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The preadolescent acne microbiome: A prospective, randomized, pilot study investigating characterization and effects of acne therapy

Carrie C. Coughlin MD[1] | Shane M. Swink MS[2] | Joseph Horwinski BS[3] | Georgia Sfyroera PhD[3] | Julia Bugayev[3] | Elizabeth A. Grice PhD[3,4] | Albert C. Yan MD[3,5]

[1]Division of Dermatology, Departments of Medicine and Pediatrics, School of Medicine, Washington University School of Medicine, St. Louis, MO, USA

[2]Philadelphia College of Osteopathic Medicine, Philadelphia, PA, USA

[3]Department of Dermatology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA

[4]Department of Microbiology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA

[5]Section of Dermatology, Children's Hospital of Philadelphia, Philadelphia, PA, USA

Correspondence
Carrie C. Coughlin, MD, 660 South Euclid Avenue, Campus Box 8123, St. Louis, MO 63110 USA.
Email: coughlinc@wustl.edu

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Abstract

Background/Objectives: Acne, a common pediatric disease, tends to be more comedonal in preadolescents, whereas older individuals are more likely to have inflammatory lesions in addition to comedones. Thus the microbiome of preadolescents may be different. In this pilot study we aimed to characterize the preadolescent acne microbiome, compare the microbiome in preadolescents with and without acne, and investigate changes in the microbiome after topical treatment with benzoyl peroxide or a retinoid in a small cohort of preadolescents.

Methods: Participants were 7-10 years of age with (intervention group) or without (control group) acne and were recruited during routine outpatient dermatology visits. Baseline questionnaires, physical examination, and pore strip application were performed for all participants. Intervention group participants were randomized to receive topical therapy with benzoyl peroxide 5% gel or cream or tretinoin 0.025% cream. Participants with acne were followed up 8-10 weeks later and pore strip application was repeated.

Results: Preadolescents with acne were colonized with a greater diversity of cutaneous bacteria than controls and the most commonly identified bacterium was *Streptococcus*. The number of bacterial species and phylogenetic diversity decreased after treatment with benzoyl peroxide and tretinoin.

Conclusion: The predominant bacteria in microbiome studies of adult acne is *Propionibacterium*, whereas in this pediatric population we saw a lot of *Streptococcus* bacteria. After treatment, the microbiomes of intervention group participants more closely resembled those of control group participants.

KEYWORDS
acne, microbiome, microbiota, pediatric, preadolescent, *Propionibacterium*, *Streptococcus*

1 | INTRODUCTION

Acne is a common dermatologic disease affecting more than 75% of children.[1,2] Benzoyl peroxide (BP) and topical retinoids are first-line therapies for children of any age with comedonal acne.[1] Investigations into the pathogenesis of acne have long implicated *Propionibacterium*,[3] which recently has been shown to be the most prevalent genus of bacteria at sebaceous sites in adults.[4,5] The microbiome

evolves with age,[6] so the preadolescent bacterial microbiota may be different from that of adolescents and adults. This study investigated the microbiome in preadolescents with and without acne and gathered data about changes in the relative abundance and diversity of bacterial microbiota after topical acne treatment.

2 | MATERIALS AND METHODS

2.1 | Participants

Children 7-10 years of age with acne that would be appropriately treated using a topical regimen were included. Age- and sex-matched healthy control participants without acne were recruited during visits to the dermatology clinic for other problems. Exclusionary criteria for all participants were recent (within 1 month) treatment with an oral or topical antibiotic, any acne treatment, or significant immunosuppression.

2.2 | Study design

We performed a prospective, interventional, randomized pilot study to evaluate the preadolescent acne microbiome. The Children's Hospital of Philadelphia (CHOP) Research Institute Institutional Review Board approved the study, which was conducted from March to August 2015. Children assented and their guardians provided consent for participation during routine visits at the CHOP outpatient dermatology clinic. At baseline, all participants answered a medical history questionnaire, were graded for acne severity on the Comprehensive Acne Severity Scale (CASS),[7] and had microbiome samples collected from five sites using pore strips[8] on intact skin: midline forehead, dorsum of the nose, medial left cheek, chin, and left retroauricular crease. Participants with acne were randomly assigned to treatment with BP 5% gel or cream or tretinoin 0.025% cream. Blocks of patients were randomized and assignments were concealed until group assignment at the baseline visit. Participants with acne returned for a follow-up visit at 8-10 weeks with repeated CASS evaluation and pore strip application.

