European Journal of Clinical Microbiology & Infectious Diseases (2019) 38:1677–1685
https://doi.org/10.1007/s10096-019-03598-9

ORIGINAL ARTICLE

Alterations in the skin microbiome are associated with disease severity and treatment in the perioral zone of the skin of infants with atopic dermatitis

Yumei Zheng[1] · Qian Wang[1] · Laiji Ma[2] · Yuanyuan Chen[2] · Ying Gao[3] · Gaolei Zhang[3] · Shumei Cui[1] · Haiyun Liang[1] · Congfen He[1] · Liya Song[1]

Received: 9 March 2019 / Accepted: 17 May 2019 / Published online: 31 May 2019

Abstract

Atopic dermatitis (AD), a chronic relapsing inflammatory pruritic skin disorder with a unique pathophysiology, has a high incidence in the perioral zone among infants. This study aimed to analyze the association of skin microfloral dynamics with disease severity and treatment of AD in 0–1-year-old infants. Based on the eczema area and severity index, subjects were divided into five groups, i.e., mild, moderate, severe, and severe post-treatment, with a healthy control group, and bacterial density at the perioral lesion, disease severity, and treatment were assessed in 0–1-year-old infants with AD. The perioral lesions were colonized predominantly by *Firmicutes*, followed in abundance by *Proteobacteria*, *Actinobacteria*, and *Bacteroidetes*. In the phylum Firmicutes, *Streptococcus* was the most predominant genus. In AD infants, the abundance of *Bacteroidetes* and *Fusobacterium* decreased significantly with an increase in disease severity ($p < 0.01$). The abundance of 6 genera, including *Prevotella*, decreased significantly with an increase in disease severity ($p < 0.05$). The abundance of *Prevotella melaninogenica* decreased gradually with an increase in disease severity and increased after treatment; this trend was reversed for *Corynebacterium simulans*. A reduction in the abundance of *Staphylococcus* and an increase in that of skin microflora including *Prevotella* spp., *Staphylococcus epidermidis*, and *Erwinia dispersa* were associated with treatment and clinical improvement. Skin bacterial composition varies with AD severity, and *Corynebacterium simulans* and *Prevotella melaninogenica* are positively and negatively correlated with AD severity, respectively. This study provides a theoretical basis to identify potential biomarkers AD occurrence and pathogenesis.

Keywords Atopic dermatitis · Perioral skin · Bacterial diversity · *Prevotella melaninogenica*

Introduction

Atopic dermatitis (AD, OMIM 603165) is a chronic, relapsing, and intensely pruritic inflammatory skin disorder. In Europe, 10 to 20% of children and teenagers are affected by AD. Approximately 50% of patients develop this disease during first year of life [1], causing grave inconvenience among the families of infected individuals. More than 50% of children with moderate-to-severe AD develop allergic rhinitis and/or asthma and atopic disorders associated with significant morbidity and sometimes death [2]. Numerous factors contribute to AD pathogenesis [3], including genetic factors, immune factors, skin barrier factors, and infection factors. AD patients experience frequent cutaneous infections, and *Staphylococcus aureus* has been frequently isolated from lesional and non-lesional skin of AD patients [4]. Downregulation of antimicrobial peptides in the skin of AD patients may further increase the susceptibility to AD [5]. While individual microbes that cause common AD-related skin infections have been identified, individual microbes often work by influencing larger bacterial communities [6, 7].

Yumei Zheng and Qian Wang contributed equally to this work.

✉ Liya Song
songly@th.btbu.edu.cn

[1] Beijing Key Lab of Plant Resources Research and Development, Beijing Technology and Business University, No. 11 Fucheng Road, Haidian District, Beijing 100048, China

[2] Shanghai Jahwa Corporation, Qingpu District, Shanghai, China

[3] Capital Institute of Pediatrics, Chaoyang District, Beijing, China

High-throughput DNA sequencing of the bacterial 16S rRNA gene revealed the presence of more diverse bacteria on healthy human skin than that revealed through culture-based methods [8]. Skin topography and microenvironment are prominent determinants of microbial community structure at particular skin sites [8]. Recently, the assessment of damaged regions including the cheeks and elbows revealed significant differences of skin microbiome between AD patients and healthy individuals, with a significant elevation in the abundance of *Staphylococcus aureus* at the lesions and a significant reduction in microbes including *Propionibacterium acnes* [9–11]. The perioral zone is a frequent site of AD occurrence in infants and young children. And continuous salivary stimulation aggravates AD and causes relapse. The perioral region of infants has the following unique physiological characteristics: (1) it is slobbery, with high humidity in the perioral areas and thus promoting microbial growth in the oral cavity; (2) it often retains breast milk, milk, fruit, and other food residues, which also influence microbial composition at the perioral site. These characteristics revealed the close associations between perioral and oral microbial compositions in infants. To understand the association between microbes and disease severity and treatment in perioral zone, this study assessed the skin bacterial diversity in the perioral region of 0–1-year-old infants with or without AD. This study showed the different disease severities and treatments are associated with microbial dynamics in this common skin disorder.

Materials and methods

Materials

Sodium chloride (NaCl) and Tween 20 were purchased from Sinopharm Chemical Reagent Co. Ltd., Beijing, China; lysozyme, Bailingwei Chemical Technology Co., Ltd., Beijing, China; the DNeasy Blood and Tissue kit, QIAGEN, Germany. Other materials used were disposable sterile cotton swabs (Jiangsu Changfeng Medical Industry Co. Ltd., Jiangsu, China), 0.5-mm glass beads (Shanghai Baili Biotechnology Co. Ltd., Shanghai, China), and Jinger FuLe repairing ointment and Mometasone Furoate Cream (the Capital Institute of Pediatrics).

Subjects and inclusion criteria

To investigate the role of bacterial communities in AD, we obtained skin microfloral specimens from 48 children with mild-to-moderate-to-severe AD and from 20 healthy controls recruited from the Capital Academy of Pediatrics (Beijing, China). This study has been approved by the Institutional Review Board (IRB) of the Capital Institute of Pediatrics, and written informed consent was obtained from the parents/guardians for the participation of infants.

