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Diversity of navel microbiome in young adults

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Abstract

Introduction. Human skin microbial communities represent a tremendous source of genetic diversity that evolves as a function of human age. Microbiota differs between regions of oily and moist skin, and appears to stabilize with age.

Aim. We have a minimal understanding of the time frame required for the stabilization of skin microbiota, and the role played by gender. In the current study, we examined the microbiota present in the navel region of college-attending young adults in the age group of 18–25 years and investigated if diversity is associated with gender (male and female).

Method. The study involved 16 female and six male subjects. Isolated DNA samples from navel swabs were processed using the Nextera XT library preparation kit and sequenced using the MiSeq platform. Data were analysed using QIIME and statistical analysis performed in R.

Results. Microbiota of navel skin is dominated by *Corynebacterium* and *Staphylococcus* and includes opportunistic pathogens like *Clostridium* and *Pseudomonas*. Also present as the major component of the flora were the organisms normally associated with the gastrointestinal tract such as *Acinetobacter*, *Campylobacter*, *Klebsiella* and organisms from the *Enterobacteriaceae* and *Moraxellaceae* families. Comparison of alpha and beta diversity of the microbiota in the male and female navel regions suggests that the flora is not statistically different ($P>0.05$). However, pairwise comparison suggests that the abundance of 12 specific genera varied with gender, including higher abundance of *Klebsiella* and *Enterobacter* in females.

Conclusion. Our findings indicate that the navel skin microbiota of young adults has a core microbiota of *Corynebacterium* and *Staphylococcus*. We also noted the presence of a significant number of opportunistic pathogens. A minor gender difference in the abundance of individual organisms was also observed.

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, such 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 micro-organisms, which are always present on the skin and reestablish themselves post-perturbation [4]. The second group are transient micro-organisms, 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 health and functioning will require science to first define the 'core' 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.

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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]. Sweat glands, hair follicles and the dermal layers of the skin also have their own microbiota associated [10]

Unlike in the gut, where microbial communities stabilize around the age of 3 years, skin microbiome is only stabilized post-puberty [11, 12]. 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 [13]. These glands produce sebum, composed of triglycerides [14]. The changes in the skin environment leads to changes in the microbial community, favouring the expansion of lipophilic micro-organisms, such as *Propionibacterium* and *Corynebacterium* [12]. Capone *et al.* [14] and Oh *et al.* [15] have indeed shown that in contrast to adult skin, prepubescent children have a greater abundance of Firmicutes bacteria, such as *Staphylococcus* and *Streptococcus*, on their skin. Thus, to establish the core microbiota for adults, it is imperative that we analyse the microbiota of subjects who have moved past the puberty stage.

In the current study, we examine the bacterial microbiota pattern from the navel swabs of college-attending young adults in the age group of 18–25 years and investigate if there are any bacterial phyla associated with the gender.

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, USA. 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 gender (male/female). Participants were instructed to swab their navel areas for 30 s 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 °C and processed within 24 h of collection.

Total DNA isolation, 16S library preparation and sequencing

Genomic DNA was extracted from the navel swabs using the Qiagen QiAamp UCP DNA micro Kit. The kit was selected considering the low amount of DNA present in the swab samples and to obtain high yield post-isolation [16, 17]. 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 Qubit 3 Fluorometer (Invitrogen Technologies). The DNA concentration in the samples ranged from 0.025 ng μl^{-1} to 19.4 ng μl^{-1}, 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 [18]. 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^{-1} of DNA was used for the 16S rRNA sequencing. Amplicons were sequenced on the MiSeq platform (Illumina, San Diego, CA, USA), using the 300 base-pair paired-end chemistry at West Chester University. Data was rarefied to 3307 reads per sample. Quantitative Insights into Microbial Ecology (QIIME Version 1.0.1) was used to process the sequence data using the QIIME pre-visualization and visualization apps on the base space platform of Illumina. The dataset is available at the NCBI under BioProject accession number PRJNA604977.

