Dynamics of the nasal microbiota in infancy: A prospective cohort study

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Background: Understanding the composition and dynamics of the upper respiratory tract microbiota in healthy infants is a prerequisite to investigate the role of the microbiota in patients with respiratory diseases. This is especially true in early life, when the immune system is in development.
Objective: We sought to describe the dynamics of the upper respiratory tract microbiota in healthy infants within the first year of life.
Methods: After exclusion of low-quality samples, microbiota characterization was performed by using 16S rDNA pyrosequencing of 872 nasal swabs collected biweekly from 47 unselected infants.
Results: Bacterial density increased and diversity decreased within the first year of life (R^2 = 0.95 and 0.73, respectively). A distinct profile for the first 3 months of life was found with increased relative abundances of Staphlyococcaceae and Corynebacteriaceae (exponential decay: R^2 = 0.94 and 0.96, respectively). In addition, relative bacterial abundance and composition differed significantly from summer to winter months. The individual composition of the microbiota changed with increasing time intervals between samples and was best modeled by an exponential function (R^2 = 0.97). Within-subject dissimilarity in a 2-week time interval was consistently lower than that between subjects, indicating a personalized microbiota.
Conclusion: This study reveals age and seasonality as major factors driving the composition of the nasal microbiota within the first year of life. A subject's microbiota is personalized but dynamic throughout the first year. These data are indispensable to interpretation of cross-sectional studies and investigation of the role of the microbiota in both healthy subjects and patients with respiratory diseases. They might also serve as a baseline for future intervention studies. (J Allergy Clin Immunol 2015;■■■:■■■-■■■.)

Key words: *Nasal microbiota, bacterial families, toddlers, cohort study, season, age*

The prevalence and incidence of asthma in children continue to increase, leading to efforts to curb the so-called epidemic, even while its causes remain ambiguous.[1] An association between bacterial colonization of the airways in children and later development of asthma has been suggested, particularly for the potentially pathogenic *Streptococcus pneumoniae*, *Moraxella catarrhalis*, and *Haemophilus influenzae*.[2] However, exposure to a wider range of microbes seems to have a protective effect on the development of asthma in children by activating the innate immune system.[3] This finding would support the hygiene hypothesis, according to which asthma is partly caused by a lack of microbial exposure early in life and its subsequent influence on the developing immune system.[4] Indeed, the presence of commensal bacteria was shown to be critical in the control of allergic airway inflammation in a mouse model.[5,6] Therefore it is assumed that the respiratory tract microbiota plays a crucial role in immune development early in life. In particular, the ages between early infancy and 3 years are suggested to be important for the long-term development of immune responses and asthmatic airway disease. Therefore the best possibility for intervention to change the natural trajectory of the disease exists within this window of time.[7]

In recent studies the respiratory tracts of healthy adult subjects were found to harbor a homogeneous microbiota that decreases in biomass from the upper to the lower respiratory tract. The bacteria present in the lungs were suggested to originate from the upper respiratory tract microbiota through microaspiration.[8,9] Therefore the upper respiratory tract is considered the entry point of commensals and potential pathogens into the airways. The nasopharynx was described as an ecologic reservoir consisting of a broad variety of commensal bacteria and potential pathogens.[10] Recent studies revealed a complex and highly variable nasopharyngeal microbiota in children,[11,12] which undergoes significant changes during disease, exposure to

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Abbreviations used
nMDS: Nonmetric multidimensional scaling
rRNA: Ribosomal RNA
SDI: Shannon Diversity Index

antimicrobials, and vaccination.[13,14] Despite these significant findings, a detailed description of the nasopharyngeal microbiota in healthy children was not previously available.

Previous studies assumed an association between the early composition of the upper airway microbiota as a marker for later immune development and subsequent development of allergy and asthma.[2] However, before any conclusions regarding the role of the microbiota on later asthma and other respiratory diseases can be drawn, this hypothesis must be tested in longitudinal measurements.

The aim of this study was to analyze biweekly serial dynamics of the nasal microbiota in healthy infants within the first year of life. In particular, we revealed the influence of age and seasonality on the personalized composition of the microbiota and investigated its dissimilarity throughout the first year of life.

METHODS

Study design

A total of 48 healthy infants from the prospective Basel Bern Infant Lung Development cohort (www.birthcohorts.net) study[15] were enrolled and followed weekly within their first year of life. Pregnant mothers were recruited from the 4 major maternity hospitals and practices of obstetricians in the agglomeration of Bern, Switzerland, through advertisements and interviews. Exclusion criteria for the study were as follows: ethnicity other than white, preterm delivery (<37 weeks' gestation), major birth defects, disease or later diagnosis of airway malformation, or specific chronic respiratory disease. The study was approved by the Ethics Committee of the Canton of Bern.

Nasal swab procedure

Nasal swabs (Verridial E. Mueller, Blonay, Switzerland) were collected biweekly by the parents of the study infants, starting in the fifth week of life. Parents were personally instructed by the study nurses on how to obtain the nasal samples in a standardized way. Immediately after acquisition, nasal swabs were sent in transport tubes and medium (UTM tubes, Verridial E. Muller) to the study center and frozen at −80°C within 10 days or less. In addition, study nurses made weekly telephone calls, during which study parents were interviewed by using a standardized questionnaire. The study infant's health status, as well as respiratory symptoms and antibiotic therapy, were assessed.[16] Nasal swabs taken during upper or lower respiratory tract infection or while the infant received antibiotic therapy were excluded from the analysis because the goal of the present study was to analyze swabs of clinically healthy infants at the time of sampling.