The subjects were 0–1-year-old infants, who had not received any topical antibiotics, hormones, any oral antibiotics, or antibacterial drugs within 1 month. All healthy subjects were examined by a physician and declared clear of any skin problems. AD severity was determined using the eczema area and severity index (EASI) local rating method, with classification carried out in accordance with local areas marked with localized and widespread disease [12]. Subjects with an AD score less than 4 were segregated into the mild group; 4–8, moderate group; greater than 8, severe group. In total, 68 subjects were segregated into four groups on the basis of EASI (Table 1): healthy control group (H, $n = 20$), mild group (ML, $n = 13$), moderate group (MD, $n = 15$), severe group (S, $n = 20$). Infants with severe AD were treated for 7–10 days with Jinger FuLe repairing ointment (many times a day) and Mometasone Furoate Cream (once a day) from the Capital Institute of Pediatrics, and constituted the post-treatment group (SPT, $n = 17$).

Specimen collection

Skin microfloral specimens were acquired from $4 \times 2\text{-cm}^2$ areas of skin on both perioral skins of each subject, by swabbing the skin 25 times with sterile cotton swabs soaked in sterile wetting solution (0.9% NaCl and 0.1% Tween 20). The cotton swabs were rotated on the skin for approximately

Table 1 Baseline characteristics of the study population

Groups	Sex:girl/total	Mode of delivery: cesarean delivery/total	Family history of allergy (parental asthma or rhinitis or AD)	Respiratory infection	Use an emollient	Wash face more than twice a day
H	14/20	4/20	3/20	0/20	10/20	5/20
ML	6/13	4/13	4/13	0/13	8/13	2/13
MD	10/15	6/15	1/15	0/15	13/15	1/15
S	6/20	6/20	10/20	0/20	8/20	3/20
SPT	6/17	5/17	8/17	0/17	8/17	3/17

AD, atopic dermatitis; *H*, healthy controls; *ML*, mild group; *MD*, medium group; *S*, severe group; *SPT*, post-treatment group

15 s and placed in collection tubes. To minimize cross-contamination, fresh sterile gloves were used for each specimen. The specimens were frozen in dry ice and stored at − 80 °C. DNA extraction was performed as soon as possible.

DNA extraction and PCR amplification

Microbial DNA was extracted from the skin samples using the DNeasy® Blood and Tissue kit (QIAGEN, Germany.) according to the manufacturer's protocol (www.qiagen.com/handbooks). The final DNA concentration and purity were determined by NanoDrop 2000 UV-Vis spectrophotometer (Thermo Fisher Scientific, Wilmington, DE), and DNA quality was checked by 1% agarose gel electrophoresis. The V1-V2 hyper-variable region of the bacterial 16S rRNA gene was amplified with primers 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 338R (5′-TGCTGCCTCCCGTAGGAGT-3′) using a thermocycler (GeneAmp 9700, ABI). PCR reactions were conducted using the following program: 3 min of denaturation at 95 °C followed by 27 cycles of 30 s at 95 °C, 30 s for annealing at 55 °C, 45 s of elongation at 72 °C, and a final extension of 10 min at 72 °C. PCR reactions were performed in triplicate using 20 μL reaction mixture containing 4 μL of 5 × FastPfu Buffer, 2 μL of 2.5 mM dNTPs, 0.8 μL of each primer (5 μM), 0.4 μL of FastPfu Polymerase, and 10 ng of template DNA. The resultant PCR products were extracted from a 2% agarose gel, further purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA), and quantified using QuantiFluor™-ST (Promega, USA) according to the manufacturer's protocol.

Illumina MiSeq sequencing

Purified amplicons were pooled in equimolar quantities and paired-end sequenced (2 × 300) on an Illumina MiSeq platform (Illumina, San Diego, CA) according to the standard protocols by Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China).

Processing of sequencing data

Raw FAST files were demultiplexed, quality-filtered by Trimmomatic, and merged by FLASH with the following criteria: (i) reads were truncated at any site receiving an average quality score < 20 over a 50-bp sliding window, (ii) primers were exactly matched allowing at the most 2 nucleotide mismatch and reads containing ambiguous bases were removed, and (iii) sequences that overlapped longer than 10 bp were merged accordingly. Operational taxonomic units (OTUs) were clustered with 97% similarity cutoff using UPARSE (version 7.1 http://drive5.com/uparse/), and chimeric sequences were identified and removed using UCHIME. The taxonomy of each 16S rRNA gene sequence was analyzed by RDP Classifier algorithm (http://rdp.cme.msu.edu/) against the Greengene (Release 13.5 http://greengenes.secondgenome.com/) 16S rRNA database using confidence threshold of 70%.

The raw data from high-throughput sequencing were collated and filtered, and the validated sequences were obtained for subsequent analysis. Approximately 3.5 million valid sequences were obtained from 85 perioral skin samples, ranging from 281 to 360 bp (average 330 bp). Samples were clustered and annotated at a similarity level of 97%. A total of 3588 operational taxonomic units (OTUs) were obtained, belonging to 31 phyla, 771 genera, and 996 species.

Statistical analysis

All data are presented as mean ± standard error, unless otherwise indicated. To analyze between-group differences, the non-parametric Wilcoxon rank-sum test was performed, while the Kruskal-Wallis H test was used for multiple group comparisons. The Simpson index and Chao index were used to determine alpha diversity. The Simpson diversity is often used in ecological studies to quantify the biodiversity of a region. The larger the Simpson index, the lower the community diversity. The Simpson index expresses the probability that individuals sampled twice consecutively from a community belong to the same species, which can well reflect species uniformity and diversity. The Chao index is often used to estimate the total number of species in ecological studies. Principal coordinate analysis (PCoA) is a non-binding data dimensionality reduction analysis method. The distance algorithm is weighted UniFrac, considering not only bacterial composition and abundance but also evolutionary relationships.

The data were analyzed using the free online Majorbio I-Sanger Cloud Platform (www.i-sanger.com).