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 [19]. 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

Presence of opportunistic pathogens in the navel region

Navel skin swabs of 22 participants were sequenced through Illumina Miseq sequencing with 16 samples from female and six male subjects. Following quality control, a total of 2180377 sequences were assigned, with an average of 99108 sequences per sample.

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% (Fig. 1) [20]. The other 13 phyla were present in a lower abundance (<1%). Comparison of the navel skin microbiota to other sites suggests that the

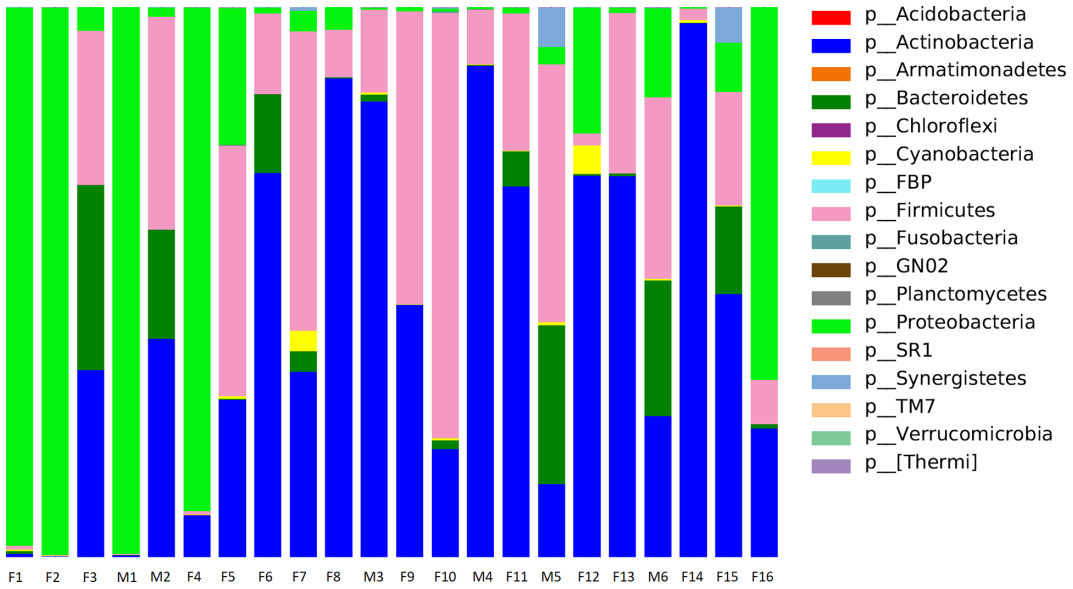

Fig. 1. Taxonomic analysis of navel skin microbiota from 16 female (F) and six male (M) subjects at phylum level.

dominant phyla are also found in high percentages in human gut, intra oral sites and human forearm [21–25].

The predominant phylum on the infants' skin is Firmicutes [14]. In contrast, adult skin is dominated by Proteobacteria [1, 11]. Results observed here (Fig. 1) show that while in some subjects Proteobacteria predominates, in the majority of samples Actinobacteria is the major phylum. Comparison against the published literature suggests that transition of microbiota may occur from Firmicutes to Actinobacteria to Proteobacteria across the infant to adult stage of human development.

At the genus level, a total of 302 bacterial genera were identified across the samples. The abundance of the top 20 bacterial genera is shown in Fig. 2. *Corynebacterium* 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

