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Exploring the Effects of Isotretinoin on the Oral Microbiome: A Comparative Study of Microbial Diversity and Composition

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SUMMARY Isotretinoin, known by its brand name Accutane, is an oral medication commonly used in the treatment of moderate to severe acne. Previous research has demonstrated the antimicrobial properties of isotretinoin, particularly in modulating cutaneous colonization by the acne linked *Cutibacterium acnes*, however, its effects on entire microbial communities are poorly understood. Therefore, the objective of this study was to investigate the effects of isotretinoin on the beta diversity of the oral microbiome. The salivary microbiomes of 7 individuals who have previously taken isotretinoin but are no longer taking it were compared to 27 control individuals with no history of isotretinoin usage. 16S sequencing followed by non-metric multidimensional scaling showed no significant difference in oral microbiome beta diversity between individuals who have taken isotretinoin and those who have not. Differential abundance analysis revealed past isotretinoin users exhibited significantly lower abundances of Actinobacteria taxa including Actinomycetales and Micrococcaceae related species. While bacterial alpha diversity was similar between study groups, oral community richness within the Actinobacteria phylum was significantly lower among isotretinoin users. These findings provide further insight into the long-term effects of isotretinoin on the human body and characterize its impact on the oral microbiome for the first time. However, additional research is needed to explore the underlying mechanisms by which isotretinoin influences microbial diversity as well as the broader implications of such alterations in microbiome composition.

INTRODUCTION

The human oral cavity provides a moist and nutrient rich environment that allows many microbial species to flourish (1). The combined genetic material of this diverse community of microorganisms, including bacteria, fungi, viruses, and protozoa, is collectively referred to as the human oral microbiome. According to the Human Oral Microbiome Database, Firmicutes, Bacteroidetes, Proteobacteria, Fusobacteria, and Actinobacteria are some of the most common phyla associated with the oral microbiome (2).

The oral microbiome impacts numerous biological processes and plays an essential role in maintaining overall human health. Some commensal microbes of the oral cavity help keep pathogenic species in check by blocking their adherence to mucosal surfaces, while other species have been shown to cause various oral diseases such as tooth decay and gum disease (2, 3). The oral microbiome's influence extends beyond the oral cavity and remains important in considering human health at a systemic level. For example, oral microbiome composition has the potential to function as a prognostic biomarker in diseases like cardiovascular disease where certain species have been associated with better cardiovascular outcomes (4) and recent evidence suggest links between oral bacteria and several other systemic diseases like diabetes, pneumonia, and stroke (2). Despite its emerging importance in relation to human health, the oral microbiome is relatively understudied and more research examining the role that medications and medical intervention have in shaping the oral microbiota is needed to fully

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understand the relationship between the oral microbiome and health outcomes. Such research will not only provide a deeper understanding of the oral microbiome but allow the development of new pharmaceutical agents and therapeutic methods capable of targeting both etiological and symptomatic components of diseases associated with disruptions in oral microflora.

Isotretinoin, commonly known by its brand name Accutane, is an oral prescription medication primarily used in the treatment of moderate to severe acne. Isotretinoin, a non-antibiotic retinoid, is the most clinically effective anti-acne drug and remains the only therapeutic able to target all major pathogenic processes of acne (5, 6). While isotretinoin's exact mechanism of action is not entirely understood, it appears to work in part by reducing colonization of the skin by *Cutibacterium acnes* (*Propionibacterium acnes*), a bacterial species involved in the pathophysiology of acne (5, 7).

Studies have shown that isotretinoin not only decreases cutaneous colonization by *C. acnes*, but also exhibits antimicrobial effects on other species found in the body, such as those inhabiting the oral cavity (8, 9). Other studies have demonstrated adverse oral effects of isotretinoin such as reduced salivary flow and buffer capacity, both of which are risk factors for dental caries (10). However, research examining the broader effects of isotretinoin in terms of the human microbiome is scarce, with the majority focusing on the skin and gut microbiomes. Therefore, the goal of this study is to examine the drug's effects on the oral microbiome specifically. Studies have found that isotretinoin treatment significantly increased beta diversity of the skin microbiome (7, 11, 12), therefore, we hypothesized that it has a similar effect on the oral microbiome. To investigate this, the salivary microbial composition of individuals with and without a history of isotretinoin usage were characterized via 16S rRNA sequencing. Non-metric multidimensional scaling (NMDS) using a Bray-Curtis distance metric revealed that the oral communities of those who have used isotretinoin in the past do not exhibit a significantly different beta diversity than non-users. In addition, alpha diversity analysis accompanied by differential abundance analysis revealed isotretinoin users express reduced oral community richness within the Actinobacteria phyla and exhibit specific alterations in taxonomic relative abundances. A better understanding of the relationship between isotretinoin and the oral microbiome can help us gain further insight into the drug's long-term side effects, how to best manage those side effects, and learn more about other ways in which isotretinoin can be used, such as in the treatment of other dysbiosis-associated diseases like periodontal disease (8).