Results

Characteristics of bacterial communities associated with AD

Rarefaction curves indicated that sampling provided sufficient coverage to analyze dominant members of the bacterial communities (data not shown). To investigate potential associations, we analyzed the association between AD severity and the Simpson diversity. Herein, the Simpson index increased with an increase in AD severity, being significantly greater in groups S (0.26 ± 0.22) and MD (0.22 ± 0.11) than in group H (0.13 ± 0.09, $p < 0.05$), indicating that the bacterial diversity in the perioral skin region in infants with moderate-to-severe AD was significantly reduced during AD progression (Fig. 1a). Furthermore, the assessment of overall bacterial richness

of infant perioral skin, revealed on the basis of the Chao index, indicated a reduction in the richness of bacterial communities in severe AD (H 491.59 ± 115.49 vs S 406.05 ± 164.39, $p < 0.05$, Fig. 1b).

Differences between skin microfloral specimens between the healthy control and the severe AD group were assessed via PCoA. PCoA revealed differences in bacterial composition and abundance between the H and S groups (Fig. 1c), with a more compact distribution in specimens obtained from healthy controls, indicating greater similarities in bacterial composition and abundance within the group. Among them, 8 specimens from group S were distributed in the same area as in group H. Hence, questionnaire survey items were compared. Six of these infants had a family history of allergies, which was speculated to further affect bacterial abundance and composition in group S.

Taxonomic analysis of community compositions

To determine differences among bacterial communities at different disease severities at a taxonomic level, we classified bacteria on the basis of their phyla, genera, and species in each group. Before investigating changes in microbiota associated with a disease state, it is important to first establish a baseline and determine normal variations in the microbiota of healthy individuals. Therefore, we first analyzed the community compositions in group H. Perioral sites in group H were dominated by *Firmicutes* (61.39%), *Proteobacteria* (14.38%), *Actinomycetes* (13.26%), and *Bacteroides* (9.20%), accounting for more than 98% (Fig. 2a). In contrast with the healthy group, *Bacteroidetes* displayed a lower abundance in AD group ($p = 0.001$). The Kruskal-Wallis H test revealed that the abundance of *Bacteroidetes* was significantly different among the four groups ($p < 0.01$), and the relative abundance

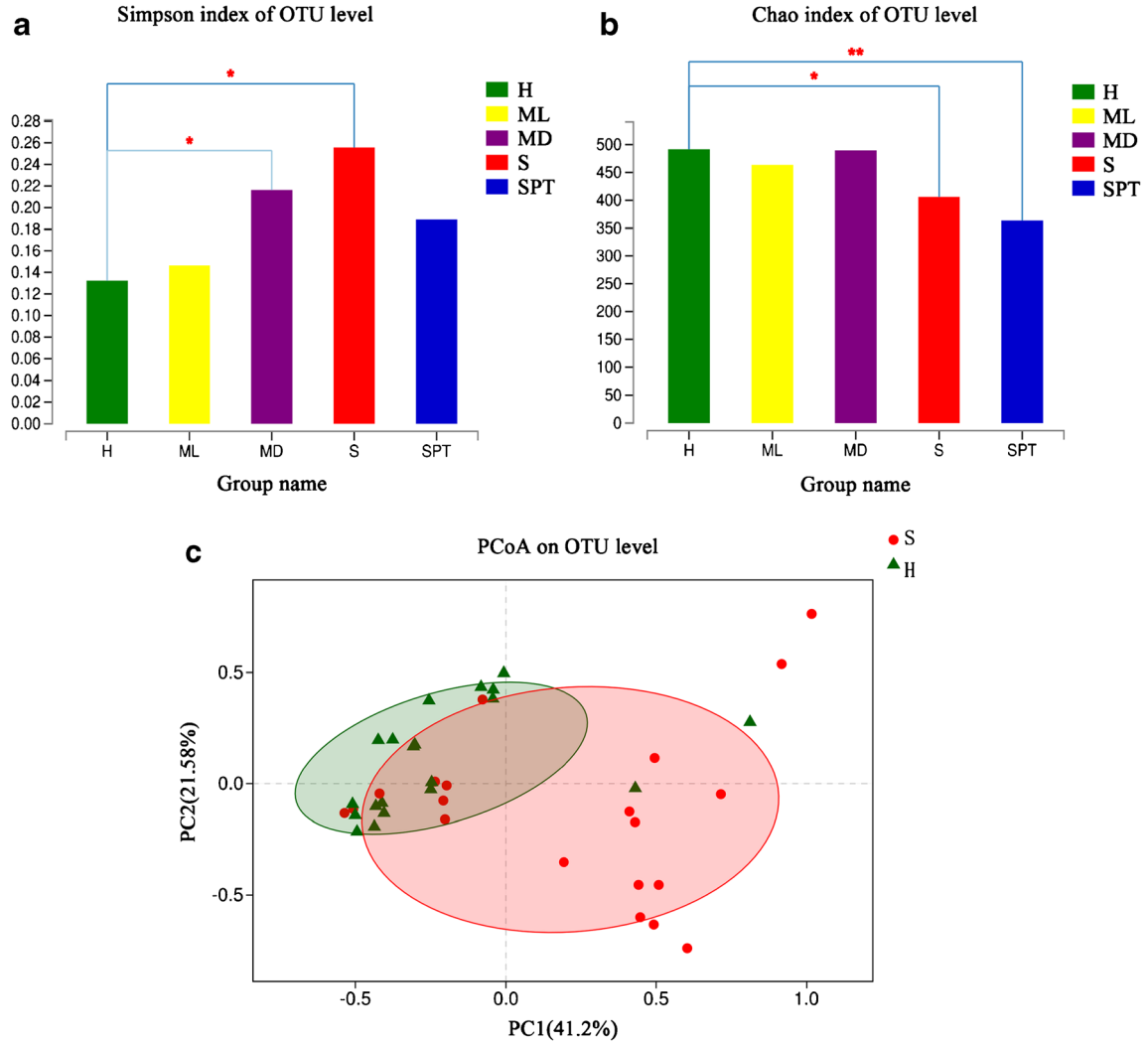

Fig. 1 Association between diversity index and atopic dermatitis (AD) severity. **a** Histogram of the average Simpson index at the level of operational taxonomic unit (OTU) level. **b** Histogram of the average Chao index at the OTU level. **c** Principal coordinate analysis (PCoA) profiles at the OTU level in healthy and severe AD groups. Green: H group (healthy controls); yellow: ML group (mild AD); purple: MD group (moderate AD); red: S group (severe AD); blue: SPT group (post-treatment severe AD). * $0.01 < p < 0.05$; ** $0.001 < p < 0.01$