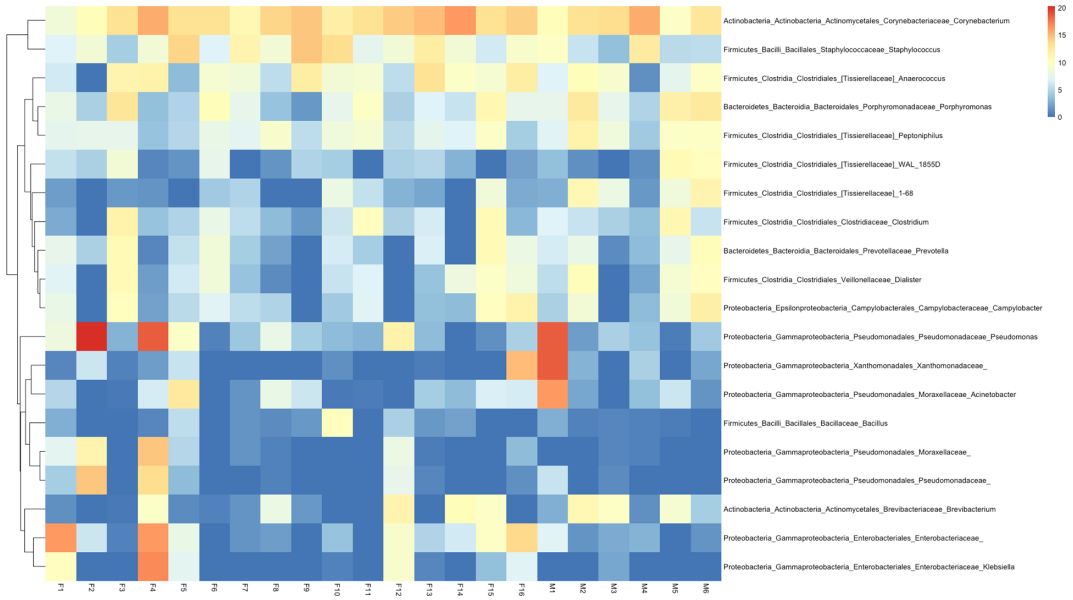

Fig. 2. Hierarchical-clustering heat map of the relative abundance of the 20 most abundant bacterial genera.

analysis of microbiota across the samples also suggested that there were few individuals with a very high concentration of the *Pseudomonas* genus and an unknown genus from the *Xanthomonadaceae* family (Fig. 2). 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 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 [26, 27]. Our results were consistent with the literature. Kwaszewska *et al.* [28] reported isolation of 155 *Staphylococcus* strains belonging to ten species and 105 strains of *Corynebacterium* belonging to nine species from the skin swabs of healthy human subjects. Coagulase-negative *Staphylococcus* and lipophilic *Corynebacterium* were the majority of organisms cultured and were found to be having a commensal relationship on the skin [28]. *Staphylococcus* have also been reported to be playing a major role in maintaining homeostatic control of skin inflammation [29] and provide resistance against *Streptococcus* skin infection [30]. Of all the *Staphylococcus* strains found in the skin, *S. aureus* has been associated with atopic dermatitis [31]. The growth of *S. aureus* is though controlled by secretion of serine proteases by *S. epidermis* [32].

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 USA, 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. *Pseudomonas* is not typically associated with skin microbiome and is linked to secondary infections of wounds [33, 34]. The knowledge of their presence in the moist skin region could allow health-care professionals to develop prophylactic measures against preventing secondary infections of wounds. 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 (Fig. 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 USA contains high percentages of organisms that are normally associated with the gastro-intestinal tract.