METHODS AND MATERIALS

Participant categorization. The salivary microbiomes of 74 human participants were considered for analysis in this study. All subjects were undergraduate students between the ages of 18-28 years old enrolled in Experimental Biology Lab at Florida State University who consented for the use of their microbiome data in this study. Participants were categorized into three groups based on their response to the following survey questions:

1. *Are you currently taking, or have you ever taken the medication isotretinoin (Accutane) orally?*
    A. *Yes, I am currently taking this medication* (ITN-X)
    B. *Yes, I have taken this medication at some point in my life* (ITN)
    C. *No, I have never taken this medication* (CTRL)
2. *Have you ever taken any kind of oral prescription acne medication in your life? Examples included Accutane (isotretinoin), doxycycline, minocycline, spironolactone, azithromycin, erythromycin, tetracycline, etc.*
    A. *Yes*
    B. *No*

Question 2 regarding prior use of oral acne medications was used to filter out individuals who had previously taken other acne medications which might confound the comparison between isotretinoin and control groups. The survey was self-reported and administered online through Google Forms and participant responses were anonymized. Those who selected option A of the first question were categorized as potential candidates for the isotretinoin-now group

(ITN-X) and represented those who were actively taking the medication at the time of sample collection. However, no participants selected option A of the first question which excluded ITN-X from this analysis. Individuals who responded with option B from the first question formed the isotretinoin-past group (ITN) and represented those who have taken isotretinoin at some point in their lifetime but are no longer taking the medication. Due to the already limited sample size, all potential ITN samples were used for analysis excluding one participant's sample whose age was close to 10 years older than all other study participants. Candidates who selected option C of the first question and responded "no" to the question asking about other acne medications were considered for the control group (CTRL). CTRL was age and sex matched to ITN and final CTRL samples were selected randomly from those who had never taken isotretinoin. In this study, the oral microbiomes of CTRL participants were used as a baseline for comparison with the microbiomes of those with a history of isotretinoin treatment.

Sample collection. Approximately 2 mL of saliva was collected in sterile vials by each participant during roughly the same week. The subjects were instructed to avoid eating, drinking, smoking, chewing gum, or brushing teeth for 30 mins prior to sample collection. A total of 74 saliva samples, one from each participant, were collected and stored on ice until processing. The samples were de-identified so that each student sample was associated with a random number.

DNA extraction and PCR amplification. To minimize potential contamination from host DNA, the HostZERO Microbial DNA Kit (Zymo Research, D4310) was used to deplete human DNA and extract bacterial DNA according to the manufacturer's protocol. The 16S rRNA gene region is largely conserved across microbial species (13), therefore, the purified microbial DNA of each sample was PCR amplified using the 341F and 806R primers to target amplification of the V3-V4 region (Table 1). The Q5 High-Fidelity DNA Polymerase, supplied by the Q5 High-Fidelity 2X Master Mix (New England BioLabs, M0492S), was utilized for amplification. Reaction setup and PCR thermocycling were performed according to the Q5 High-Fidelity 2X Master Mix protocol. Successful amplification of the DNA was verified using gel electrophoresis on 2% agarose. Amplicons between 400-500 bps were generated for each sample. Microbial DNA was recovered and purified from the PCR amplicons using the NEBNext Sample Purification Beads (New England BioLabs, E7104) per manufacturer protocol.

TABLE. 1 PCR primer sequences.

Primer	Sequence
341F1	CCTACGGGDGGCWGCAG
341F2	CCTAYGGGGYGCWGCAG
806R	GACTACNVGGGTMTCTAATCC

Library preparation and next-generation sequencing. Before sequencing, libraries were constructed from the resulting amplicons using the NEBNext Ultra II DNA Library Prep Kit for Illumina (New England BioLabs, E7645) according to the manufacturer's manual. Library concentration was determined using the Qubit Fluorometer (ThermoFisher Scientific). To evaluate library size and adaptor dimer abundance, 1.5 uL of each completed library was ran on 2% agarose gel. Libraries approximately 600 bps were expected. The finished libraries were then pooled together into a final library pool using 250 ng of DNA from each sample. The final library pool was size selected and size distribution was assessed using TapeStation (Agilent) for additional quality control. The final library was then submitted for KAPPA quantification and Illumina next-generation sequencing (NGS) via the NovaSeq 6000 system (Illumina) in the FSU College of Medicine Translational Science Laboratory.

Sequence processing and quality control. Sequence data generated by deep sequencing was demultiplexed by CASAVA to separate individual libraries based on index sequences. Sequencing reads were reported in fastq format and data quality was assessed using the

FastQC software reports (Babraham Bioinformatics). Phred quality (mean sequence quality), GC content, per base sequence content, and adapter content were used to evaluate sequence and sample quality, all of which were provided by the FastQC reports. Poor quality reads were trimmed from sequences using Trimmomatic (v0.32) (14) with the following parameters: leading and trailing bases with scores less than 20 were removed as were any bases with a combined score less than 20 over a four base sliding window. Samples exhibiting overall insufficient Phred quality were dropped and replaced with age and sex matched samples that met the QC criteria.