PCR amplification of 16S ribosomal RNA genes and 454 amplicon sequencing

Amplification by using PCR and amplicon sequencing was described elsewhere.[13] In brief, DNA was extracted with 200 μL of transport medium, followed by amplification of the bacterial 16S ribosomal RNA (rRNA) variable regions V3 to V5 by using the multiplex identifier tagged primer pair 341F/907R. PCR reactions were eluted by 40 μL of double-distilled water, and the concentration was measured with the Agilent 2100 Bioanalyzer (Agilent Technologies, Basel, Switzerland). PCR products with a concentration of less than 1.0 ng/μL were excluded from the study. This corresponds to less than 1 pg/μL bacterial DNA, as evaluated by using quantitative PCR of pneumococcal DNA at different dilutions (data not shown). A minimum of 1 pg/μL bacterial DNA was recently recommended as the threshold when working with low-density materials.[17] Of each purified PCR product, 40 ng/μL was pooled, whereas every multiplex identifier was used once, resulting in 8 amplicon pools.[13] The amplicon pools were sequenced with the 454 sequencing platform. The reads were submitted to the National Center for Biotechnology Information Sequence Read Archive (accession no. SRP041616). Analysis of sequencing products was performed by using PyroTagger, which comprises the definition of operational taxonomic units based on 97% sequencing identity, estimation of chimeras, and taxonomic assignments.[18]

Quality control

In addition to the exclusion of PCR products with a concentration of less than 1 ng/μL, we performed the following quality control steps. First, a cutoff value of 70 reads per sample was defined. Second, 2 negative control samples were sequenced, and those samples displaying greater than 5% sequence reads identical to these negative control samples were excluded.

Bacterial density and α diversity calculations

Bacterial density was estimated based on the concentration of the PCR product. α Diversity, which was referred to as within-community diversity,[19] was assessed by using the Shannon Diversity Index (SDI). The SDI was calculated in R, version 3.02 (http://www.R-project.org.), by using the function "diversity" of the "vegan" package. For analyses of bacterial density and SDI, samples were binned according to the date of acquisition, either referring to the month of the year or to the age of the study infant, resulting in 1 to 3 samples per infant for every month.

β Diversity analyses

β Diversity, which is referred to as community comparison,[19] was calculated by using the function "vegdist" of the "vegan" package in R. We used the Manhattan-type dissimilarity Jaccard to calculate the weighted beta diversity indices (abundance-based). For each study infant, Jaccard dissimilarity was calculated by means of pairwise comparison of 2 samples. Time intervals (Δt) between the samples were subsequently increased, ranging from 2 to 44 weeks. Values from each infant were binned according to the time interval between the samples, as previously described.[20] Short-term within-subject dissimilarity includes all pairwise comparisons of samples from a subject in 2-week intervals. Resulting values were grouped according to the month of age or season. Between-subject dissimilarity includes pairwise comparisons of all samples from 1 subject with all samples from all the other subjects within the corresponding month. "metaMDS" of the "vegan" package was used for nonmetric multidimensional scaling (nMDS), as previously described.[13] As an input matrix, we used the abundance-based Jaccard dissimilarities, as described above. Statistical analysis on nMDS clustering was done with the function "adonis" of the "vegan" package. Vectors were fitted by using "envfit": the resulting arrows point to the direction of the most rapid change in the variable, whereas the length indicates the strength of this gradient.[21] Age in months and month of sampling (seasonality) was investigated for clustering.

Calculation of relative bacterial abundances

Prevalence and relative abundances of bacterial families were obtained based on the taxonomic assignment of PyroTagger, as described previously.[13,18] For every bacterial family, the mean and 95% CI per sample were calculated. The 5 most abundant families were analyzed separately, whereas all the remaining families were grouped as "others." Again, samples were binned according to the date of acquisition, either referring to the month of the year or to the age of the study infant.

Additional statistical analyses

Regression models were then fitted for the relative bacterial abundance, bacterial density, SDI value, and β diversity by using either a linear or an exponential function for age and a second-order polynomial function for the

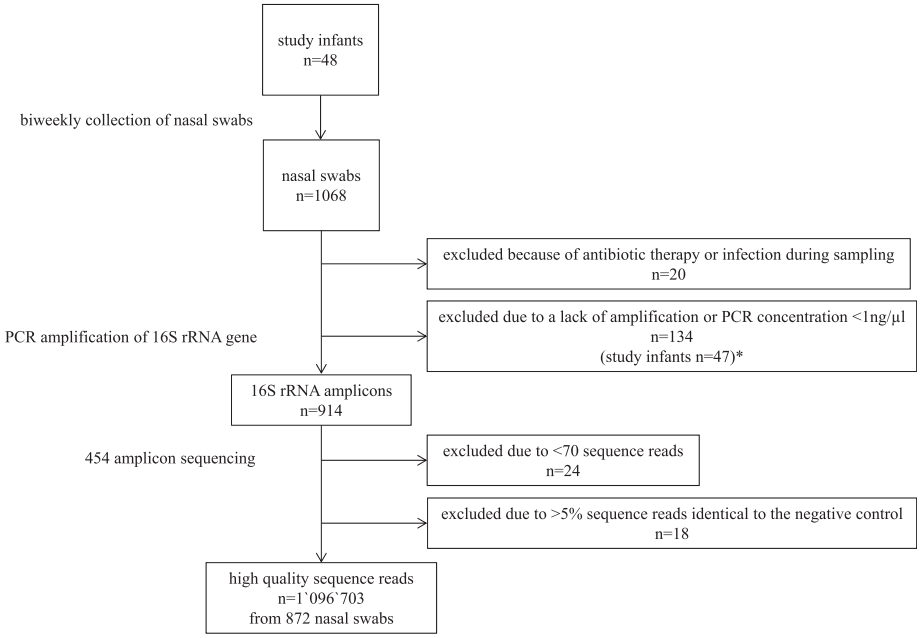

FIG 1. Flow chart of study infants and nasal swab processing, including information on excluded samples. Infections were defined as upper or lower respiratory tract infections. *Twenty-one of 134 excluded nasal swabs were obtained from 1 study infant and therefore samples from that infant were fully excluded from the study, resulting in 47 infants included.

season. Statistical analyses and graphic representations were generated with GraphPad Prism software (version 6.03; GraphPad Software, La Jolla, Calif). The model with the best goodness of fit (R^2) was chosen and interpreted as accurate if the R^2 value was greater than 0.4.

