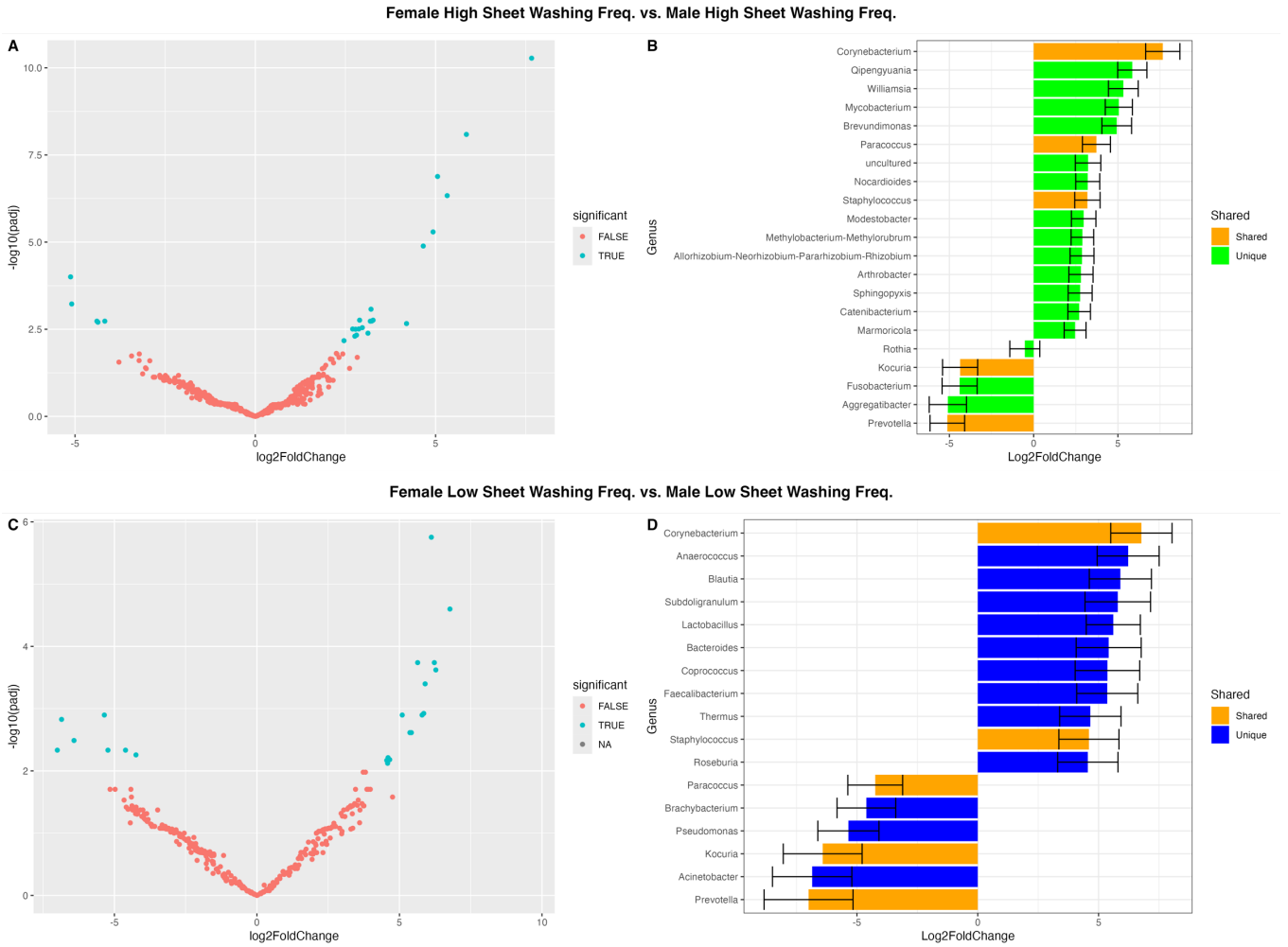

FIG. 4 Across high and low sheet washing frequencies, females have a higher number of upregulated genera than men. (A, B) DESeq2 plots representing high sheet washing frequency in females vs. males. (C, D) DESeq2 plots representing low sheet washing frequency in females vs. males. (A, C) DESeq2 analysis showing volcano plot when comparing (A) high and (C) low sheet washing frequency between female and male sexes (p-value of 0.01, log2FoldChange > 2, and baseMean > 1). (B, D) Bar plot at the genus level comparing (B) high sheet washing frequency and (D) low sheet washing frequency between females and males. Genera present on the right-hand side (positive Log2FoldChange) are genera that are more abundant in the female group while the genera present on the left-hand side (negative Log2FoldChange) are more abundant in the male group.