2.3 | Sample collection, sequencing, and analysis

Pore strips were cut into six pieces of equal size and moistened with certified DNA-free water (MoBio Laboratories, Carlsbad, CA, USA) and five pieces were applied to skin sites to be sampled. One piece was a control. Strips were allowed to harden for 10-15 minutes, removed from the skin, and placed in a tube with 300 μL of yeast cell lysis buffer (Epicentre Biotechnologies, Madison, WI, USA). At each sampling event, negative control samples (pore strips moistened with the DNA-free water and air dried without application to the skin and tubes of buffer without any sample) were collected, processed, and analyzed exactly as the experimental samples were to control for background reagent contamination. Ready-Lyse Lysozyme Solution (Epicentre Biotechnologies) 0.5 μL was added to each sample for a final concentration equivalent to 20 mg/mL. Samples were incubated at 37°C for 1 hour at 600 RPM and then vortexed at high speed for 10 minutes with glass beads (G-3290-3, MoBio Laboratories). Samples were then incubated at 65°C for 30 minutes at 600 RPM followed by 5 minutes on ice. Strips and liquid were removed to a spin column to extract fluid and spun for 1 minute at 10 000 *g*. Downstream isolation was performed as previously described.[9]

The V1-V3 region of the 16S rRNA gene was amplified from each sample in duplicate using the Accuprime kit (Invitrogen, Carlsbad, CA, USA), primers 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 534R (5′-ATTACCGCGGCTGCTGG-3′) barcoded as previously,[10] and 32 cycles of polymerase chain reaction (PCR) (94°C for 45 seconds, 50°C for 60 seconds, 72°C for 90 seconds). PCR products were purified and normalized using the SequalPrep Normalization Kit (Invitrogen) and standard protocol. Sequencing was performed on the Illumina MiSeq using 300–base pair paired-end chemistry at the PennCHOP Microbiome Center. The analysis workflow was performed as described in detail in Meisel and colleagues.[9] Because uneven sampling can affect diversity metrics, sequences were subsampled randomly to 10 000 sequences per sample.

3 | RESULTS

Eight participants with acne were randomized to treatment with BP (n = 5; 3 girls, 2 boys) or tretinoin (n = 3 girls). The average age in each group was 9 years (range 7-10). Control participants (n = 8) were age and sex matched. Three participants were lost to follow-up; we collected post-treatment data on three children treated with BP and two treated with tretinoin an average of 8.6 weeks (range 7-10 weeks) after their baseline visits. Average baseline CASS values were 1.4 (BP), 1.3 (tretinoin), and 0 (controls). In control participants, *Streptococcus* was the genus present in highest relative abundance, followed by *Propionibacterium* (Figure 1). Participants with acne also had high relative abundances of *Streptococcus* but had more *Staphylococcus* and *Propionibacterium* than controls at all sites before treatment. Alpha diversity, as measured according to the number of observed species and phylogenetic diversity (Figure 2), was higher at baseline in participants with acne than in controls at all skin sites except the retroauricular crease. In patients treated with BP, the relative abundances of *Staphylococcus* (four sites, excluding the nose) and *Propionibacterium* (all sites) decreased, but the change was not statistically significant. Average CASS values decreased to 1 (BP and tretinoin). Treatment significantly decreased the alpha diversity of the skin microbiome in participants with acne to levels similar to those in control participants as measured according to the number of observed species and phylogenetic diversity (both $P < .001$; paired Wilcoxon rank sum test).