Analyses were repeated with a different multivariable linear regression model to evaluate robustness and to assess whether age and season were independently associated with outcomes. A multilevel model with a random effect to correct for clustering on the individual level was used. Outcome parameters were bacterial density, SDI value, and the relative abundance of the 5 most abundant bacterial families and "others." Because univariable regression models showed varying and mainly nonlinear associations, we included month as an indicator variable for both exposure variables, age and season, in the model. Results are represented as coefficients with 95% CIs and *P* values. Analyses were performed with STATA version 12.0 software for Windows (STATA Corp, College Station, Tex).

RESULTS

Study population and sample processing

A total of 1068 nasal swabs were collected. After exclusion of low-quality swabs and those obtained during respiratory tract infection or antibiotic therapy, 872 samples from 47 infants were included in the final analysis (Fig 1). This corresponds to 19 (range, 11-25) swabs per infant and resulted in 1,096,703 (mean ± SD, 1258 ± 952) high-quality sequence reads. Infants were considered healthy at the time of sampling. All infants were breast-fed for a mean ± SD duration of 8.9 ± 2.6 months. Forty-one study infants obtained 2 doses of pneumococcal conjugate vaccine within the first year of life.[22] Detailed information on the study population is listed in Table I and Table E1 in this article's Online Repository at www.jacionline.org.

TABLE I. Characteristics of the study population (n = 48 infants)

Characteristic	Summary statistic
Anthropometrics	
Boys, no. (%)	24 (50.0)
Gestational age at birth (wk), mean (SD)	39.7 (1.6)
Length at birth (cm), mean (SD)	49.8 (2.0)
Birth weight (kg), mean (SD)	3.4 (0.5)
Diet	
Breast-feeding,* no. (%)	48 (100.0)
Pneumococcal vaccine†	
First dose: weeks after birth, mean (SD)	10.1 (3.5)
Second dose: weeks after birth, mean (SD)	18.9 (4.1)
Season of birth	
Spring, no. (%)	12 (25.0)
Summer, no. (%)	13 (27.1)
Fall, no. (%)	12 (25.0)
Winter, no. (%)	11 (22.9)
Sampling	
Nasal swabs included,‡ mean per infant (SD)	18.6 (3.1)

*Infants were breast-fed for a mean ± SD duration of 8.9 ± 2.6 months.
†Value for the first dose represents the mean number of weeks for 42 infants. Six infants did not receive any dose. The second dose was received by 41 infants. None of the study infants received the third dose before the 12th month of life. Detailed information is available in Table E1.
‡Mean and SD of 47 infants.

Dynamics of bacterial density and diversity

Bacterial density increased within the first year of life, which was best modeled by an exponential fit (R^2 = 0.95; Fig 2, *A*, and see Table E2 in this article's Online Repository at www.jacionline.org). In contrast, no seasonal trends were detected for bacterial density (Fig 2, *B*). This was confirmed by using the multivariable regression model (see Table E3 in this article's Online Repository at www.jacionline.org).

The SDI, which is based on operational taxonomic units of 97% sequence identity, decreased exponentially by age and was lowest at the last month of the first year of life (R^2 = 0.73; Fig 2, *C*, and see Table E4 in this article's Online Repository at

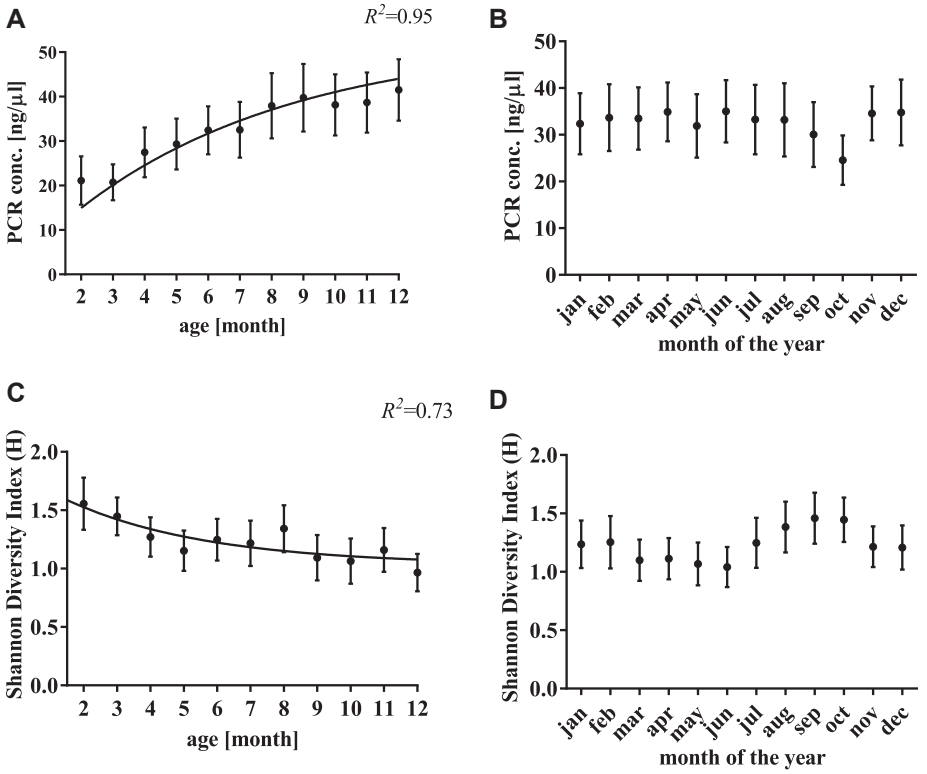

FIG 2. Bacterial density and SDI values representing means and 95% CIs. *Lines* are shown if R^2 values are greater than 0.4. Best fits are indicated. **A** and **B,** There is an exponential association of bacterial density by age (R^2 = 0.95; Fig 2, *A*); however, there was no trend of SDI values by season (Fig 2, *B*). **C** and **D,** SDI values decreased exponentially by age (R^2 = 0.73; Fig 2, *C*) and increased in late summer (Fig 2, *D*).