have been shown to impact unweighted UniFrac beta diversity metrics, aligning with our findings, as well as weighted UniFrac beta diversity metrics (37). Overall, this data suggests that sex is a greater driver of hand microbial community diversity than sheet washing frequency but that sheet washing frequency can still influence microbial presence and abundance. Based on the significant beta diversity results, our subsequent analyses focused on evaluating between-community differences rather than within-community differences and exploring the underlying causes behind these differences.

When comparing the relative abundance of phyla, an evident trend is that less sheet washing results in greater variation in the abundance of phyla when comparing females and males. This trend is specifically apparent in Actinobacteriota and Firmicutes, where the difference in relative abundance between males and females is more pronounced in low sheet washing frequency compared to high sheet washing frequency samples (Figure 2B, Figure 2C). Previous literature suggests a decades-long decline in microbial diversity and ancestral microbes within the human population due to an increase in hygiene practices, which could explain the reduction in variation of relative abundance of phyla between sexes in high sheet washing frequency compared to low sheet washing frequency samples (38). Taxa bar plot analysis also suggests that while the dominant phyla present are shared among the various

conditions, some phyla are unique. Such phyla include Verrucomicrobiota, which is only observed in female low sheet washing frequency samples (Figure 2A). These findings are consistent with the literature which states that females have a higher abundance of Verrucomicrobia in the gut microbiome compared to males (39). The lack of Verrucomicrobiota in high sheet washing frequency samples supports the trend that low sheet washing frequency results in higher microbial variation due to decreased hygiene practice. Additionally, taxa bar plot analysis identifies the dominant genera constituting the population of each phylum. *Corynebacterium* (Figure 2B) has a higher relative abundance in males than females in both high and low sheet washing frequency in the Actinobacteriota phylum. These findings are validated by previous research which states that females have higher concentrations of vaginal microbiota, including Eneterbacterales and Lactobacillaceae, whereas males have higher concentrations of Cutibacterium and Corynebacterium (40).

Core Microbiome analysis aimed to investigate the correlation between the sheet washing frequency and sexes, while also examining the shared and unique genera associated with each group. Our results revealed that there is greater diversity in hand microbial composition when bed sheets are washed infrequently (Figure 3). The observation aligns with the taxa bar plot analysis, which highlighted increased variation between sexes in the abundance of specific phyla under infrequent sheet washing conditions (Figure 2). While there is a lack of previous literature on sheet washing and its impact on the skin microbiome, it is well-established that sanitation practices reduce the overall microbial load on abiotic surfaces (1). Therefore, the infrequent washing of bed sheets likely leads to a greater accumulation of various microorganisms on the fabric. When individuals encounter these bed sheets, they are more likely to pick up a diverse subset of microorganisms onto their hands. Factors such as the shedding of skin cells, various bodily fluids, and other elements like pets or foods on beds contribute to the breeding of bacteria (41–43). The combination of warmth, darkness and the presence of moisture provides a suitable habitat for bacteria to thrive and reproduce (44). Furthermore, our analysis revealed distinct core microbial composition between sexes, especially when sheets are washed less frequently. This is expected as sex-specific properties of skin are known to have differences in skin thickness, the number of hairs, sweat production, and hormone production (40). In the core microbiome of males with low washing frequency, the most abundant genera unique to this group are identified as *Kocuria*, *Streptococcus*, and *Acinetobacter* (Figure S5). Conversely, females with infrequent sheet washing display a core microbiome dominated by *Lactobacillus*, *Faecalibacterium*, and *Dialister*, unique to this group (Figure S6). The abundance of these genera only represents those unique to their respective groups, which is why other bacteria with higher prevalence are not listed as they are also present in other groups. While all of these identified genera are primarily non-pathogenic, they can act as opportunistic pathogens, causing infections under conditions of weakened immunological response or in individuals with debilitated health (45, 46). For instance, *Kocuria* has been found in many infections including urinary tract infections, cholecystitis, brain abscesses, and meningitis (45). Additionally, *Acinetobacter* is known to be a contributor to nosocomial infections (46). Overall, infrequent sheet washing increases genera in the core microbiome of the skin that can act as opportunistic pathogens.