Taxonomic profiling and community analysis. Once quality samples were selected and trimmed, the microbial sequencing data was analyzed using Mothur (v.1.48.0) 16S rRNA analysis software (15). All non-unique reads were removed from samples using the Mothur unique.sequences command to reduce PCR or sequencing based overrepresentation errors. Microbial community composition was determined by aligning sequences with reference genomes of the SILVA V4.138 rRNA database (16) and assigning OTUs. All non-bacterial reads and reads unclassified at the phylum level were removed from the analysis for all samples using the Mothur align.seqs command. Aligned OTU and ASV data was further analyzed in the phyloseq R package (17). Relative abundance at all phylogenetic levels were calculated in R using rarefied data scaled to the sample with the lowest read count. Additionally, bacterial abundance was analyzed at the genus level within the Actinobacteria phylum given the important relationship between isotretinoin and dermal *C. acnes*, an established member of Actinobacteria. Differentially abundant OTUs for ITN were called with the DESeq2 R package using test="Wald", fitType="parametric". An alpha value of 0.05 was used to distinguish significantly enriched taxa between the groups. The diversity characteristics and differential taxa abundances of the control and treatment groups were then compared to measure the potential effects that isotretinoin may have on oral microbiome community composition. Alpha diversity for CTRL and ITN samples was calculated using Chao1, Abundance-based Coverage Estimator (ACE), Shannon Simpson, and Inverse Simpson methods within the phyloseq package. Significance was determined with Wilcoxon rank sum tests using an alpha of 0.05. Beta diversity was evaluated through non-metric multidimensional scaling (NMDS) using the Bray-Curtis distance metric in phyloseq. The alpha and beta diversity of the ITN and CTRL groups were compared at all phylogenetic levels and for the Actinobacteria phylum to evaluate whether the medication's observed effect on skin microbiome diversity translated to the oral community. Statistical significance of observed differences in beta diversity were determined using permutational multivariate analysis of variance (PERMAMOVA).

RESULTS

Survey response and sample size. To compare the oral communities of those with and without a history of isotretinoin usage, participants were categorized as active isotretinoin users (ITN-X), previous isotretinoin users (ITN), or non-users (CTRL) based on their survey feedback. Of the 74 participants, 8 (11%) reported "yes, I have taken isotretinoin" and 66 (89%) reported "no, I have never taken isotretinoin." While we had originally planned for three groups, 0 participants responded with "yes, I am currently taking this medication" meaning only the oral microbiomes of those who have previously used isotretinoin but are no longer taking it (ITN) could be compared with CTRL. A total of 34 oral communities were analyzed. All participants with a past history of isotretinoin were utilized for ITN excluding one participant who was nearly 10 years older than all other participants. The final cohorts consisted of seven ITN samples and a sex matched and randomly selected set of 27 CTRL samples. As the treatment group was 43% female, the controls were also selected to be 44% females to match as closely as possible (Fisher exact test, $p > 0.05$). The demographic information for all samples used in the study are presented in Table 2.

TABLE. 2 Participant demographics.

	ITN (n = 7)	CTRL (n = 27)	p
Age (years)	18-28		N/A
Sex (% female)	43	44	1

Previous isotretinoin users exhibited lower relative abundances of oral Actinobacteria at the phylum level. Using 16S rRNA sequence analysis, a total of 7 bacterial phyla were detected at relative abundances greater than 1% across the 34 oral microbiomes sampled (Figure 1). On average, ITN samples were dominated by Firmicutes

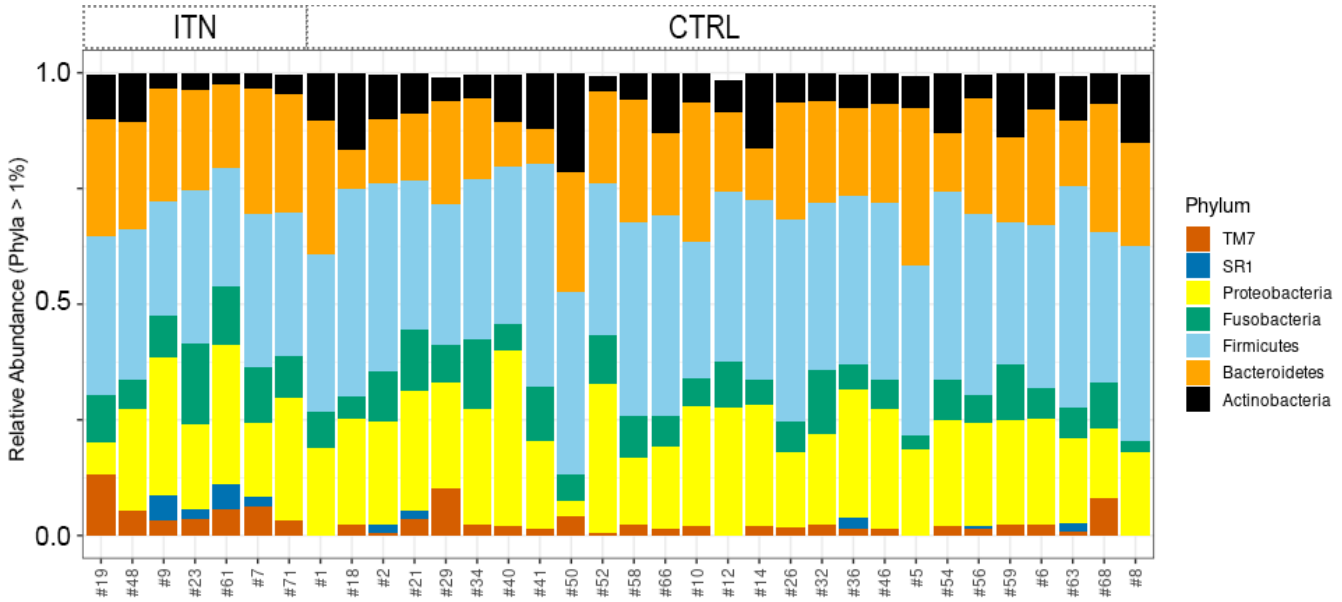

FIG. 1 Oral microbiome taxonomic abundance varied with isotretinoin usage history at the phylum level. Comparison of bacterial relative abundance by phylum showed higher oral levels of Bacteroidetes, Fusobacteria, TM7, and SR1 across ITN samples but lower abundances of Firmicutes, Proteobacteria, and Actinobacteria compared to CTRL. Only phyla with relative abundances greater than 1% in any given sample are shown. Sample number is denoted along x-axis.