www.jacionline.org). A trend toward increased SDI values in the late summer months was found (August, September, and October; Fig 2, *D*, and see Table E4). We did not observe significant differences for SDI values between vaccinated (n = 41) and unvaccinated (n = 7) infants at ages of 2 and 4 months (first and second dose of pneumococcal vaccine, respectively).

β Diversity: Jaccard dissimilarity measurements

The composition of samples within different time intervals ranging from 2 to 44 weeks was pairwise compared to investigate the dissimilarity of the microbiota. Dissimilarity increased exponentially with increasing time interval (R^2 = 0.97; Fig 3, *A*), suggesting a higher stability over a short time period. The time interval of 2 weeks was then chosen to investigate the influence of age and season on the dissimilarity of the microbiota. Within-subject dissimilarity was consistently lower than between-subject dissimilarity within the first year of life (Fig 3, *B*). This suggests a distinct personalized microbiota throughout the first year of life.

Also, this stability was observed during seasonal changes, except in the month of July, when the within-subject dissimilarity was not significantly different from the between-subject dissimilarity (P = .17, multiple t tests; Fig 3, *C*). Thus the microbiota is most distinct and individualized during the winter months.

β Diversity: Whole microbial community comparison by using nMDS

Next, nMDS analyses were used as the ordination method for whole microbial community comparison. These analyses allowed investigation of the phylogenetic variation among all samples according to age and season. Significant differences in the second and third months of life were found (P = .001 and .002, respectively; Fig 4, *A*), indicating a distinctly different microbiota at this age. Regarding seasonal changes, we observed the months of December, January, February, and March, which are opposite to July and August (Fig 4, *B*). Significant differences were shown for March, August, and October (P = .04, .01, and .02, respectively). This suggests distinctly different microbiota profiles for the summer and winter months.

Dynamics of bacterial families

The dynamics of the 5 most abundant bacterial families (Moraxellaceae, Streptococcaceae, Corynebacteriaceae, Pasteurellaceae, and Staphylococcaceae) and all remaining families ("others") showed a heterogeneous picture for both age and season.

Streptococcaceae showed no clear trend by age (Fig 5, *A*), whereas the opposite was found for Moraxellaceae, Corynebacteriaceae, Pasteurellaceae, Staphylococcaceae, and "others" (Fig 5, *A-C*). These trends by age were best modeled by exponential functions (see Table E2). Regarding seasonality, Streptococcaceae, Moraxellaceae, and Staphylococcaceae showed no clear trend (Fig 5, *D* and *E*). In contrast, the Pasteurellaceae family was more abundant in the winter months, whereas the opposite was true for Corynebacteriaceae and "others" (Fig 5, *E* and *F*). These seasonal trends were best modeled by using a nonlinear polynomial function (see Table E2). The multivariable models confirmed that associations

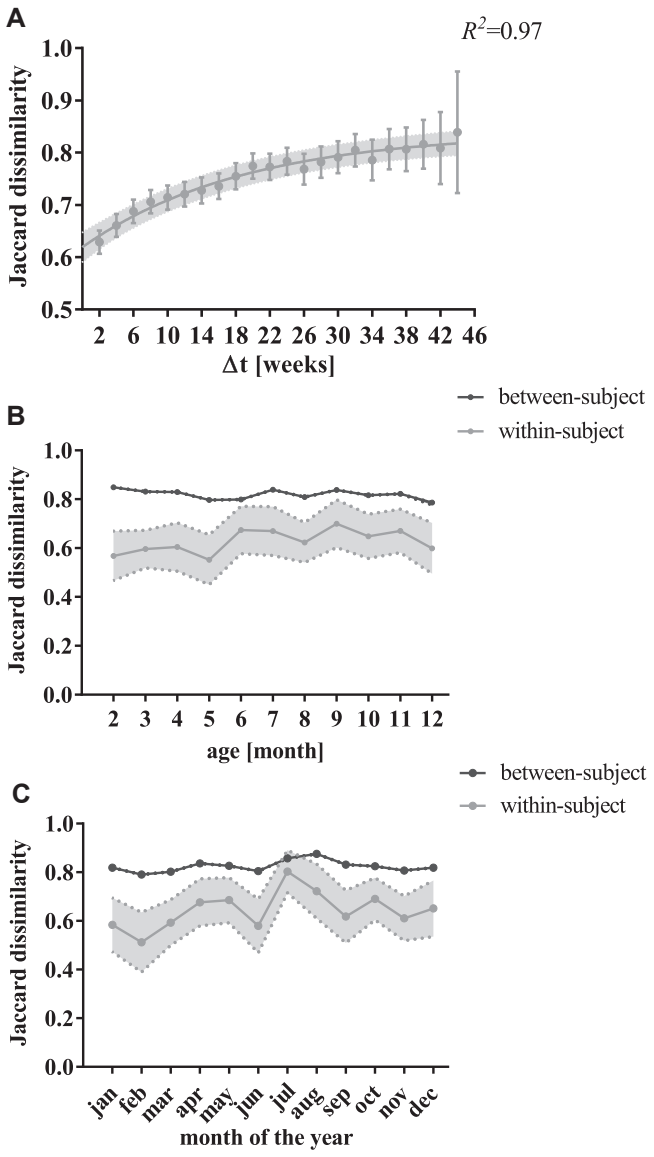

FIG 3. Jaccard dissimilarity measurements. **A,** Exponentially increasing dissimilarity by increasing time intervals between samples (R^2 = 0.97). Shown are mean values, the 95% prediction band, and the *line* indicating best fit. **B,** There is consistently lower within-subject than between-subject dissimilarity of samples in 2-week intervals for the first 12 months of age (means and 95% CIs are indicated). **C,** There was no significant difference in within-subject and between-subject dissimilarity in July (P = .17, multiple t tests), but differences were significant for all other months (means and 95% CIs are indicated).