DESeq2 analysis revealed there were five shared genera between the two sexes for both high and low sheet washing frequency (Figure 4B, Figure 4D). A shared genus that was the most upregulated for females across both sheet washing frequency groups was *Corynebacterium* (47), which is typically found on the skin microbiome (Figure 4B, Figure 4D). The most upregulated genus for males was the *Prevotella* genus which was also shared with females and is a genus typically found in the oral microbiome (Figure 4B, Figure 4D) (48). The genus that switches from being upregulated from the female side to the male side as sheet washing frequency decreases is *Paracoccus* (Figure 4B, Figure 4D). This genus contains several hundred species and is found in a variety of pristine and polluted environments, indicating that it may have been brought in from an outside environment (49). *Staphylococcus* was upregulated in females across both sheet washing species and is typically present on mucus membranes and skin of humans (Figure 4B, Figure 4D) (50). The shared genus abundant on the male side for both sheet washing frequencies was the *Kocuria* genus which research has shown to be part of the normal flora of skin and oral cavities of humans (Figure 4B, Figure 4D) (45). As expected, all five shared genera were common to both sexes

(Figure 4B, Figure 4D). Interestingly, this DESeq2 analysis contradicts a previous study that showed higher abundances of *Corynebacterium* on male hands (51). However, the study reinforced our finding of a higher abundance of *Lactobacillus* in the female low sheet washing group as compared to the other sex-specific sheet washing groups (51). These results are consistent with the core microbiome results in Figure 3 which show shared genera between sex-specific sheet washing groups; however, each sheet washing group in conjunction with sex had unique genera present.

The unique genera found to be significantly abundant for females and males changed depending on sheet washing frequency; however, females had more unique species in both cases. These results suggest that hygiene practices and sex influence the abundance of genera found on the human skin.

Limitations Our study explored differences between males and females; however, the samples from our dataset only looked at biological sex and not gender, therefore our conclusions only account for biological and behavioural sex differences. Our study is also unable to distinguish whether behavioural differences or genetic and physiological differences between sexes are driving the observed differences. Additionally, sheet washing frequency may serve as a reflection of an individual's overall hygiene habits and external factors such as hand washing frequency may be confounding variables that impact the microbial composition of individuals as hand washing frequency has been previously shown to impact microbial diversity (37). Various factors such as age, health, lifestyle, and environment also impact the human microbiome (40). While our investigation focused on sex and sheet washing frequency, additional variables included in the dataset such as time spent outside, time spent with windows open, and/or roommates, could be further confounding variables contributing to the observed differences. The original dataset only sampled a single environment, a college dormitory, which generally consists of individuals within a limited age range. Differences in age and environments, such as urban or rural settings, have previously been demonstrated to impact skin microbial composition (35). While our results showed that differences in both sex and sheet washing frequency drive variation in microbial composition, our conclusions are specific to the context of our study and cannot be generalized to other age groups and environmental settings. Additionally, the dataset had small sample sizes, particularly for the female low sheet washing frequency group, making it difficult to draw generalizations about this group as well as the others. Further research with larger sample sizes is needed to validate our findings. The specificity of our results was also limited to genus-level identification for taxonomic, core microbiome, and differential abundance analyses as many samples in the dataset lacked species-level taxonomic information.