(31%), Bacteroidetes (24%), and Proteobacteria (21%), with smaller relative coverage of Fusobacteria (11%), TM7 (5.8%), Actinobacteria (5.3%), and SR1 (2.3%). CTRL samples, on average, were composed of Firmicutes (38%), Proteobacteria (22%), Bacteroidetes (20%), Actinobacteria (9.4%), Fusobacteria (8.1%), TM7 (2.3%) and SR1 (0.5%). Five of the seven ITN samples (#9, #23, #61, #7, and #71) showed an overall lower relative abundance of Actinobacteria at the phylum level (Figure 1). On average, the relative abundance of this phylum was 44% lower across all ITN samples compared to that of CTRL. Four of the five ITN samples with reduced Actinobacteria abundance (#9, #23, #61, and #7) also exhibited elevated levels of SR1 at the phylum level (Figure 1). On average, SR1 was 393% more abundant in ITN then CTRL. TM7 was also markedly elevated among ITN samples with a 152% average increase in relative abundance compared to CTRL. Differential abundance analysis with DESeq2 showed limited increases in Firmicutes, Bacteroidetes, Fusobacteria, TM7, and SR1 OTUs in the ITN cohort coupled with losses of Firmicutes and Actinobacteria (padj < 0.05) (Figure 2). This indicates that the oral microbial communities of isotretinoin users have lower taxonomic abundances of Actinobacteria but higher levels of bacteria belonging to other phyla like Bacteroidetes, Fusobacteria, TM7, and SR1.

Decreases in oral Actinobacteria of previous isotretinoin users were driven by reductions in Actinomycetales and Micrococcaceae related species. Oral taxonomic changes associated with isotretinoin usage were further characterized by analyzing bacterial genera abundance within the Actinobacteria phylum. A total of 13 genera of the Actinobacteria phylum were detected across all study samples (Figure 3). ITN samples were dominated by Actinomycetales_unclassified (50%), Micrococcaceae_unclassified (18%), *Actinomyces* (18%), *Atopobium* (5.3%), Actinobacteria_unclassified (3.4%), and Actinomycetaceae_unclassified (3.1%). CTRL samples were similarly dominated by Actinomycetales_unclassified (51%), Micrococcaceae_unclassified (27%), *Actinomyces* (13%), Actinobacteria_unclassified (2.8%), *Atopobium* (2.3%), and Actinomycetaceae_unclassified (2.2%). While statistically insignificant, elevated levels of *Actinomyces*, *Atopobium*, Actinobacteria_unclassified, and Actinomycetaceae_unclassified

