DIVERSITY OF HUMAN NAVEL MICROBIOME IN YOUNG ADULTS

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ABSTRACT

Human skin microbial communities represent a large source of genetic diversity, and the diversity evolves as a function of human age. In the current study, we examine the microbial diversity present in the navel region of college-attending young adults in the age group of 18-25 years and investigate if the microbial diversity is associated with the sex of young adults. We characterized bacterial species in navel swab samples from 22 individuals. Comparison of alpha and beta diversity of the microbiota in the male and female navel region suggests that the flora is not statistically different ($p > 0.05$). Organisms from the genera *Corynebacterium* and *Staphylococcus* were the most dominant bacteria. Also present as the major component of the flora were the organisms normally associated with the gastro-intestinal tract such as *Acinetobacter* sp., *Bacteroidia* sp., *Campylobacter* sp., and organisms from the Enterobacteriaceae and Moraxellaceae families. *Klebsiella* and *Pseudomonas* were also found to be part of the navel skin microbiota of the young adults. Our findings indicate that the skin microbiota continues to evolve beyond the young adult age group. Epidemiological implication of the observed results is discussed in the report.

KEYWORDS

Microbiome; skin; navel region; Corynebacterium; staphylococcus; human microbiota

INTRODUCTION

Skin is the largest and one of the most complex organs of the human body in surface area and weight (1). Skin is composed of 1.8 m^2 of diverse habitat with an abundance of folds, invaginations and specialized niches (1). Its three major functions include protection against environmental factors, regulation of body temperature, and sensation of environmental conditions. Along with skin structures, sich as hair follicles and glands, each of the niches has its own combination of pH, temperature, moisture and sebum content (2). These allow for unique microbiota to be established in each of the skin niches (3). Skin microbiota is generally composed of two groups. The first group are the residential microorganisms which are always present on the skin and reestablish themselves post-perturbation (4). The second group are transient microorganisms, which arise from the environment, do not establish themselves permanently on the skin, and only remain on the skin for time periods ranging from hours to days (4). Both groups of organisms are normally non-pathogenic in nature and, in many cases, provide protective functions against invasion by pathogenic organisms and in education of our immune system (1). As our understanding of the human genome and interaction with the human microbiome increases, more functions will almost certainly come to light.

Determining the human microbiota's role in human health and functioning will require science to first define the "baseline" microbiota. Many studies have already been reported on the microbial communities associated with various sites across the digestive system (5,6) and their critical role in maintaining human health. While much attention has been devoted to the microbiota present in the oral cavity and the gut region, skin microbiota has not received much attention. Studies published thus far have suggested that the bacteria present is dependent on the physiology of the skin site, with specific bacteria being associated with moist, dry, and sebaceous

microenvironments (4, 7-9). *Propionibacterium* spp. has been shown to be the dominant genus in the sebaceous areas of the skin (1). In contrast, moist skin areas have been primarily dominated by bacteria from *Staphylococcus* and *Corynebacterium* genera (1). The most diverse skin sites are the dry areas, with a mixed presence of the organisms from Actinobacteria, Proteobacteria, Firmicutes, and Bacteriodetes phyla (7-9).

Unlike in the gut, where microbial communities stabilize around the age of three years, skin microbiome is only stabilized post-puberty (10,11). During puberty, the androgen level rises in the body, leading to the stimulation of terminal hair growth and to the beginning of the functioning of the apocrine sweat glands (12). These glands produce sebum, composed of triglycerides (12). The changes in the skin environment leads to changes in the microbial community, favoring the expansion of lipophilic microorganisms, such as *Propionibacterium* and *Corynebacterium* (11). Capone et al. (13) and Oh et al. (14) have indeed shown that in contrast to adult skin, pre-pubescent children have a greater abundance of Firmicutes bacteria, such as *Staphylococcus* and *Streptococcus*, on their skin. Thus, to establish the baseline microbiota for adults, it is imperative that we analyze the microbiota of subjects who have moved past the puberty stage.

In the current study, we examine the bacterial biota pattern from the navel swabs of college-attending young adults in the age group of 18-25 years. We try to answer whether there is a baseline bacterial biota present in all adults in the studied age group, and if there are any bacterial phyla associated with the sex of young adults.

METHODOLOGY

Study Set Up: The recruitment of subjects was carried out in a junior-level class (third year) at the West Chester University, West Chester, PA. This was strategically done to ensure that the

research subjects were old enough to be beyond puberty. Participation in the study was limited to subjects between the ages of 18 and 25. Swabs from ESK Environmental Sampling Kit by Puritan Medical Products were distributed to participants, along with a short demographic survey to indicate their sex (male/female). Participants were instructed to swab their navel areas for 30 seconds right before shower and then to return the swab to the authors. The swab samples received from 22 volunteers contained measurable DNA and demographic information for use in the current study. The swabs were stored at 4^{o}C and processed within 24 hours of collection.