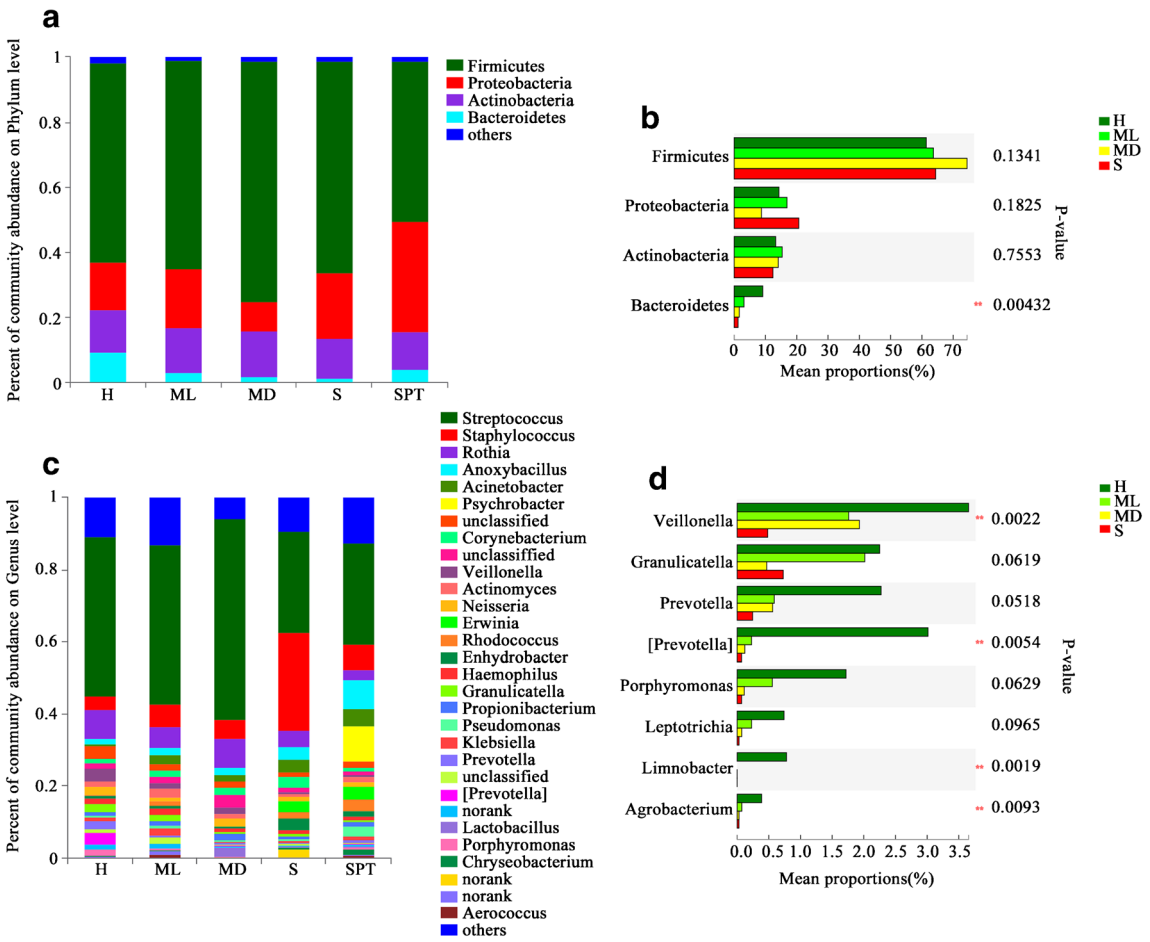

Fig. 2 Community composition and variation among different atopic dermatitis (AD) states. **a** Histogram of relative abundance of community compositions for every group at the phylum level. **b** Analysis of phylum differences across four groups revealed via the Kruskal-Wallis H test. **c** Histogram of the relative abundance of community compositions for every group at the genus level. **d** Differential analysis of genera varying with disease severity across four groups revealed through the Kruskal-Wallis H test. The abundance of all classes was greater than 1%

decreased significantly with an increase in disease severity (Fig. 2b). Furthermore, the abundance of *Fusobacterium* also decreased significantly ($p = 0.004$) with an increase in disease severity (data not shown).

Analysis of the major microbial populations at the genus level revealed a predominance of *Streptococcus*, which accounted for up to 44% of the total skin bacteria in H group, with over 26 genera constituting the remaining populations. Thus, 25 genera with predominance > 1% constitute 80–85% of the total population on average (Fig. 2c). Perioral sites in healthy infants were dominated by *Streptococcus* (44.05%), *Rothia* (7.92%), *Staphylococcus* (3.42%), *Veillonella* (3.61%), *Prevotella* (3.11%), *Neisseria* (2.38%), *Prevotella* (2.23%), *Granulicatella* (2.22%), *Porphyromonas* (1.72%), *Haemophilus* (1.60%), *Anoxybacillus* (1.51%), *Actinomyces* (1.44%), *Corynebacterium* (1.32%), and others (23%, including unnamed genera). Compared to H group, the abundance of *Staphylococcus* increased in AD, the increase being significant in the severe group ($p = 0.003$), whereas the abundance of *Streptococcus* decreased in the severe group ($p = 0.026$). An increase in the abundance of some genera, including *Anoxybacillus*, *Acinetobacter*, *Corynebacterium*, and *Erwinia*, was accompanied by a reduction in the abundance of some genera, including *Veillonella*, *Prevotella*, [*Prevotella*], *Granulicatella*, and *Porphyromonas* (Fig. 2d). Furthermore, some genera with an abundance less than 1% were also detected, primarily *Pseudomonas*, *Micrococcus*, and *Paracoccus*.