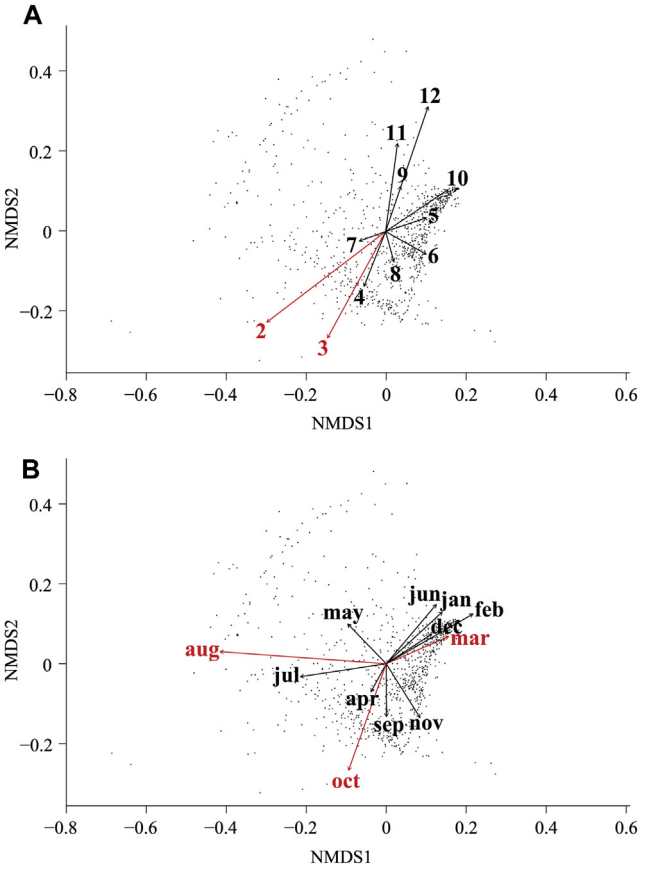

FIG 4. Weighted β diversities represented by using nMDS. *Arrows* indicate clustering of samples by age and season. Significance is indicated in red. **A,** There is a significant difference by age at months 2 and 3 ("adonis" function of R; P = .001 and .002, respectively). **B,** There is a significant difference by season for March, August, and October ("adonis" function of R; P = .04, .01, and .02, respectively).

of the dynamics for both age and season were independent of each other (see Tables E5-E10 in this article's Online Repository at www.jacionline.org).

DISCUSSION

This prospective cohort study includes high-quality data of 872 nasal swabs from 47 unselected healthy infants and describes dynamic changes of the upper respiratory tract microbiota within the first year of life.

Our results showed an increasing bacterial density, which is opposed by a decreasing SDI value, by age. This indicates the development of a distinct microbiota profile toward the end of the first year of life. In addition, we found an increasing dissimilarity of the microbiota with an increasing time interval between sampling dates. Also, within-subject dissimilarity was consistently lower than that between subjects, indicating a personalized nasal microbiota. Furthermore, there were distinct bacterial compositions at age 2 to 3 months compared with the following months. The same was true for the microbiota composition in summer compared with winter months.

A consistently lower within-subject dissimilarity than between subjects was also recently shown in a microbiome study at different body sites.[23] Thus a subject's microbiota is more stable compared with the microbiota of a study population over age and season. This stability might be a result of host-environment equilibrium. If true, the individual microbiota can be considered a "fingerprint" biomarker, which could be used for research in future large cross-sectional or longitudinal trials investigating immune and asthma development.

Although this is the first study on the dynamics of the nasal microbiota in healthy infants within the first year of life with such a dense sampling, comparable studies were performed on the infant's gut microbiota. In contrast to our findings, a gradual increase in microbial diversity by age was shown.[24] However, superimposed onto these findings are the effects of certain life