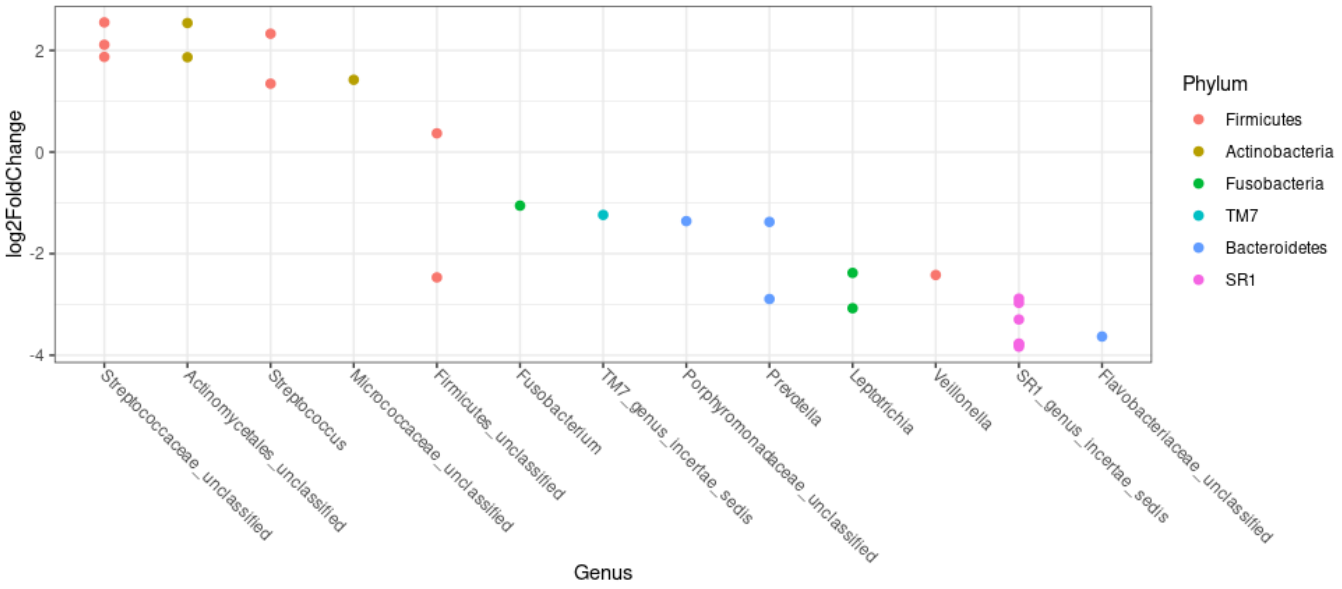

FIG. 2 Differential abundance analysis with DESeq2 revealed key differences in oral microbiome OTUs between isotretinoin users and non-users. Each dot represents a unique OTU found to be significantly differentially abundant between study groups and is colored according to phylum. Those with positive log2 fold change values indicate an OTU with a significantly lower relative abundance in ITN samples compared to CTRL while those with a negative log2 fold change value indicate an OTU with a significantly higher abundance in ITN compared to CTRL (padj < 0.05).

were detected among ITN samples (padj > 0.05) (Figure 3). However, further differential abundance analysis via DESeq2 revealed significantly lower relative abundances of two Actinomycetales_unclassified OTUs and one Micrococcaceae_unclassified OTU in the ITN cohort compared to CTRL (padj < 0.05) (Figure 2, 3). Additional analysis at the genus level showed high variation across samples for low abundance genera including *Rothia*, *Scardovia*, *Propionibacteriaceae*, *Olsenella*, *Corynebacterium*, and *Bifidobacterium* (padj > 0.05) (Figure 3).

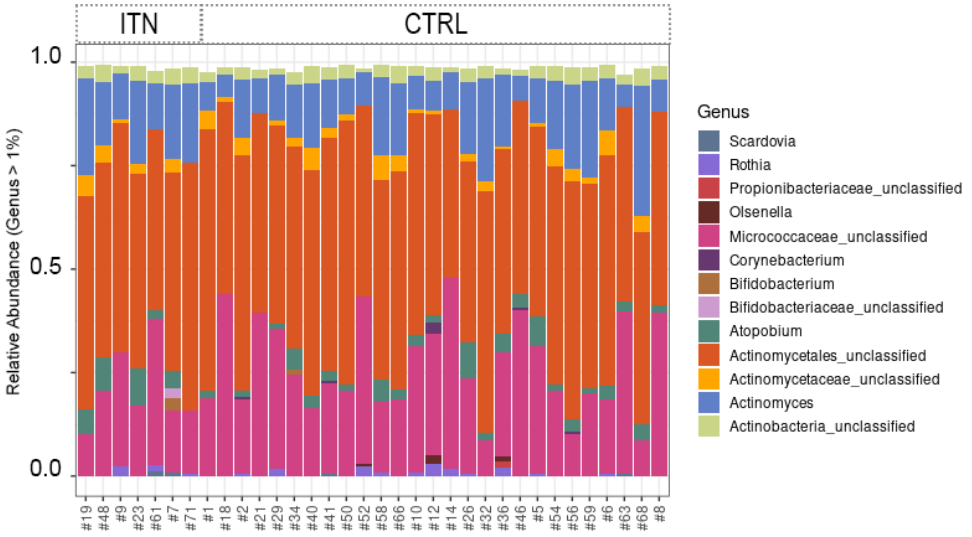

FIG. 3 Oral microbiome taxonomic abundance varied with isotretinoin usage at the genus level within the Actinobacteria phylum. Comparison of Actinobacteria genera relative abundance showed significantly reduced levels of Actinomycetales_unclassified and Micrococcaceae_unclassified across ITN samples compared to CTRL. Minor elevations of *Actinomyces*, *Atopobium*, Actinobacteria_unclassified, and Actinomycetaceae_unclassified were also detected in ITN. Low abundance genera including *Rothia*, *Scardovia*, *Propionibacteriaceae*, *Olsenella*, *Corynebacterium*, and *Bifidobacterium* showed high variation across study samples. Only Actinobacteria genera with relative abundances greater than 1% in any given sample are shown. Sample number is denoted along x-axis.

Previous isotretinoin users exhibited reduced oral community richness within the Actinobacteria phylum. To characterize the effects of isotretinoin on oral alpha diversity, community richness was quantified through Observed richness, Chao1, and ACE Indexes and community diversity was assessed using Shannon, Simpson, and Inverse Simpson Indexes. There was no significant difference between the ITN and CTRL groups as measured by any of the alpha diversity metrics (Wilcoxon rank-sum test, $p > 0.05$) when all phyla were considered (Figure 4). However, analysis of richness and diversity within the Actinobacteria phylum showed reduced richness by Observed richness, Chao1, and ACE metrics (Wilcoxon rank-sum test, $p < 0.05$) and mixed results for diversity with a significant increase in the Simpson Index (Wilcoxon rank-sum test, $p < 0.05$) but not in the Shannon and Inverse Simpson Indexes (Wilcoxon rank-sum test, $p > 0.05$) (Figure 5).