To further determine the potential pathogenic bacteria and the unbalanced bacterial flora in infants with AD, we analyzed the differences in bacteria between H group and S group (Table 2). Fifteen different bacterial genera were screened out. Besides *Staphylococcus*, the abundance of *Rhodococcus*, *Alloiococcus*, and *Tepidimonas* in group S was significantly higher ($p < 0.05$) than that in group H. While the predominance of *Streptococcus* and *Rothia* was significantly decreased ($p < 0.05$). The abundance of the less predominant genera (< 5%) including *Prevotella*,

Table 2 Different genera of average relative abundance ≥ 0.1% in H and S groups

Species name	H (%)	ML (%)	MD (%)	S (%)	*p* value (H&S)	SPT (%)
Streptococcus	44.07 ± 17.37	41.69 ± 18.44	56.35 ± 19.89	28.6 ± 23.64*	0.0256	28.25 ± 27.68
Staphylococcus	3.83 ± 5.71	7.97 ± 18.46	5.22 ± 5.92	26.01 ± 29.56**	0.0031	7.21 ± 10.19
Rothia	8.09 ± 8.11	6.66 ± 8.16	7.94 ± 7.21	4.31 ± 6.40*	0.0294	2.76 ± 6.30
Veillonella	3.66 ± 3.30	1.76 ± 1.61	1.93 ± 2.38	0.48 ± 0.79***	0.0007	0.55 ± 0.96
[Prevotella]	3.01 ± 4.56	0.23 ± 0.43	0.12 ± 0.22	0.07 ± 0.21**	0.0021	0.19 ± 0.47
Granulicatella	2.25 ± 2.27	2.02 ± 1.84	0.47 ± 0.87	0.73 ± 1.38*	0.0466	0.52 ± 1.13
Prevotella	2.27 ± 3.64	0.59 ± 1.05	0.56 ± 1.37	0.25 ± 0.48*	0.0121	0.95 ± 3.61
Rhodococcus	0.15 ± 0.35	1.43 ± 2.36	0.00 ± 0.01	1.96 ± 3.12**	0.0030	3.25 ± 5.79
Porphyromonas	1.72 ± 3.23	0.56 ± 1.07	0.11 ± 0.27	0.07 ± 0.12*	0.0334	0.54 ± 2.02
Limnobacter	0.78 ± 2.40	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00*	0.0140	0.00 ± 0.00
Leptotrichia	0.74 ± 1.62	0.23 ± 0.40	0.07 ± 0.21	0.03 ± 0.05*	0.0173	0.02 ± 0.02
Agrobacterium	0.39 ± 0.92	0.07 ± 0.09	0.03 ± 0.04	0.03 ± 0.04**	0.0040	0.08 ± 0.08
Alloiococcus	0.03 ± 0.04	0.22 ± 0.53	0.05 ± 0.14	0.29 ± 0.82*	0.0263	0.13 ± 0.30
Atopobium	0.22 ± 0.66	0.07 ± 0.15	0.21 ± 0.40	0.07 ± 0.20*	0.0101	0.16 ± 0.48
Tepidimonas	0.00 ± 0.00	0.05 ± 0.10	0.00 ± 0.00	0.16 ± 0.33***	0.0000	0.25 ± 0.46

AD, atopic dermatitis; *H*, healthy controls; *ML*, mild group; *MD*, medium group; *S*, severe group; *SPT*, post-treatment group. All *p* values were calculated using the two-sided Wilcoxon rank-sum test. *$p < 0.05$; **$p < 0.01$;***$p < 0.001$. All data are represented as mean ± standard error values

Porphyromonas, *Limnobacter*, *Leptotrichia*, and *Agrobacterium* ($p_{(H\&S)} < 0.05$) also decreased. In addition, *Limnobacter* was detected only in specimens from group H.

Nine species displaying an abundance of more than 1% were detected in four groups. Group H contained *Rothia mucilaginosa* (7.64%), *Prevotella melaninogenica* (2.04%), *Staphylococcus haemolyticus* (2.01%), *Anoxybacillus kestanbolensis* (1.51%), and *Staphylococcus epidermidis* (1.21%). The abundance of *Anoxybacillus kestanbolensis* was increased slightly but not significantly with disease severity, whereas the opposite was true for *Prevotella melaninogenica* ($p = 0.034$). The abundance of *Staphylococcus epidermis* in the ML group was not different from that in the H group; however, it slightly increased in the MD and S groups (H 1.179 ± 1.036%, ML 1.045 ± 2.004%, MD 2.248 ± 2.601%, S 3.15 ± 4.977%). As expected, *Staphylococcus aureus* dominated group S. Significant differences in bacterial communities at the species level between group H and group S are shown in Table 3.

Effects of drug interventions on the bacterial communities in AD

In this study, clinical symptoms in 17 cases of severe AD improved after 7–10 days of treatment, while 3 were lost to follow-up. After drug interventions, the bacterial diversity in severe AD group began to increase slightly toward the level of healthy group. However, it did not differ significantly after treatment (Fig. 1a). Bacterial richness also did not differ significantly (Fig. 1b). Taxonomic composition analysis revealed that the abundance of *Bacteroidetes* increased slightly after treatment, more similar to that in group H (S 1.26 ± 1.52%, SPT 4.21 ± 7.42%, H 9.14 ± 9.34%; Fig. 2a). In the previous results, the abundance of *Bacteroidetes* was significantly different between H and S groups. Furthermore, we compared the changes in perioral skin bacteria before and after treatment of severe AD at the genus level (Fig. 2c). No significant difference was observed in most bacterial communities, except *Staphylococcus*, *Brevibacillus*, *Bacillus*, and *Peptoniphilus* (Fig. 3a). The significant reduction after treatment revealed that normal levels of *Staphylococcus* may be associated with disease recovery (S 26.01 ± 29.56%, SPT 7.21 ± 10.19%, H 3.83 ± 5.71%, $p_{(S\&SPT)} = 0.04$). Eight genera whose abundance was significantly different between the S and H groups were changing with the trend of disease recovery (Table 2). Furthermore, the predominant skin bacterium that was changing with the trend of disease recovery was observed in Fig. 3b. The abundance of more than 50 genera including *Anoxybacillus*, *Psychrobacter*, *Veillonella*, *Actinomyces*, *Prevotella*, *[Prevotella]*, *Propionibacterium*, *Klebsiella*, *Porphyromonas,* and *Micrococcus* was increasing after treatment.