Conclusions The objective of our study was to investigate the influence of sex and the hygiene factor of sheet washing frequency on the hand skin microbial composition of individuals in a shared living environment. We found that both sex and sheet washing frequency impacted hand skin microbiome diversity and composition. More specifically, we first found that sex is a greater driver of microbial composition than sheet washing frequency as there were greater differences in beta diversity metrics between sex groups than within sex groups, and sex impacted the abundance of genera similarly at high and low sheet washing frequency. However, sheet washing frequency still influences hand microbial representation, with bacterial genera representation at both low and high sheet washing frequency found to be mostly different. More specifically, we observed that less frequent sheet washing is associated with greater differences in hand microbial composition load between sexes since there were greater variations in abundance of certain phyla, as well as more unique genera when sheets were washed less frequently. The presence and abundance of genera in each group indicate that a higher frequency of hygiene practices, like sheet washing, can decrease microbiome differences between sexes and reduce opportunistic pathogens. Altogether, our study contributes to the continued field of research on the differential composition of microbiota between sexes but uniquely discusses how sex intersects with hygiene practices like sheet washing to influence microbial diversity and abundance. Our study therefore acts

as a platform for future research on how hygiene habits influence the human microbiome and health outcomes in a sex-specific manner.

Future Directions To address one of the limitations previously discussed, this study could be re-run and expanded to include possible confounding variables found in the dataset. Such variables could include time spent with windows open, time spent outside, and/or roommates. Specifically, previous research on the same dataset has shown that having one or more roommates induces significant changes in the microbiome (10). Other previous research has also demonstrated the effect of time spent outside where after spending time outdoors, microbial richness and phylogenetic diversity increase and the skin microbiome becomes more similar to soil microbiota (40). Therefore, these practices should be explored to see if they correlate with sex or sheet washing and whether they could be contributing to the microbial composition differences observed in this study. Additionally, as mentioned in the discussion of limitations, sheet washing frequency could be linked to general hygiene practices so it could therefore be the sum of these actions, instead of solely sheet washing, that influence microbial composition. To address this, future studies could research the contribution of different hygiene practices to overall microbial composition differences.

To address another previously mentioned limitation, future studies could aim to increase the scope of the research on hygiene practices, sex, and microbial composition. One way to increase the scope could be to investigate the effects of various hygiene practices. Hand washing has already been shown to impact microbial composition, but other hygiene practices like showering or teeth brushing are not as well characterized (4). Additionally, other sample types could be explored, such as the abiotic surface samples available in the data set. Finally, the study took place in a single college dormitory with individuals of similar age and likely socioeconomic status, so including more diverse populations in future research could allow the findings to be extrapolated to the general population to a greater degree.

Finally, since our study was limited to examining taxa-level differences, we were unable to explore whether different species were pathogenic. Future studies could delve into species-level impacts of sheet washing frequency and sex, in addition to researching whether the microbial diversity differences observed in this study are associated with differential short- and/or long-term health outcomes.

ACKNOWLEDGEMENTS

We would like to thank the MICB 475 teaching team, specifically Dr. Evelyn Sun and Christopher Lee, for their support and guidance during this project. We would also like to thank the UBC Department of Microbiology and Immunology for the funding and resources for this project. Finally, we would like to acknowledge Richardson *et al.* for their work that generated the study data set. We would also like to thank two anonymous reviewers for constructive feedback on this manuscript.

CONTRIBUTIONS

Co-authorship should be considered equal between Lina Anwari (LA), Abigail Cho (AC), Dahyeon (Betty) Hong (DH), Màiri MacAulay (MM), and Juliet Malkowski (JM) for this manuscript. LA analyzed and generated figures for taxa bar plots analysis. AC generated phyloseq objects, performed alpha diversity analysis, and contributed to writing limitation sections. DH performed initial processing in QIIME2, as well as analyzed and generated figures for core microbiome analysis. MM analyzed and generated figures for beta diversity analysis, as well as contributed to writing conclusions and future directions. JM analyzed and generated figures for DESeq2 analysis and contributed to writing the introduction. All authors contributed to writing the abstract, methods, results, discussion, and supplemental sections. All authors edited the draft version of the manuscript.