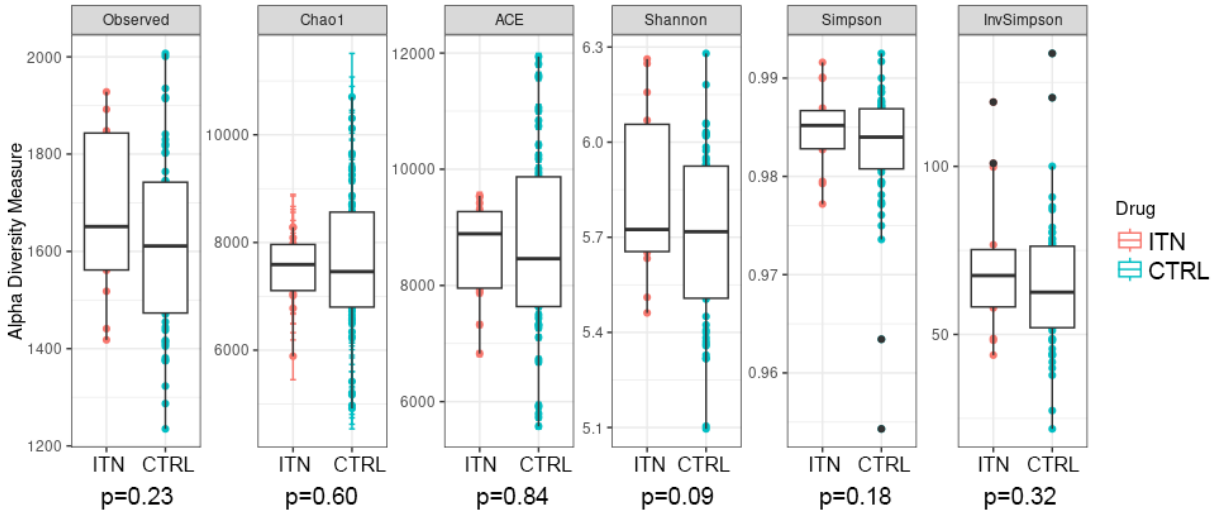

FIG. 4 Oral microbiome species diversity did not differ significantly between isotretinoin users and non-users as indicated by alpha diversity metrics. Species richness, quantified through Observed richness, Chao1, and ACE Indexes, for ITN samples was not statistically different from that of CTRL samples ($p > 0.05$). Species diversity, quantified through the Shannon, Simpson, and InvSimpson Indexes, was not statistically different across ITN and CTRL samples ($p > 0.05$). Error bars indicate mean ± SD.

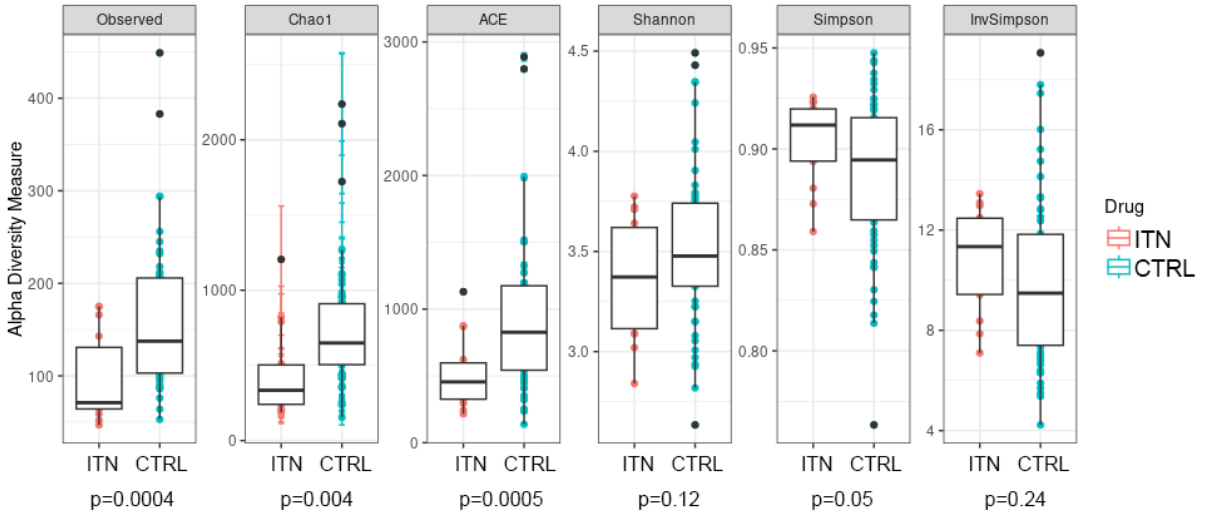

FIG. 5 Oral microbiomes of isotretinoin users exhibited lower Actinobacteria richness as indicated by alpha diversity metrics. Oral community richness quantified through Observed richness, Chao1, and ACE metrics was significantly reduced in ITN compared to CTRL ($p < 0.05$). Community diversity quantified through the Simpson, Shannon, and Inverse Simpson Indexes yielded mixed results with a significant increase in the Simpson Index ($p < 0.05$) but not in the Shannon or Inverse Simpson Indexes ($p > 0.05$). Error bars indicate mean ± SD.