Comparison of species-level differences between groups S and SPT revealed a reduction in the abundance of *Staphylococcus* primarily owing to the reduction in *Staphylococcus aureus* (S: 21.70 ± 31.17%, SPT: 1.51 ± 2.50%, H: 0.36 ± 1.27%). The abundance of *Staphylococcus epidermidis*, *Anoxybacillus kestanbolensis, Prevotella melaninogenica* increased slightly but not significantly after treatment (Table 3). Furthermore, *Erwinia dispersa*, which displayed no prominent in group S, displayed a significant increase in abundance after treatment (SPT: 2.06 ± 7.70%, S: 0.05 ± 0.15%, $p = 0.012$).

Table 3 Different species of average relative abundance ≥ 0.1% in H and S groups

Species name	H (%)	ML (%)	MD (%)	S (%)	*p* value (H&S)	SPT (%)
Staphylococcus aureus	0.36 ± 1.27	6.20 ± 18.14	2.09 ± 3.73	21.70 ± 31.17	0.00001***	1.51 ± 2.50
Rothia mucilaginosa	7.84 ± 8.16	6.63 ± 8.18	7.78 ± 7.29	4.28 ± 6.40	0.0337*	2.68 ± 6.04
Prevotella melaninogenica	2.08 ± 3.56	0.51 ± 0.98	0.53 ± 1.36	0.18 ± 0.45	0.0302*	0.92 ± 3.61
Prevotella nanceiensis	0.14 ± 0.26	0.03 ± 0.09	0.01 ± 0.03	0.02 ± 0.10	0.0392*	0.00 ± 0.01
Corynebacterium simulans	0.03 ± 0.11	0.02 ± 0.05	0.37 ± 1.05	0.56 ± 2.24	0.0177*	0.02 ± 0.04
Sphingomonas yabuuchiae	0.05 ± 0.11	0.09 ± 0.12	0.01 ± 0.03	0.19 ± 0.39	0.0336*	0.29 ± 0.39

AD, atopic dermatitis; *H*, healthy controls; *ML*, mild group; *MD*, medium group; *S*, severe group; *SPT*, post-treatment group. All *p* values were calculated using the two-sided Wilcoxon rank-sum test. *$p < 0.05$; ***$p < 0.001$. All data are represented as mean ± standard error values. *Corynebacterium simulans* is positively correlated with the severity of AD, while *Prevotella melaninogenica* is negatively correlated with the severity of ADs

Discussion

The perioral zone is a frequent site of AD occurrence in infants and young children. In the present study, 0–1-year-old infants with or without AD were segregated into five groups, and the bacterial density at the perioral site was determined at different disease severity and upon treatment. We explored the baseline levels of microbiota of healthy individuals. In contrast with adult skin predominated by *Proteobacteria*, *Actinobacteria*, and *Firmicutes* [8], infants were colonized predominantly by *Firmicutes*, followed in abundance by *Proteobacteria*, *Actinobacteria,* and *Bacteroidetes*. *Streptococcus* was detected as the most predominant genera in the perioral area of infants, followed by *Rothia.* While the abundance of other genera was not different from that at other sites [13].

Consistent with previous reports [2], the overall skin bacterial richness and diversity of AD infants decreased with an increase in disease severity, which was associated with the marked increase in the abundance of *Staphylococcus,* primarily *Staphylococcus aureus*. Interestingly, the abundance of *Veillonella*, *[Prevotella]*, *Prevotella* (*Prevotella melaninogenica*), *Porphyromonas*, *Leptotrichia*, and *Agrobacterium* gradually decreased with an increase in disease severity and increased after treatment, while the reverse is true for *Corynebacterium simulans*. In addition to *S. aureus*, *C. simulans* can also be considered an important source of infection in AD. These results suggest that these communities may serve as potential biomarkers to help determine the disease severity; however, further studies with larger sample sizes are required to further validate these findings. *Limnobacter* only existed on perioral skin in healthy infant, while *Tepidimonas* was present only in infants with AD. The obliteration of some bacteria was accompanied by the appearance of some other bacteria, which may be an important indicator of AD occurrence and may also be an important source of infection; this warrants further verification.

In contrast with previous reports of the increase in the abundance of *Staphylococcus* being positively correlated with an increase in AD severity, *Staphylococcus* abundance in the moderate group was not higher than that in the mild group, probably resulting from antagonism between *Streptococcus* and *Staphylococcus*. The abundance of *Streptococcus* was reportedly associated with AD [14], being the highest in the moderate group in the present study. Variation in the relative abundance of *Staphylococcus* and *Rhodococcus*, that was associated with immunodeficiency diseases [15], was accompanied by an increase and reduction in the abundance of other bacteria such as *Streptococcus* and *Atopobium*. The association between such bacteria and AD pathogenesis cannot be explained probably because of synergistic and antagonistic effects among the flora. This suggested that the presence of specific microorganisms may be just as important as the absence of others.

After 7 to 10 days of treatment, the EASI of all infants improved, and the bacterial diversity increased slightly but not significantly; however, bacterial richness remained unchanged. Concurrent with a previous report [16], clinical improvement was reportedly associated with a reduction in the abundance of *S. aureus.* Moreover, the increase in the abundance of *A. kestanbolensis* and *S. epidermidis* may play an important role in restoring skin microflora by antagonizing other potential pathogenic bacteria [17]. The reduction in the abundance of pathogenic bacteria, such as *S. aureus*, would further promote the colonization of other bacteria, e.g., the abundance of *Erwinia dispersa* increased from 0.05 to 2.06%. However, the specific interactions need to be further verified in vitro in future studies. Skin microflora including *Actinomyces*, *Propionibacterium* [2], *Porphyromonas*, and *Klebsiella* also increased gradually, indicating that the recovery of skin diseases was accompanied by the recovery of skin microflora.