4 | DISCUSSION

With the recent focus on the appropriate duration of antibiotic therapy,[11] characterization of the microbiome is important to

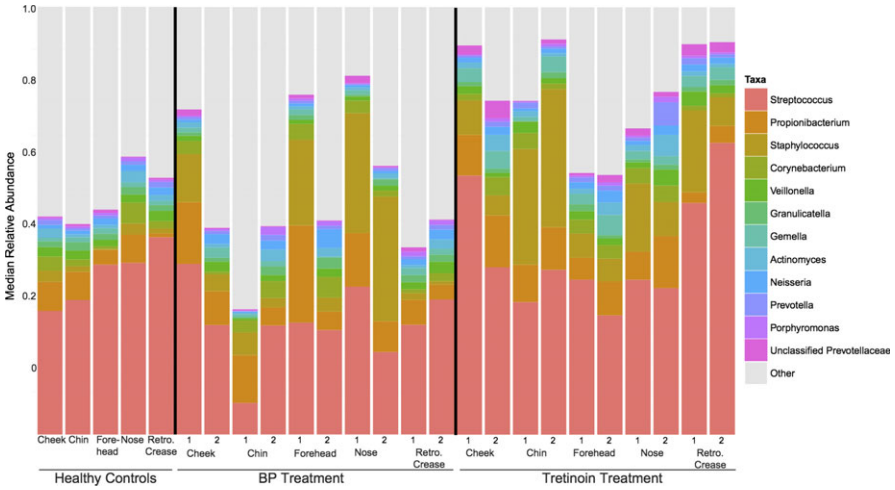

FIGURE 1 Median relative abundance of the top 12 genus-level taxa colonizing skin sites in healthy controls and individuals with acne before and after treatment. All other less abundant taxa are grouped in the category "Other." The *y*-axis denotes median relative abundance (%). The number on the *x*-axis denotes time point, with 1 indicating pretreatment and 2 indicating posttreatment where relevant

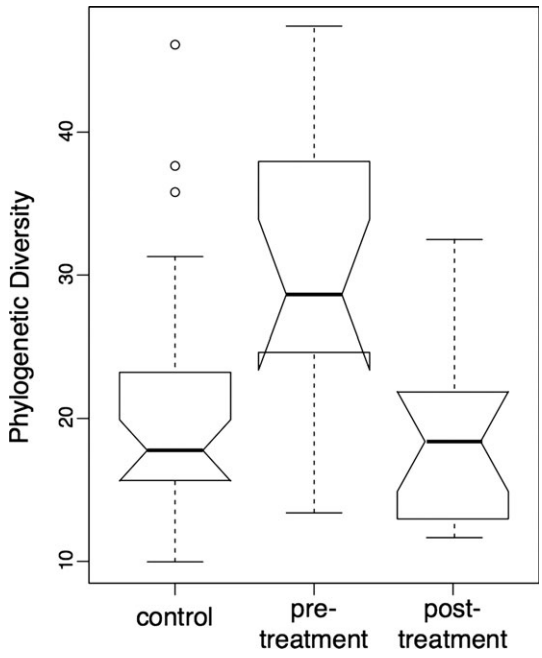

FIGURE 2 Boxplot comparing the median phylogenetic diversity of (from left to right) healthy control skin, pretreatment acne skin, and posttreatment acne skin. The thick line in the middle of the box indicates the median; the upper and lower box hinges correspond to the first and third quartiles; whiskers extend to the highest and lowest values within 1.5 times the interquartile range (IQR). Outliers of the IQR are dots above or below the whiskers

increase the efficacy of antibiotic therapy and inform investigations of alternative therapies. Moreover, describing the effect of acne treatment on the microbiome is central to refining the pathogenesis of acne, evaluating mechanisms underlying effects of therapy, and identifying potential unintended consequences of therapy.