REFERENCES

1. **Vandegrift R, Bateman AC, Siemens KN, Nguyen M, Wilson HE, Green JL, Van Den Wymelenberg KG, Hickey RJ.** 2017. Cleanliness in context: reconciling hygiene with a modern microbial perspective. Microbiome **5**:76.
2. 2022. How Infections Spread | Infection Control | CDC. https://www.cdc.gov/infectioncontrol/spread/index.html. Retrieved 3 December 2023.

3. **Al-Rifaai JM, Al Haddad AM, Qasem JA.** 2018. Personal hygiene among college students in Kuwait: A Health promotion perspective. J Educ Health Promot **7**:92.
4. **Fierer N, Hamady M, Lauber CL, Knight R.** 2008. The influence of sex, handedness, and washing on the diversity of hand surface bacteria. Proc Natl Acad Sci U S A **105**:17994–17999.
5. **Pinon A, Gachet J, Alexandre V, Decherf S, Vialette M.** 2013. Microbiological Contamination of Bed Linen and Staff Uniforms in a Hospital. 7. Adv Microbiol **3**:515–519.
6. **Meadow JF, Altrichter AE, Bateman AC, Stenson J, Brown G, Green JL, Bohannan BJM.** 2015. Humans differ in their personal microbial cloud. PeerJ **3**:e1258.
7. **Chen YE, Tsao H.** 2013. The skin microbiome: current perspectives and future challenges. J Am Acad Dermatol **69**:143-155.e3.
8. **Eriksson K, Dickins TE, Strimling P.** 2022. Global sex differences in hygiene norms and their relation to sex equality. PLOS Glob Public Health **2**:e0000591.
9. **Richardson M, Gottel N, Gilbert JA, Lax S.** 2019. Microbial Similarity between Students in a Common Dormitory Environment Reveals the Forensic Potential of Individual Microbial Signatures. mBio **10**:e01054-19.
10. **Luongo A, MacPherson K, Meloche C, Roberge S.** 2022. Cohabitation impacts the microbial diversity and taxonomic makeup of the biotic and abiotic environments in a college dormitory. Undergrad J Exp Microbiol Immunol 27.
11. **Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet CC, Al-Ghalith GA, Alexander H, Alm EJ, Arumugam M, Asnicar F, Bai Y, Bisanz JE, Bittinger K, Brejnrod A, Brislawn CJ, Brown CT, Callahan BJ, Caraballo-Rodríguez AM, Chase J, Cope EK, Da Silva R, Diener C, Dorrestein PC, Douglas GM, Durall DM, Duvallet C, Edwardson CF, Ernst M, Estaki M, Fouquier J, Gauglitz JM, Gibbons SM, Gibson DL, Gonzalez A, Gorlick K, Guo J, Hillmann B, Holmes S, Holste H, Huttenhower C, Huttley GA, Janssen S, Jarmusch AK, Jiang L, Kaehler BD, Kang KB, Keefe CR, Keim P, Kelley ST, Knights D, Koester I, Kosciolek T, Kreps J, Langille MGI, Lee J, Ley R, Liu Y-X, Loftfield E, Lozupone C, Maher M, Marotz C, Martin BD, McDonald D, McIver LJ, Melnik AV, Metcalf JL, Morgan SC, Morton JT, Naimey AT, Navas-Molina JA, Nothias LF, Orchanian SB, Pearson T, Peoples SL, Petras D, Preuss ML, Pruesse E, Rasmussen LB, Rivers A, Robeson MS, Rosenthal P, Segata N, Shaffer M, Shiffer A, Sinha R, Song SJ, Spear JR, Swafford AD, Thompson LR, Torres PJ, Trinh P, Tripathi A, Turnbaugh PJ, Ul-Hasan S, van der Hooft JJJ, Vargas F, Vázquez-Baeza Y, Vogtmann E, von Hippel M, Walters W, Wan Y, Wang M, Warren J, Weber KC, Williamson CHD, Willis AD, Xu ZZ, Zaneveld JR, Zhang Y, Zhu Q, Knight R, Caporaso JG.** 2019. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. 8. Nat Biotechnol **37**:852–857.