In this study, reduction in richness of bacteria after treatment may be associated with the bactericidal effects of drug intervention. The primary component of Mometasone Furoate Cream is mometasone furoate. It has been reported in the literature that there were antibacterial activities of mometasone furoate against *Streptococcus pneumoniae*, *Streptococcus viridans*, and *S. aureus* in vitro [18]. In this

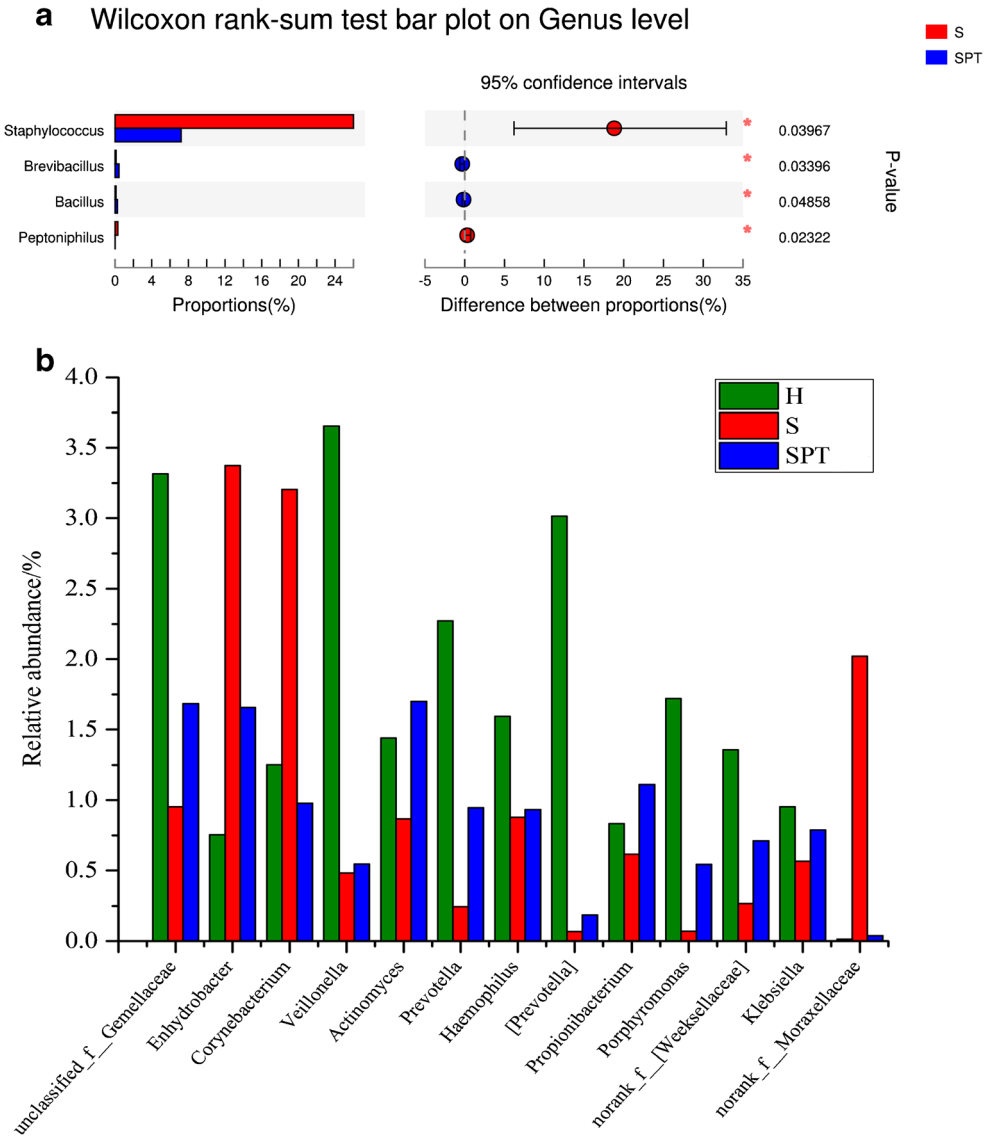

Fig. 3 Effects of drug interventions on bacterial communities in atopic dermatitis (AD). **a** The Wilcoxon rank-sum test bar plot of different genera between the severe group (red, S) and the post-treatment group (blue, SPT); the abundance of all genera was greater than 0.1%. **b** Histogram of the relative abundance of genera with a tendency to recover; abundance of all genera was greater than 1%.*$0.01 < p < 0.05$

study, the decrease of *S. aureus* may be related to the antibacterial effect of drug intervention. However, no significant change was observed in *Streptococcus* before and after treatment. Perhaps, the treatment duration was too short; hence, not many species displayed a significant variation after treatment. After AD treatment, the abundance of some bacterial communities increased, such as *Brevibacillus*, probably owing to better nutrition provided by the emollient (Jinger FuLe repairing ointment) for growth of the bacterial community. From the microbiological perspective, the treatment of AD does not merely involve blind sterilization, but rather restoration of the normal balance of skin flora.

Recent studies have reported that AD is also correlated with intestinal flora. Since a low abundance of *Lactobacillus* and *Bifidobacteria* or the lack of species-specific *Bifidobacteria* is considered to be strongly associated with AD, AD may be prevented by increasing the abundance of these intestinal bacteria [19–21]. Similarly, the recovery of AD may also be related to skin microbiome. Manipulation aiming at an increase in the number of selected, potentially beneficial microorganisms using special nutrients (prebiotics) or even living microorganisms (probiotics) has presented a novel avenue. Emollients are the first-line treatment for AD, and supplementation of probiotics seems to enhance the therapeutic effect [22–26]. The creams containing a 5% lysate of the non-pathogenic bacterium *Vitreoscilla filiformis* can markedly improve the patient SCORAD index and pruritus [27]. The present study provides a theoretical basis for the development of probiotics and microecological preparations related to AD treatment.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This study has been approved by the Institutional Review Board (IRB) of the Capital Academy of Pediatrics.