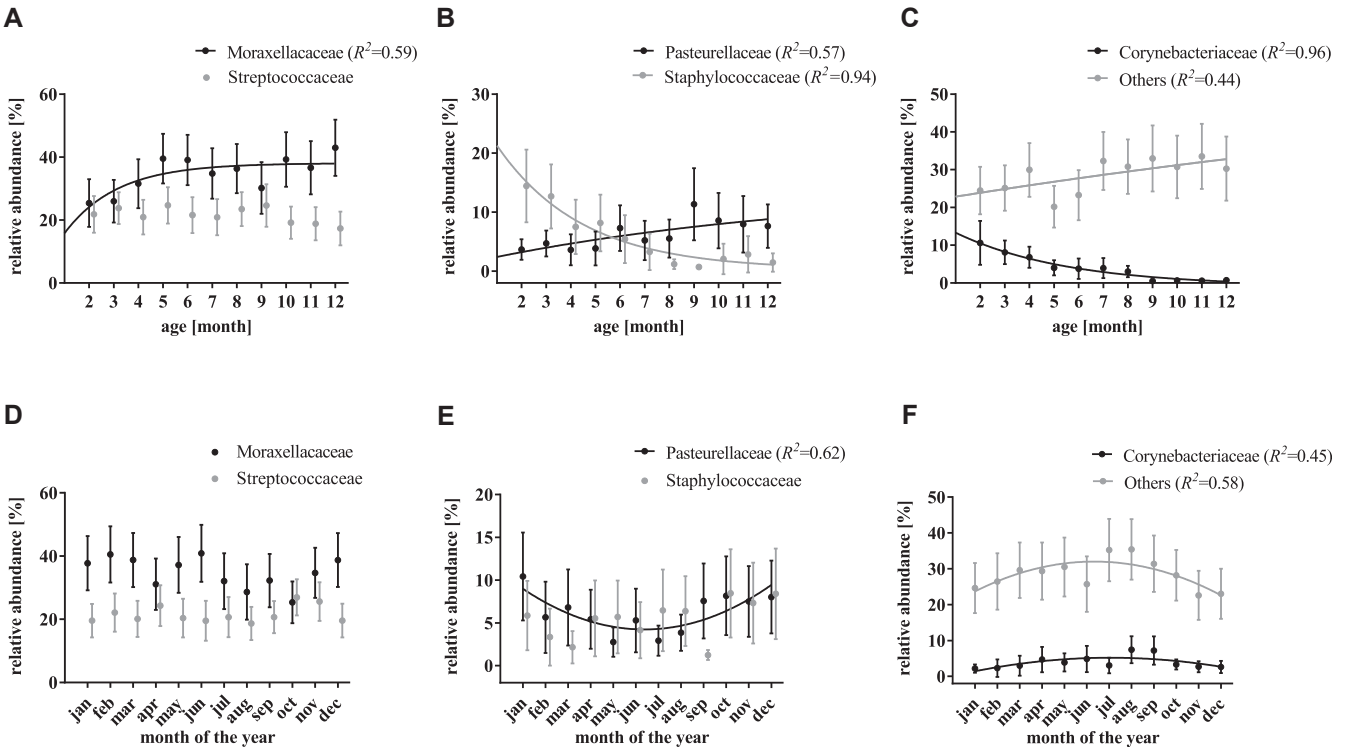

FIG 5. Relative abundance of bacterial families (means and 95% CIs). *Lines* indicate R^2 values of greater than 0.4. Best fits are indicated. **A,** Regarding age, Moraxellaceae levels exponentially increased (R^2 =0.59), but there were no associations for Streptococcaceae. **B,** Exponential association for Staphylococcaceae (R^2 = 0.94) and Pasteurellaceae (R^2 = 0.57). **C,** Exponential decay for Corynebacteriaceae (R^2 = 0.96) and "others" (R^2 = 0.44). **D,** Regarding season, there were no associations for Moraxellaceae and Streptococcaceae. **E** and **F,** Polynomial association for Pasteurellaceae (R^2 = 0.62; Fig 5, *E*), Corynebacteriaceae (R^2 = 0.45), and "others" (R^2 = 0.58; Fig 5, *F*).

events in early infancy, such as the change to solid food, which directly affect the diversity of the gut microbiota.[24-26] Thus findings of density and diversity in our study suggest a diverse and transient colonization in the first few months of life. Also, by using nMDS plots, a distinct clustering of the second and third months of age apart from the following months was observed. In a similar vein a recent study demonstrated an association between the colonization of the upper airways by bacterial pathogens at the age of 6 months and cough and wheezing episodes within the first year of life.[27] An immune response induced by the microbiota was suggested, which activates and possibly influences maturation of the adaptive immune system.[28] Therefore we hypothesize that the nasal microbiota is sensitive to disturbances, especially in the first months of life. The rather high relative abundance of Corynebacteriaceae and Staphylococcaceae in the first few months resembles the microbial composition found on the skin in this age group.[29] Therefore we assume that the nasal microbiota is similar to that on the skin in the first months of life, probably because of the close contact of the infant to the mother's skin during breast-feeding. The infant later had a "characteristic" respiratory tract microbiota over time. It should be noted that similar studies were performed in The Netherlands.[30,31] In particular, the authors of those studies discovered higher levels of Staphylococcus and Corynebacterium in the nasopharynx regardless of feeding type at the age of 6 weeks compared with 6 months.[30] They hypothesized that this might be explained by environmental encounters. Staphylococcaceae and Corynebacteriaceae levels were also clearly increased in the first 2 to 4 months of life in our study, which caused us to hypothesize that our findings from Switzerland are also applicable to infants in different countries with a similar climate. Moraxellaceae and Streptococcaceae showed the highest relative abundance per sample, which accords with previous studies in which these families were found as part of the healthy nasopharyngeal microbiota.[11,13,14] Earlier studies with conventional culture methods also showed a high prevalence of Moraxellaceae in nasopharyngeal samples, which confirms the results obtained by using next-generation sequencing methods.[32,33]

Regarding seasonal changes, we found an increase of Corynebacteriaceae and "others" in the summer months. In contrast, Pasteurellaceae were more abundant during winter. This was also expected for Moraxellaceae and Streptococcaceae because a previous study showed a peak colonization incidence of *M catarrhalis* and *H influenzae* in healthy children in fall/winter and winter/spring, respectively.[34] Associations between *S pneumoniae*, *H influenzae*, and *M catarrhalis* colonization and the presence of multiple respiratory tract viruses in the nasopharynx of otherwise healthy children were also found.[35] This suggests an outgrowth of potential pathogens, which is probably driven by the presence of winter viruses. In line with this hypothesis, a recent study in a mouse model showed a decrease in microbial diversity on introduction of *S pneumoniae* in the upper respiratory tract and a temporary dominance of pneumococci in the microbiota.[36] However, because the Moraxellaceae and

Streptococcaceae families consist of potential pathogenic, as well as commensal, species, we assume inverse seasonal patterns for these species, which would result in their cancelling each other out.