The cutaneous microbiome changes with treatment exposures and age and differs according to body site.[4,6,12] Studies of the adult acne microbiome have focused on the association between different strains of *Propionibacterium* and patients' acne and on *Malassezia* species.[3-5,13] In this study, preadolescents with acne had a greater diversity of cutaneous bacteria than controls on facial and retroauricular skin. This diversity decreased with BP and tretinoin treatment. The high relative amount of *Streptococcus* seen in all participants is particularly interesting because reports of the adult acne microbiome indicate that *Propionibacterium* dominates,[3,4] although our findings are consistent with prior studies of cutaneous bacteria in prepubertal children.[12] This is important when considering targeted therapies to treat acne and suggests that preadolescents may require a different treatment approach than older individuals. Studies of the microbiome have also provided insights into alternative mechanisms of therapy, such as probiotics,[14] and different influences on microbial communities, such as B12.[15] The decrease in bacterial diversity with both treatments in this study is notable because BP and tretinoin are known as comedolytic, but tretinoin is not typically thought of as having antibacterial properties like BP.[16] This suggests that topical retinoids may have an effect on the local microbiome through alterations in the cutaneous microenvironment rather than having direct effects on resident microorganisms. Additional studies with more participants are needed to explore the acne microbiome further, evaluate differences between and similarities in bacteria in individuals of different ages, and investigate treatment targets.

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REFERENCES

1. Eichenfield LF, Krakowski AC, Piggott C, et al. Evidence-based recommendations for the diagnosis and treatment of pediatric acne. *Pediatrics*. 2013;131(Suppl 3):S163-S186.
2. Lucky AW, Biro FM, Huster GA, Leach AD, Morrison JA, Ratterman J. Acne vulgaris in premenarchal girls. An early sign of puberty associated with rising levels of dehydroepiandrosterone. *Arch Dermatol*. 1994;130:308-314.

3. Fitz-Gibbon S, Tomida S, Chiu BH, et al. Propionibacterium acnes strain populations in the human skin microbiome associated with acne. *J Invest Dermatol*. 2013;133:2152-2160.
4. Grice EA, Kong HH, Conlan S, et al. Topographical and temporal diversity of the human skin microbiome. *Science*. 2009;324:1190-1192.
5. Bek-Thomsen M, Lomholt HB, Kilian M. Acne is not associated with yet-uncultured bacteria. *J Clin Microbiol*. 2008;46:3355-3360.
6. Johnson CL, Versalovic J. The human microbiome and its potential importance to pediatrics. *Pediatrics*. 2012;129:950-960.
7. Tan JK, Tang J, Fung K, et al. Development and validation of a comprehensive acne severity scale. *J Cutan Med Surg*. 2007;11:211-216.
8. Craft N, Li H. Response to the commentaries on the paper: propionibacterium acnes strain populations in the human skin microbiome associated with acne. *J Invest Dermatol*. 2013;133:2295-2297.
9. Meisel JS, Hannigan GD, Tyldsley AS, et al. Skin microbiome surveys are strongly influenced by experimental design. *J Invest Dermatol*. 2016;136:947-956.
10. Fadrosh DW, Ma B, Gajer P, et al. An improved dual-indexing approach for multiplexed 16S rRNA gene sequencing on the Illumina MiSeq platform. *Microbiome*. 2014;2:6.
11. Lee YH, Liu G, Thiboutot DM, Leslie DL, Kirby JS. A retrospective analysis of the duration of oral antibiotic therapy for the treatment of acne among adolescents: investigating practice gaps and potential cost-savings. *J Am Acad Dermatol*. 2014;71:70-76.
12. Oh J, Conlan S, Polley EC, Segre JA, Kong HH. Shifts in human skin and nares microbiota of healthy children and adults. *Genome Med*. 2012;4:77.
13. Akaza N, Akamatsu H, Numata S, et al. Microorganisms inhabiting follicular contents of facial acne are not only *Propionibacterium* but also *Malassezia* spp. *J Dermatol*. 2016;43:906-911.
14. Wang Y, Kuo S, Shu M, et al. *Staphylococcus epidermidis* in the human skin microbiome mediates fermentation to inhibit the growth of *Propionibacterium acnes*: implications of probiotics in acne vulgaris. *Appl Microbiol Biotechnol*. 2014;98:411-424.
15. Kang D, Shi B, Erfe MC, Craft N, Li H. Vitamin B12 modulates the transcriptome of the skin microbiota in acne pathogenesis. *Sci Transl Med*. 2015;7:293ra103.
16. Leyden JJ. A review of the use of combination therapies for the treatment of acne vulgaris. *J Am Acad Dermatol*. 2003;49:S200-S210.

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