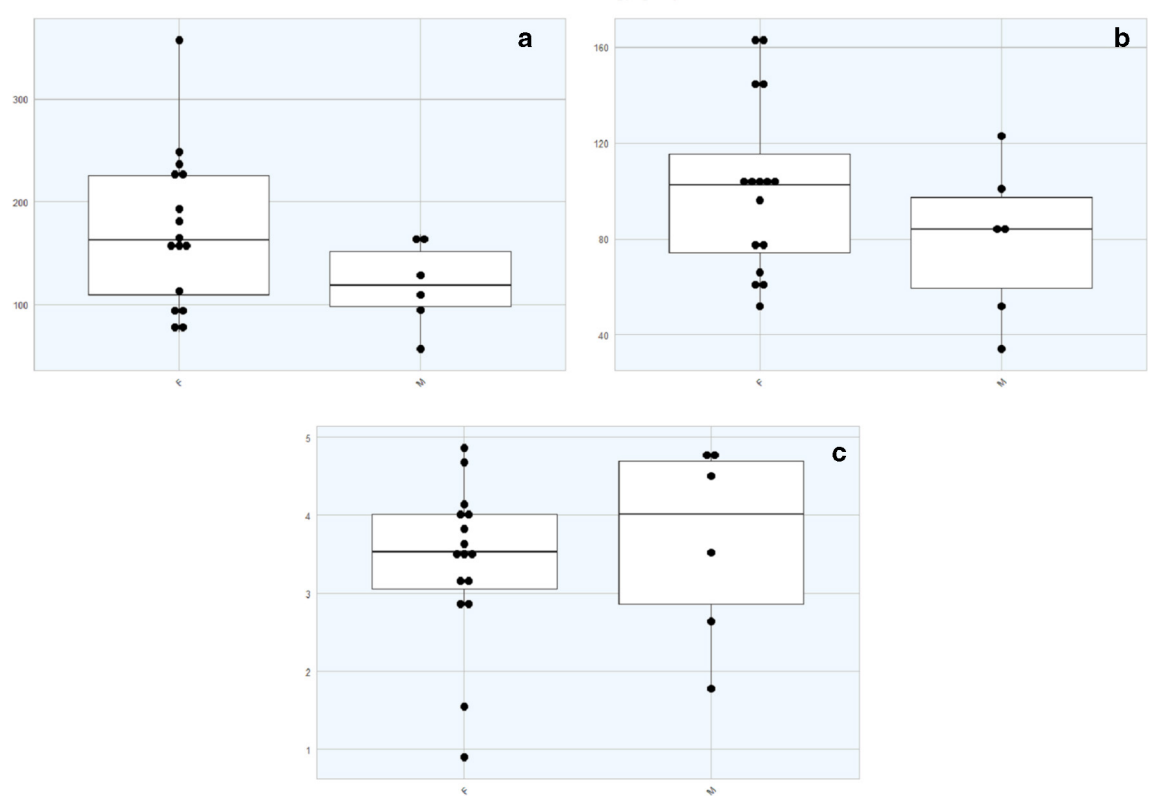

Fig. 3. Box plots representing comparison between the diversity indices [(a) Chao1; (b) observed species; (c) Shannon] for female (F) and male (M) navel skin swab samples.

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 and 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 [35]. 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 [36, 37]. While many consumers may follow unsafe food-handling practices at home [37, 38], 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.* [39], 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 there was no presence of *Klebsiella* [40]. 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 [40]. Staudinger *et al.* [41] reported that Gram-positive bacteria are more abundant than Gram-negative 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 year olds 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.

Gender difference in the abundance of microbiota

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 [42]. Shannon diversity index showed no statistical difference between the male and female microbiota in terms of species richness and evenness (Fig. 3a, P=0.64). Chao1 index also indicated that the species richness is statistically similar in the compared microbiota (Fig. 3b, P=0.052). While samples from females seem to have higher microbial diversity than male samples (Fig. 3c), the difference is not statistically significant (P=0.20). The ability of samples to be separated by gender was also assessed by analysing the beta diversity. PCoA plots, based on the weighted Unifrac distance matrices, showed that the skin microbiota does not differ significantly between male and female populations (Fig. 4a–c). The samples were clustered together across all the analysed plots.

Studies have shown that overall gut microbiota is gender-specific and the observed differences in the microbiota could act as potential determinant of gender predisposition of diseases [43, 44]. In contrast, our results show that skin microbiota in the navel region seems to be gender-independent in the young-adult stage. We would like to caution that further research is warranted considering the sample size for the number of males in our study was limited (n=6).