Oral microbiome beta diversity was not significantly different between previous isotretinoin users and non-users. To determine the impact of isotretinoin on oral microbiome beta diversity, beta diversity of ITN and CTRL samples was visualized through NMDS using a Bray-Curtis distance metric and statistical significance was estimated using PERMANOVA. NMDS analysis revealed no significant difference in sample clustering between ITN and CTRL when considering all bacterial phyla (PERMANOVA, $p > 0.05$) (Figure 6A). This trend continued for taxa within the Actinobacteria phyla as no significant difference in ITN and CTRL sample clustering was observed (PERMANOVA, $p > 0.05$) (Figure 6B). This indicates that the oral microbiomes of individuals with a history of isotretinoin usage are similar to those of non-users in this study population.

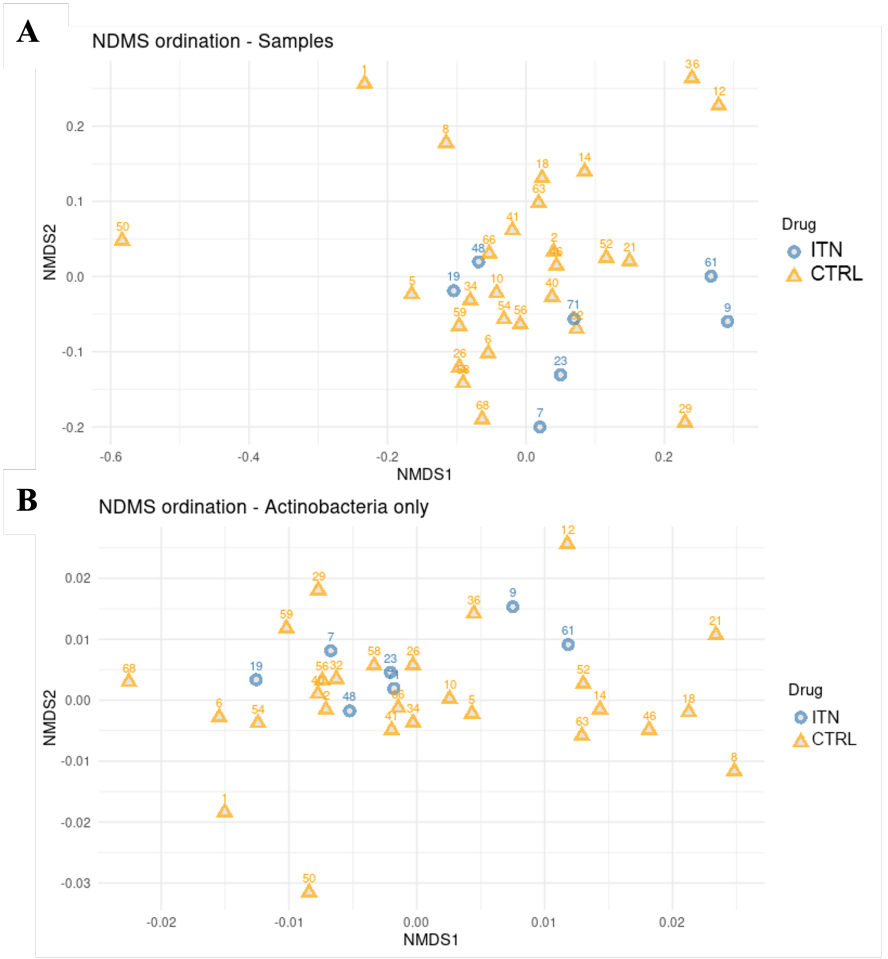

FIG. 6 NMDS revealed no significant difference in oral microbiome beta diversity between isotretinoin users and non-users. (A) Analysis of microbial beta diversity through NMDS using a Bray-Curtis distance metric showed no significant variation in ITN and CTRL sample clustering (PERMANOVA, $p > 0.05$). (B) Further NMDS analysis continued to show no significant difference in sample clustering when only considering Actinobacteria taxa (PERMANOVA, $p > 0.05$). Number above plot point indicates sample number.

DISCUSSION

Previous research has shown that orally administered isotretinoin increases the beta diversity of the skin microbiome and influences microbial abundances throughout the human body (7, 11, 12). However, the medication's effect specifically on oral microbiome composition and diversity remained unclear. To help address this, we characterized the salivary microbiomes of 34 college students and compared levels of microbial abundance and diversity between those who have and have not taken isotretinoin.

Differential abundance analysis of bacterial taxa revealed that the oral microbiomes of past isotretinoin users had a significantly higher relative abundance of OTUs belonging to Bacteroidetes, Fusobacteria, TM7, and SR1 but lower abundances of Actinobacteria compared to non-users. Superficially, this may suggest that isotretinoin treatment enhances oral abundance of Bacteroidetes, Fusobacteria, TM7, and SR1 while reducing abundances of Actinobacteria; however, this study did not address any causal relationships between medication and bacterial colonization. This observed decline in Actinobacteria is consistent with previous research as isotretinoin is known to have antimicrobial effects on *C. acnes* (18), the bacteria species linked to acne pathogenesis and a well-known member of the

Actinobacteria phylum. Further analysis at the genus level within the Actinobacteria phylum revealed a significant drop in three Actinobacteria OTUs, two unclassified Actinomycetales members and one unclassified Micrococcaceae. This indicates that the observed decline in Actinobacteria in isotretinoin users was driven by the significant loss of these three Actinobacteria related species and may suggest that isotretinoin plays a role decreasing their oral abundance. Furthermore, these findings may indicate that isotretinoin treatment targets decolonization of a subgroup of Actinobacteria-related species, rather than just *C. acnes* alone and may suggest the existence of a unifying characteristic or bacterial feature that confers this group's susceptibility to isotretinoin. Future studies exploring the vulnerability of Actinobacteria members to isotretinoin are needed and may have the potential to foster a better understanding of isotretinoin's mechanism of action.