12. **Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP.** 2016. DADA2: High resolution sample inference from Illumina amplicon data. Nat Methods **13**:581–583.
13. **Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, Peplies J, Glöckner FO.** 2013. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res **41**:D590–D596.
14. **McMurdie PJ, Holmes S.** 2013. phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. PLoS ONE **8**:e61217.
15. **Paradis E, Schliep K.** 2019. ape 5.0: an environment for modern phylogenetics and evolutionary analyses in R. Bioinformatics **35**:526–528.
16. **Wickham H, Averick M, Bryan J, Chang W, McGowan L, François R, Grolemund G, Hayes A, Henry L, Hester J, Kuhn M, Pedersen T, Miller E, Bache S, Müller K, Ooms J, Robinson D, Seidel D, Spinu V, Takahashi K, Vaughan D, Wilke C, Woo K, Yutani H.** 2019. Welcome to the Tidyverse. J Open Source Softw **4**:1686.
17. **Dixon P.** 2003. VEGAN, a package of R functions for community ecology. J Veg Sci **14**:927–930.
18. **Whittaker RH.** 1972. Evolution and Measurement of Species Diversity. TAXON **21**:213–251.
19. **Chao A.** 1984. Nonparametric Estimation of the Number of Classes in a Population. Scand J Stat **11**:265–270.
20. **Chao A, Lee S-M.** 1992. Estimating the Number of Classes via Sample Coverage. J Am Stat Assoc **87**:210–217.
21. **Shannon CE.** 1948. A mathematical theory of communication. Bell Syst Tech J 27:379–423.
22. **Simpson EH.** 1949. Measurement of Diversity. 4148. Nature **163**:688–688.
23. **Fisher RA, Corbet AS, Williams CB.** 1943. The Relation Between the Number of Species and the Number of Individuals in a Random Sample of an Animal Population. J Anim Ecol **12**:42–58.
24. **Fisher RA.** 1992. Statistical Methods for Research Workers, p. 66–70. *In* Kotz, S, Johnson, NL (eds.), Breakthroughs in Statistics: Methodology and Distribution. Springer, New York, NY.
25. **Lozupone C, Knight R.** 2005. UniFrac: a New Phylogenetic Method for Comparing Microbial Communities. Appl Environ Microbiol **71**:8228–8235.
26. **Lozupone CA, Hamady M, Kelley ST, Knight R.** 2007. Quantitative and Qualitative β Diversity Measures Lead to Different Insights into Factors That Structure Microbial Communities. Appl Environ Microbiol **73**:1576–1585.
27. **Jaccard P.** 1912. The Distribution of the Flora in the Alpine Zone. New Phytol **11**:37–50.