Pairwise comparison of the microbiota between samples from male and female at the genus level shows only 11 genera to be present in a statistically significant amount (Table 1). Seven genera were found to be present in a statistically higher

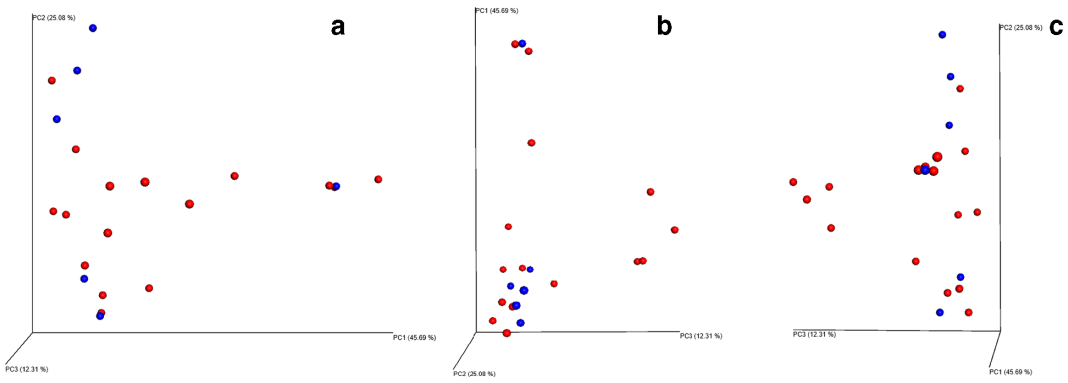

Fig. 4. PCoA analysis of human navel skin swab samples based on weighted UniFrac distances. The three images show the clustering across two different coordinates. Red points represent female samples and blue points represents male samples.

Table 1. List of bacterial genera that were found to differ statistically ($P<0.05$) in abundance between male and female samples. Negative log2fc values indicate that the genus was found higher in female samples and a positive value indicates the genus was found higher in male samples

Phylum	Class	Order	Family	Genus	log2fc	*P* value
Proteobacteria	Gammaproteobacteria	Pseudomonadales	*Moraxellaceae*		−8.66	0.00
Proteobacteria	Gammaproteobacteria	Enterobacteriales	*Enterobacteriaceae*	*Klebsiella*	−7.47	0.00
Proteobacteria	Gammaproteobacteria	Enterobacteriales	*Enterobacteriaceae*	*Enterobacter*	−5.89	0.01
Actinobacteria	Actinobacteria	Actinomycetales	*Actinomycetaceae*	*Actinobaculum*	−5.02	0.04
Firmicutes	Bacilli	Lactobacillales	*Lactobacillaceae*	*Lactobacillus*	−4.65	0.03
Actinobacteria	Actinobacteria	Actinomycetales	*Microbacteriaceae*	*Rathayibacter*	−4.32	0.03
Proteobacteria	Alphaproteobacteria	Sphingomonadales	*Sphingomonadaceae*		−4.00	0.03
Proteobacteria	Alphaproteobacteria	Sphingomonadales	*Sphingomonadaceae*	*Sphingomonas*	−2.95	0.02
Actinobacteria	Actinobacteria	Actinomycetales	*Microbacteriaceae*		3.93	0.04
Firmicutes	Clostridia	Clostridiales	*[Tissierellaceae]*	*1–68*	4.12	0.01
Proteobacteria	Betaproteobacteria	Burkholderiales	*Oxalobacteraceae*		4.72	0.02
Firmicutes	Clostridia	Clostridiales	*[Tissierellaceae]*		8.55	0.00

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 (>eightfold higher abundance), *Klebsiella* ap. (>sevenfold higher abundance) and *Enterobacter* (>fivefold 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 (Table 1). Other organisms present in higher abundance in males includes unknown genera from *Oxalobacteraceae* and *Microbacteriaceae* families. 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 [41]. One of the limitations of the current study is the limited sample size ($n=22$). Further studies, with larger sample sizes would need to validate the results observed in this report.

In conclusion, we have demonstrated that *Corynebacterium* and *Staphylococcus* are the core microbiota present in the navel region of young adults and the overall diversity is similar in male and female young adults with varying abundance in genera. The navel skin microbiota of the young adults also has 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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Author contributions
V.S. and S.S. conceived and designed the experiments; V.S., S.S. and T.D.R. were responsible for sample collection; S.S. and V.S. performed the experiments; S.S. and V.S. were responsible for analysis of data; V.S., S.S. and T.D.R. were responsible for the preparation of manuscript.

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

Ethical statement
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.

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