In addition to beta diversity, we found no significant difference in oral microbiome alpha diversity between isotretinoin users and non-users when considering all bacterial phyla. However, we did find significant reductions in oral alpha diversity richness across isotretinoin users when considering only Actinobacteria taxa. This suggest that isotretinoin treatment may not significantly alter mean domain-wide bacterial diversity but influences oral community richness of Actinobacteria. These findings compliment the results of our differential abundance analysis which revealed significant drops in the relative abundance of three Actinobacteria OTUs: two unclassified Actinomycetales and one unclassified Micrococcaceae. Overall, this further supports the idea that Actinobacteria related species are impacted by isotretinoin. In comparison with other studies, research examining isotretinoin-associated alterations in skin microbiome alpha diversity are less coherent (19) and virtually non-existent for the oral microbiome. Consistent with this project, one study found that alpha diversity of the skin microbiome was not significantly impacted by isotretinoin compared to pretreatment (11). In contrast, a 2021 study found that alpha diversity significantly increases as a function of isotretinoin treatment time and remains elevated months after treatment (7). However, these studies examine diversity changes in the skin microbiome specifically, therefore, their findings may also be indicative of the large variation in microbial composition between the skin and oral communities (20, 21). Our present study represents the first to characterize isotretinoin-associated changes in microbiome diversity of the oral community. As such, more studies investigating the drugs effect at the oral level are necessary to expand on the findings of this study.

Through beta diversity analysis, we found that the oral microbiomes of participants who have taken isotretinoin in the past do not exhibit a significantly different microbiome beta diversity than those who have never taken the drug. This indicates that the oral microbiomes of post-treatment isotretinoin users do not differ in between community diversity than non-users and suggest that isotretinoin does not increase oral community beta diversity post-treatment. Our findings are inconsistent with preexisting studies of the skin microbiome which have shown greater dissimilarity between microbial communities in isotretinoin users (7, 11, 12). As previously mentioned, it is essential to note that these studies focus on the skin microbiome and this present study represents the first to examine the effects of isotretinoin on oral diversity. While we had predicted a similar effect on the oral microbiome to that of the skin, it is logical that these effects did not translate as there is extensive variation in the microbial makeup and physiology of the skin and oral microbiomes (20, 21). While the mechanisms for which isotretinoin operates and the extent to which the drug influences microbes is likely multifactorial and remains an area of active research, our findings alone may indicate that the beta diversity alterations previously seen in the skin microbiome with isotretinoin usage are dependent on skin-exclusive pathways or ones that are less prevalent in the oral cavity. For example, one theory is that isotretinoin targets *C. acnes* decolonization of the skin by decreasing skin sebum, the main nutrient source of *C. acnes* (22). However, more extensive research characterizing isotretinoin's mechanisms of action and effects on human microbiome constituents are needed before any such conclusions can be made.

Limitations First, the oral microbiomes of the participants were analyzed using saliva samples only. While the organisms inhabiting saliva account for 99.9% of all bacteria in the oral cavity, differences still exist between saliva and other parts of the oral cavity (23), therefore, the results of this study may not accurately reflect the entire oral microbiome.

Additionally, the use of 16S rRNA sequencing provides good coverage for bacteria, however, this region is not present in all microorganisms and fails to identify some species of bacteria and excludes viruses, protists, and fungi, which are all vital components of the microbiome. A substantial portion of bacterial reads were unclassified at the genus level meaning insight provided by taxonomic classification could not fully considered. It is also important to emphasize that this study examined correlative data and additional studies are required to establish causative relationships between isotretinoin and bacterial abundances in the oral microbiome. The unavoidably small subject sample size ultimately limits the statistical power of this study, and our research was restricted to undergraduate students enrolled in Experimental Biology Lab at Florida State University. Furthermore, a multitude of factors were not considered in this study's participant categorization, like drug dosage, treatment duration, medical history, and lifestyle elements like diet and exercise which are factors known to play important roles in shaping the microbiome and likely influence isotretinoin's microbial effects (1, 7, 18, 24, 25, 26).

Conclusions Isotretinoin is a powerful medication known to exhibit antimicrobial effects on human commensals, such as *C. acnes*, the bacterial species linked to acne pathogenesis. While the drug's effects on entire microbial communities are poorly understood, recent studies have demonstrated isotretinoin increases beta diversity of the skin microbiome. As such, we sought to characterize the effects of isotretinoin on salivary microbiome diversity. Here we demonstrate that oral microbiome beta diversity was not significantly different between post-treatment isotretinoin users and non-users. Differential abundance analysis parried with alpha diversity metrics revealed an overall decrease in Actinobacteria richness which suggests that isotretinoin targets decolonization of Actinobacteria-related species rather than just *C. acnes* alone. Our findings provide further evidence confirming that isotretinoin's reach extends beyond *C. acnes* and the skin microbiome and describes its impact on oral diversity for the first time. Future well-designed studies examining isotretinoin's mechanisms at the molecular and microbial level and a deeper understanding of microbiome features linked to human health are warranted to verify and expand on the results of this study.

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