28. **Bray JR, Curtis JT.** 1957. An Ordination of the Upland Forest Communities of Southern Wisconsin. Ecol Monogr **27**:326–349.
29. **Anderson MJ.** 2001. A new method for non-parametric multivariate analysis of variance. Austral Ecol **26**:32–46.
30. **Wickham H.** 2016. Data Analysis, p. 189–201. *In* Wickham, H (ed.), ggplot2: Elegant Graphics for Data Analysis. Springer International Publishing, Cham.
31. **Wickham H.** 2016. ggplot2. Springer International Publishing, Cham. http://link.springer.com/10.1007/978-3-319-24277-4. Retrieved 9 December 2023.
32. **Lahti L, Shetty S.** 2023. microbiome: Microbiome Analytics (1.24.0). Bioconductor version: Release (3.18).
33. **Gao C-H, Yu G, Cai P.** 2021. ggVennDiagram: An Intuitive, Easy-to-Use, and Highly Customizable R Package to Generate Venn Diagram. Front Genet 12.
34. **Love MI, Huber W, Anders S.** 2014. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol **15**:550.
35. **Ying S, Zeng D-N, Chi L, Tan Y, Galzote C, Cardona C, Lax S, Gilbert J, Quan Z-X.** 2015. The Influence of Age and Gender on Skin-Associated Microbial Communities in Urban and Rural Human Populations. PLOS ONE **10**:e0141842.
36. **Robert C, Cascella F, Mellai M, Barizzone N, Mignone F, Massa N, Nobile V, Bona E.** 2022. Influence of Sex on the Microbiota of the Human Face. Microorganisms **10**:2470.
37. **Vindenes HK, Drengenes C, Amin H, Bertelsen RJ.** 2023. The impact of alcohol-based hand sanitiser and hand washing with soap and water on bacterial skin microbiota composition. JEADV Clin Pract **2**:764–774.
38. **Finlay BB, Amato KR, Azad M, Blaser MJ, Bosch TCG, Chu H, Dominguez-Bello MG, Ehrlich SD, Elinav E, Geva-Zatorsky N, Gros P, Guillemin K, Keck F, Korem T, McFall-Ngai MJ, Melby MK, Nichter M, Pettersson S, Poinar H, Rees T, Tropini C, Zhao L, Giles-Vernick T.** 2021. The hygiene hypothesis, the COVID pandemic, and consequences for the human microbiome. Proc Natl Acad Sci U S A **118**:e2010217118.
39. **Valeri F, Endres K.** 2021. How biological sex of the host shapes its gut microbiota. Front Neuroendocrinol **61**:100912.
40. **Skowron K, Bauza-Kaszewska J, Kraszewska Z, Wiktorczyk-Kapischke N, Grudlewska-Buda K, Kwiecińska-Piróg J, Wałecka-Zacharska E, Radtke L, Gospodarek-Komkowska E.** 2021. Human Skin Microbiome: Impact of Intrinsic and Extrinsic Factors on Skin Microbiota. 3. Microorganisms **9**:543.
41. **Grice EA, Segre JA.** 2011. The skin microbiome. Nat Rev Microbiol **9**:244–253.
42. **Kates AE, Jarrett O, Skarlupka JH, Sethi A, Duster M, Watson L, Suen G, Poulsen K, Safdar N.** 2020. Household Pet Ownership and the Microbial Diversity of the Human Gut Microbiota. Front Cell Infect Microbiol **10:**73.
43. **Mahmud MdR, Akter S, Tamanna SK, Mazumder L, Esti IZ, Banerjee S, Akter S, Hasan MdR, Acharjee M, Hossain MdS, Pirttilä AM.** Impact of gut microbiome on skin health: gut-skin axis observed through the lenses of therapeutics and skin diseases. Gut Microbes **14**:2096995.
44. **Dannemiller KC, Weschler CJ, Peccia J.** 2017. Fungal and bacterial growth in floor dust at elevated relative humidity levels. Indoor Air **27**:354–363.
45. **Kandi V, Palange P, Vaish R, Bhatti AB, Kale V, Kandi MR, Bhoomagiri MR.** Emerging Bacterial Infection: Identification and Clinical Significance of Kocuria Species. Cureus **8**:e731.
46. **Almasaudi SB.** 2018. Acinetobacter spp. as nosocomial pathogens: Epidemiology and resistance features. Saudi J Biol Sci **25**:586–596.
47. **Aoki T, Kitazawa K, Deguchi H, Sotozono C.** 2021. Current Evidence for Corynebacterium on the Ocular Surface. Microorganisms **9**:254.
48. **Könönen E, Gursoy UK.** 2022. Oral Prevotella Species and Their Connection to Events of Clinical Relevance in Gastrointestinal and Respiratory Tracts. Front Microbiol 12.
49. **Lasek R, Szuplewska M, Mitura M, Decewicz P, Chmielowska C, Pawłot A, Sentkowska D, Czarnecki J, Bartosik D.** 2018. Genome Structure of the Opportunistic Pathogen Paracoccus yeei (Alphaproteobacteria) and Identification of Putative Virulence Factors. Front Microbiol **9**:2553.
50. 2023. Staphylococcus | Description, Characteristics, Diseases, & Antibiotic Resistance | Britannica. https://www.britannica.com/science/Staphylococcus. Retrieved 6 December 2023.
51. **Edmonds-Wilson SL, Nurinova NI, Zapka CA, Fierer N, Wilson M.** 2015. Review of human hand microbiome research. J Dermatol Sci **80**:3–12.
52. **Eriksson K, Dickins TE, Strimling P.** 2022. Global sex differences in hygiene norms and their relation to sex equality. PLOS Glob Public Health **2**:e0000591.