Seasonal changes were also observed in the β diversity analysis by using nMDS plots, in which samples obtained in warmer months group differently to samples from colder months. In a recent study a causal role of winter viral infection during infancy and early childhood asthma was found, wherein infants born approximately 4 months before the winter virus peak carried the highest risk.[37] This supports our finding of decreased diversity in the winter months, as well as our hypothesis that infants experience a higher sensitivity to disturbances in the first months of life, as discussed above. Also, this underlines the importance of both age and season on the dynamics of the microbiota.

Our study has some major strengths. We performed a dense sampling of 48 infants within the first year of life, resulting in more than 800 analyzed samples. Such detailed longitudinal data are not yet available but are necessary to illustrate the dynamics of the microbiota over age and season. In fact, we believe that an even denser sampling would be necessary to better understand the dynamic changes of the microbiota. In a preliminary analysis we looked at the microbiota from 2 consecutive nasal swabs of 10 infants taken within 3 hours. First results showed lower within-subject dissimilarity of these samples compared with the shortest time interval in this study (2 weeks). An additional strength of this study is the fact that the infants' dates of birth are equally distributed throughout the year, minimizing the misinterpretation of results based on skewed distribution of months of birth. We also only included infants who were breast-fed, and all were chosen from a rather homogeneous sampling area. This is an advantage because it makes this study less prone to other influencing factors, such as climate and ethnicity.

However, there are some limitations of this study. Because the nasal cavities are not yet fully developed in infants, taking nasopharyngeal swabs bears the risk of trauma or perforation. Therefore we performed nasal sampling and considered nasal swabs as representative of the upper respiratory tract microbiota, as was recently shown.[10] Rapola et al[38] also demonstrated that isolation rates of the otitis media pathogens *S pneumoniae* and *H influenzae* do not differ between nasal and nasopharyngeal swabs. In addition, the nasal swabs were taken by the parents of the study infants, and thus we cannot exclude a potential sampling bias because an inadequately obtained swab possibly interferes with sample processing, particularly given that we were analyzing low-density communities.[17] Therefore for quality control, we excluded all samples containing a bacterial DNA concentration of less than 1 pg/μL or with greater than 5% sequences being identical to those of a negative control, as recently recommended.[39] We also omitted samples at the time of infection but not 2 or more weeks after. Preliminary analyses investigating Jaccard dissimilarity shortly before, during, and after infection revealed that this is a justified approach. However, more samples and a different study design are needed to investigate the effect of infection on the microbiota composition.

Finally, we analyzed the microbiota data of this study in a pooled way rather than for each infant individually. This might mask considerable changes within a subject's microbiota. Because there are many host and environmental factors that might explain these changes, analyses of these factors would go beyond the scope of this study. Mean values are likely to reduce the effect of such factors, including the occasional inadequate sampling, and should therefore reveal the influence of age and season more accurately.

In conclusion, in this study we were able to demonstrate the dynamics of the nasal microbiota in healthy infants within the first year of life. Furthermore, we demonstrated the need for age and seasonal variability to be taken into account when assessing microbiota data. Interestingly, a relative uniqueness of a subject's microbiota was found, which is most pronounced in the winter months (ie, a personalized microbiota). Overall, these data serve as essential baseline information for intervention studies in infants and are necessary for future investigation of the influence of disease, environmental, and/or host factors on a subject's microbiota.

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Key messages

- **Nasal microbiota shows higher intraindividual than interindividual similarity.**
- **Dissimilarity of the microbiota increases with increasing time intervals between samples.**
- **There is a distinct bacterial composition in the first 3 months of life.**
- **Age and seasonality are major factors driving the dynamics of the nasal microbiota.**

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TABLE E1. Overview of study infants and numbers of swabs

		Pneumococcal vaccine*			Excluded swabs		
ID	Date of birth	First dose	Second dose	Included swabs	Infection/antibiotics	"Others"†	Total
1	02.2010	9.7	17.7	16	1	5	22
2	04.2010	8.7	17.9	17	0	8	25
3	04.2010	16.4	27.1	20	1	3	24
4	04.2010	9.7	18.6	23	0	0	23
5	05.2010	10.4	21.6	15	1	1	17
6	06.2010	8.6	14.6	17	0	5	22
7	06.2010	8.4	18.0	15	0	9	24
8	07.2010	9.0	17.7	22	3	0	25
9	07.2010	9.6	18.6	18	0	4	22
10	07.2010			20	1	1	22
11	07.2010			18	1	2	21
12	07.2010	8.3	17.3	21	0	2	23
13	08.2010			19	0	1	20
14	08.2010	10.0	20.0	19	1	1	21
15	08.2010	9.6	19.7	18	1	5	24
16	09.2010	9.7	19.9	22	0	2	24
17	09.2010	8.1	18.1	19	0	0	19
18	09.2010	9.0	18.0	16	2	6	24
19	10.2010	9.3	19.6	13	0	10	23
20	10.2010	8.6	18.0	11	0	5	16
21	10.2010	8.0	17.0	14	0	7	21
22	11.2010			15	1	7	23
23	11.2010	9.7	18.1	23	1	0	24
24	11.2010			0	0	21	21
25	12.2010	9.3		22	0	2	24
26	12.2010	10.3	20.4	16	0	4	20
27	01.2011	8.4	17.7	19	1	1	21
28	01.2011	31.0	40.1	18	0	6	24
29	01.2011	9.3	9.3	16	0	7	23
30	02.2011	8.9	18.7	15	1	5	21
31	03.2011	8.9	17.7	17	0	8	25
32	03.2011	10.6	19.6	20	0	3	23
33	03.2011	9.7	18.0	19	0	3	22
34	06.2011	10.9	18.9	17	0	0	17
35	07.2011	10.3	17.4	21	1	0	22
36	10.2011	9.7	18.7	23	0	0	23
37	10.2011			15	0	6	21
38	12.2011	9.9	18.3	21	0	2	23
39	04.2012	9.9	19.9	12	0	12	24
40	04.2012	8.7	17.1	19	1	4	24
41	05.2012	9.6	15.6	20	0	2	22
42	08.2012	9.4	17.4	20	0	2	22
43	10.2012	10.6	18.7	22	1	0	23
44	01.2013	8.0	17.0	25	0	0	25
45	01.2013	8.6	17.9	21	0	1	22
46	02.2013	9.7	19.0	19	0	2	21
47	05.2013	12.7	19.3	21	1	1	23
48	07.2013	9.0	20.0	23	0	0	23
Total				872	20	176	1068

*Indicated is the number of weeks after birth. The recommended vaccination schedule for pneumococcal conjugate vaccine is 2, 4, and 11 to 15 months.[E1] None of the study infants received the third dose before the 12th month of life.