The sample collection protocol for this exploratory microbiome study was approved by the Institutional Review Board committee at West Chester University (Protocol ID 20190430C). All participants were provided with informed consent forms, which were signed by everyone who participated in the study.

Total DNA Isolation, 16S Library Preparation and Sequencing: Genomic DNA was extracted from the navel swabs using the Qiagen QiAmp UCP DNA micro Kit. For control, a blank swab sample was used for DNA extraction to determine the background microbial signal. The DNA concentration in all of the samples was determined using the Qbit 3Fluorometer (Invitrogen Technologies). The DNA concentration in the samples ranged from 0.025 ng/µl to 19.4 ng/ µl, except for the control sample, which was below detection limit.

A dual-index amplicon sequencing method was used for PCR amplification of the V3-V4 region of the 16S rRNA gene (15). All of the samples were processed by using the NexteraXT Library Preparation Kit (Illumina) in accordance with the manufacturer's protocol for 16S metagenomic sequencing, except for the concentration of the input DNA. In the current study, 0.02 ng/µl of DNA was used for the 16S rRNA sequencing. Amplicons were sequenced on the MiSeq platform (Illumina, San Diego, CA), using the 300base pair paired-end chemistry at West Chester

University. Data was rarefied to 3307 reads per sample. Quantitative Insights into Microbial Ecology (QIME, version 1) was used to process the sequence data using the QIME pre-visualizaiton and visualization apps on the base space platform of Illumina.

The dataset is available at the NCBI under accession number xyz (*awaiting the number and will be added in the revision stage).*

Statistical Analysis: The relative abundance (%) of individual taxa within each community was estimated by comparing the number of sequences assigned to a specific taxon to the number of total sequences obtained for that sample. The starting input file consisted of raw count of genus abundance per sample per condition, and samples were annotated as having 16 female and 6 male experimental conditions. Differential expression and normalized abundance on raw counts data was performed using the DEseq2 package in R (16). Significance was determined using an alpha-significance level of 0.05. Clustering was performed using the k-means algorithm and five-group initiation. Normalization was done using a log2 transformation.

RESULTS AND DISCUSSION

Navel skin swabs of 22 participants were sequenced through Illumina Miseq® sequencing resulting, with 16 sixteen samples from female subjects and 6 samples from male subjects. Following quality control, a total of 2,180,377 sequences were assigned, with an average of 99,108 sequences per sample.

Alpha diversity of the male and female skin microbiota was compared to evaluate the phylogenetic composition of bacterial communities. Shannon diversity index, Chao1 index, and observed species were used to compare the alpha diversity. Shannon diversity index showed no statistical difference between the male and female microbiota in terms of species richness and evenness (Figure 1a, $p = 0.64$). Chao1 index also indicated that the species richness is statistically

similar in the compared microbiota (Figure 1b, $p = 0.052$). While female samples seem to have higher diversity than male samples in the observed species comparison (Figure 1c), the difference is not statistically significant ($p = 0.20$).

The ability of samples to be separated by sex was assessed by analyzing the beta diversity. PCoA plots, based on the weighted Unifrac distance matrices, showed that the skin microbiota do not differ significantly between male and female populations (Figure 2a-c). The samples were clustered together across all the analyzed plots.

A total of 17 phyla were identified in the bacterial community of the evaluated navel samples. Actinobacter, Bacterioidetes, Firmicutes, and Proteobacteria were the dominant phyla, having a relative abundance of >5% (Figure 3) (17). The other 13 phyla were present in a lower abundance (<1%). Analyzing the data at genus level, a total of 302 bacterial genera were identified across the samples via taxonomic summary (Supplementary Table 1). The abundance of top 20 bacterial genera is shown in Figure 4. *Cornynebacterium* and *Staphylococcus* genera were the most dominant bacteria across all of the samples. *Anaerococcus, Klebsiella, Porphyromonas* and an unknown genus from *Enterobacteriaceae* were the other prominent genera present in the navel skin microbiota. The analysis of microbiota across the samples also suggested that there were certain individuals with a very high concentration of the *Pseudomonas* genus and an unknown genus from the Xanthomonadaceae family (Figure 4). It is important to note that 21 of the 22 samples contained *Pseudomonas* as a major component of the microbiota. The Gram-negative organisms from the Bacteroidia class and the spore forming gram-positive organisms from the Clostridia and Bacilli classes made for the other organisms that were present in the top 20 bacterial genera present.