Informed consent The written informed consent was obtained from the guardians for the participation of infants and to acquire photographs. Before inclusion in the study, the investigator provided the volunteers with all the information about the study. When the parents/guardians of infants give them consent, them and the investigator signed and dated the consent form.

References

1. Kanchongkittiphon W, Gaffin JM, Phipatanakul W (2015) Child with atopic dermatitis [J]. Annals of Allergy Asthma & Immunology Official Publication of the American College of Allergy Asthma & Immunology 114(1):6–11
2. Kong HH, Oh J, Deming C et al (2012) Temporal shifts in the skin microbiome associated with disease flares and treatment in children with atopic dermatitis [J]. Genome Res 22(5):850–859
3. Leung DY, Boguniewicz M, Howell MD et al (2004) New insights into atopic dermatitis [J]. J Clin Investig 113(5):651–657
4. Leyden JJ, Marples RR, Kligman AM (1974) Staphylococcus aureus in the lesions of atopic dermatitis [J]. Br J Dermatol 90(5):525–530
5. Ong PY, Ohtake T, Brandt C et al (2002) Endogenous antimicrobial peptides and skin infections in atopic dermatitis [J]. N Engl J Med 347(15):1151–1160
6. Dybboe R, Bandier J, Skov L et al (2017) The role of the skin microbiome in atopic dermatitis: a systematic review [J]. Br J Dermatol 177(5):1272
7. Byrd Allyson L, Belkaid Yasmine, Segre Julia A (2018) The human skin microbiome [J]. Nat Rev Microbiol 16:143–155
8. Grice EA, Kong HH, Conlan S et al (2009) Topographical and temporal diversity of the human skin microbiome [J]. Science 324(5931):1190–1192
9. Cundell Anthony M (2018) Microbial ecology of the human skin [J]. Microb Ecol 76: 113–120
10. Dekio I, Sakamoto M, Hayashi H et al (2007) Characterization of skin microbiota in patients with atopic dermatitis and in normal subjects using 16S rRNA gene-based comprehensive analysis [J]. J Med Microbiol 56(12):1675–1683
11. Byrd AL, Deming C, Skb C et al (2017) Staphylococcus aureus and Staphylococcus epidermidis strain diversity underlying pediatric atopic dermatitis [J]. Sci Transl Med 9(397)
12. Zhao Bian (2004) Evaluation of eczema area and severity index [J]. Chinese journal of dermatology 37(1):3–4. (in Chinese)
13. Capone KA, Dowd SE, Stamatas GN et al (2011) Diversity of the human skin microbiome early in life [J]. J Investig Dermatol 131(10):2026–2032
14. Shi B, Bangayan NJ, Curd E et al (2016) The skin microbiome is different in pediatric versus adult atopic dermatitis [J]. J Allergy Clin Immunol 138(4):1233–1236
15. Hua G, Guo J (2003) Progress in the study on the classification and application of *Rhodococcus* [J]. Bulletin of microbiology 30(4): 107–111
16. Thomas Charlotte L, Fernández-Peñas P (2017) The microbiome and atopic eczema: more than skin deep [J]. Australas J Dermatol 58(1):18–24
17. Iwase T, Uehara Y, Shinji H et al (2010) Staphylococcus epidermidis Esp inhibits Staphylococcus aureus biofilm formation and nasal colonization [J]. Nature 465(7296):346–349
18. Castañeda SS, Liu PLA, Agbay RLMC (2010) In vitro antibacterial activity of mometasone furoate, fluticasone propionate and fluticasone furoate nasal preparations against Streptococcus pneumoniae, Haemophilus influenzae, Streptococcus viridans, Staphylococcus aureus, Pseudomonas aeruginosa, and Escherichia coli [J]. Philippine Society of Otolaryngology Head & Neck Surgery Inc
19. Kalliomäki M, Salminen S, Arvilommi H et al (2001) Probiotics in primary prevention of atopic disease: a randomised placebo-controlled trial [J]. Lancet 357:1076–1079
20. Kalliomäki M, Salminen S, Poussa T et al (2003) Probiotics and prevention of atopic disease: 4-year follow-up of a randomised placebo-controlled trial [J]. Lancet 361(9372):1869–1871
21. Isolauri E, Arvola T, Sütas Y et al (2010) Probiotics in the management of atopic eczema [J]. Clin Exp Allergy 30(11):1605–1610
22. Drago L, De Vecchi E, Toscano M et al (2014) Treatment of atopic dermatitis eczema with a high concentration of Lactobacillus salivarius LS01 associated with an innovative gelling complex: a pilot study on adults [J] .J Clin Gastroenterol 48 Suppl 1: S47–S51
23. Glatz M, Jo JH, Kennedy EA et al (2018) Emollient use alters skin barrier and microbes in infants at risk for developing atopic dermatitis [J]. PLoS One 13(2):e0192443
24. Catherine Mack Correa M, Nebus J (2012) Management of patients with atopic dermatitis: the role of emollient therapy [J]. Dermatol Res Pract 2012:836931
25. Haahtela T (2014) What is needed for allergic children? [J]. Pediatric Allergy & Immunology 25(1):21–24
26. Hon KL, Pong NH, Wang SS et al (2013) Acceptability and efficacy of an emollient containing ceramide-precursor lipids and moisturizing factors for atopic dermatitis in pediatric patients [J]. Drugs in R & D 13(1):37–42
27. Gueniche A, Knaudt B, Schuck E et al (2010) Effects of nonpathogenic gram-negative bacterium Vitreoscilla filiformis lysate on atopic dermatitis: a prospective, randomized, double-blind, placebo-controlled clinical study [J]. Br J Dermatol 159(6):1357–1363