The navel region in a human being is a moist site and the literature is replete with data showing *Corynebacterium* and *Staphylococcus* as the major component of the microbiota in such sites (18-19). Our results were consistent with the literature. However, of clinical significance was the prevalence of high concentrations of opportunistic pathogens, such as *Pseudomonas* and *Klebsiella*. The samples were collected in September from college attending students between the ages of 18-25. In the United States, the climate during the duration of this study (Fall 2019) normally prevents outdoor water-based activities. This would discount contamination of the navel microbiota from water based activities. Further, considering the organisms were almost uniformly present across all of the samples suggests that their presence is an integral part of the microbiota for this age group (Figure 3). When one adds the presence of *Acinetobacter*, *Bacteroidia,* *Campylobacter*, and the unknown genus from Enterobacteriaceae and Moraxellaceae as other major organisms in the microbiota, a clear picture emerges. The navel region of 18 to 25-year-old human subjects in the United States contains high percentages of organisms that are normally associated with the gastro-intestinal tract.

Based on the current study, it is not possible to ascertain if the presence of high levels of organisms that are normally associated with the gastro-intestinal tract is due to a lack of personal hygiene or if the organisms are part of the evolving normal flora in the navel region. Nevertheless, the data strongly suggests that to further the health of the community, in particular to decrease the cases of human-spread diseases, the washing of hands should be strongly recommended after touching the navel region.

According to the United States Labor Department, 3,683,000 people in the age group of 16-24 work in the restaurant industry across the country (20). With the large number of gastro-interstitial opportunistic pathogens being part of the normal flora in this workforce, food-handling

and personal hygiene discussions should include the recommendation to wash hands after touching the navel area. Numerous studies have highlighted the need for the improvement of the hygiene and sanitation practices in the commercial food service environment (21-23). While many consumers may follow unsafe food-handling practices at home (23, 24), we believe that improving the practices at restaurants could have a significant impact on public health. This would be particularly relevant in restaurants and food-handling facilities employing teens and young adults.

Aiolfi et al. (25), in their study of the microbiome from umbilicus samples collected prior to laparoscopic surgery, reported the presence of many of the gram-negative opportunistic pathogens reported here. *Hulcr* et al. reported that in the adult population of North Carolina, USA, the navel skin microbiota did contain *Enterobacter,* but no presence of *Klebsiella* (26). In their study, since the human subjects participating in the research were participants in an online meeting of science communicators, one can assume that the subjects were older than 25 years old (26). Staudinger et al. (27) reported that gram-positive bacteria are more abundant than gram-negative the bacteria on superficial human skin of subjects in the age group of 22-29 years. Comparing our results to those in the literature, we conclude that the microbiota of 18 to 25 years old human being differs from older individuals. While the population of *Corynebacterium* and *Staphylococcus* has increased to levels found in older subjects, the high level of gram-negative bacteria suggests that somewhere during the young adult to matured adult stages, the microbial community stabilizes. Further studies are warranted to better understand the changes in microbiota on human skin as a function of age and the factors influencing the change.

Pairwise comparison of the microbiota between male and female samples at the genus level shows 11 genera to be present in a statistically significant amount (Table 1). Seven genera were found to be present in a statistically higher abundance in females ($p < 0.05$). Of these seven genera,

five were gram-negative organisms and two were gram-positive organisms. The organisms present in a higher abundance in females include opportunistic pathogens from the Moraxellaceae family (> 8 fold higher abundance), *Klebsiella* ap. (>7 fold higher abundance) and *Enterobacter* (>5 fold higher abundance). In contrast, four genera were present in a higher abundance in males ($p < 0.05$), including spore forming gram-positive organisms from the Tissierellaceae family. Understanding the relationship between the microenvironment in the navel region of the male vs the female could allow further insight into the evolution of microbiota. Previous studies have reported that skin cleansers and skin cosmetics like moisturizers do not impact microbiota and thus can be discounted as the reason for the observed differences (27).

In conclusion, we have demonstrated that the human skin microbiota is not fully established until the young adult stage, and that it continues to evolve beyond. It is still yet to be explored how ethnicity, race, environment and other variables play a role in the maturation of the microbiota. The navel skin microbiota of the young adults and older teenagers have a significantly higher abundance of opportunistic pathogens. It needs to be determined if the observed abundance has any biological or clinical significance.

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CONFLICT OF INTEREST

The authors declare they have no actual or potential competing financial interests.

AUTHOR CONTRIBUTIONS

VS and SS conceived and designed the experiments; VS, SS and TDR were responsible for sample collection; SS and VS performed the experiments; SS and VS were responsible for analysis of data; VS, SS, and TDR were responsible for the preparation of manuscript.

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