†Other reasons for exclusion were no amplification or less than 1 ng/μL PCR product, less than 70 sequence reads, or greater than 5% sequence reads identical to the negative control sample.

TABLE E2. Regression analyses

	Age	Season
Outcome variable	**Exponential fit***	**Polynomial fit**
Bacterial density	**0.95**	0.05
	$Y = 9.9 + (47.7 - 9.9) * (1\text{-}exp[-0.1 * X])$	$Y = 34.3 - 0.4 * X + 0.02 * X^2$
SDI	**0.73**	0.18
	$Y = 0.8 * exp(-0.2 * X) + 0.1$	$Y = 1.2 + 0.004 * X + 0.001 * X^2$
Streptococcaceae	Not converged	0.08
		$Y = 20.7 - 0.1 * X + 0.02 * X^2$
Moraxellaceae	**0.59**	0.3
	$Y = 2.5 + (38.1 - 2.5) * (1 - exp[-0.5 * X])$	$Y = 43.2 - 2.6 * X + 0.2 * X^2$
Staphylococcaceae	**0.94**	0.32
	$Y = 26.5 * exp(-0.3 * X) + 0.1$	$Y = 4.8 - 0.4 * X + 0.06 * X^2$
Corynebacteriaceae	**0.96**	**0.45**
	$Y = 17.8 * exp(-0.2 * X) - 0.9$	**$Y = 0.06 + 1.5 * X - 0.1 * X^2$**
Pasteurellaceae	**0.57**	**0.62**
	$Y = 1.5 + (14.2 - 1.5) * (1\text{-}exp[-0.1 * X])$	**$Y = 10.9 - 2.1 * X + 0.2 * X^2$**
"Others"	**0.44**	**0.58**
	$Y = 21.8 + (62.4 - 21.8) * (1\text{-}exp[-0.03 * X])$	**$Y = 20.3 + 3.7 * X - 0.3 * X^2$**
Jaccard dissimilarity†	**0.97**	
	$Y = 0.62 + (0.84 - 0.62) * (1\text{-}exp[-0.05 * X])$	

Data were derived from regression analysis. Shown is the goodness of fit as R^2 values for all outcome variables according to age and season. If the R^2 value if greater than 0.4, the corresponding equation of the best fit is indicated in boldface.

*Additionally, linear regression was fitted for age; however, this was found to be inferior to the exponential fit for all outcome variables.

†Shown is goodness of fit according to the time interval Δt in weeks.

TABLE E3. Adjusted analysis of bacterial density

	Adjusted analysis		
Bacterial density	**Coefficient**	**95% CI**	***P* value**
Age (mo)			
3	1.3	−6.4 to 9.0	.7
4	6.5	−1.4 to 14.4	.1
5	8.6	1.0 to 16.3	<.05
6	10.2	2.2 to 18.1	<.05
7	13.1	5.3 to 20.9	<.05
8	18.7	10.9 to 26.6	<.05
9	18.4	10.4 to 26.4	<.05
10	18.4	10.6 to 26.3	<.05
11	16.5	8.6 to 24.5	<.05
12	21.5	13.5 to 29.6	<.05
Month of year			
February	−0.8	−9.0 to 7.4	.9
March	−0.3	−8.3 to 7.6	.9
April	1.0	−7.1 to 9.1	.8
May	−0.5	−8.5 to 7.4	.9
June	0.1	−8.1 to 8.2	1.0
July	−0.1	−8.3 to 8.1	1.0
August	−1.2	−9.4 to 7.0	.8
September	−2.6	−10.8 to 5.5	.5
October	−7.0	−15.0 to 0.9	.1
November	1.1	−6.7 to 9.0	.8
December	3.8	−4.2 to 11.9	.4

Data are derived from linear regression analysis. Coefficient of adjusted analysis was used for all variables. Reference variables were age of 2 months and January.

TABLE E4. Adjusted analysis of SDI values

	Adjusted analysis		
SDI value	**Coefficient**	**95% CI**	***P* value**
Age (mo)			
3	−0.1	−0.3 to 0.1	.3
4	−0.3	−0.6 to −0.1	<.05
5	−0.4	−0.6 to −0.1	<.05
6	−0.2	−0.5 to 0.01	.1
7	−0.4	−0.6 to −0.2	<.05
8	−0.3	−0.5 to −0.03	<.05
9	−0.4	−0.7 to −0.2	<.05
10	−0.5	−0.7 to −0.3	<.05
11	−0.4	−0.6 to −0.2	<.05
12	−0.6	−0.8 to −0.3	<.05
Month of year			
February	0.0	−0.2 to 0.2	1.0
March	−0.1	−0.4 to 0.1	.3
April	−0.1	−0.4 to 0.1	.3
May	−0.1	−0.4 to 0.1	.2
June	−0.1	−0.4 to 0.1	.3
July	0.1	−0.2 to 0.3	.7
August	0.2	−0.1 to 0.4	.2
September	0.2	−0.05 to 0.4	.1
October	0.2	−0.05 to 0.4	.1
November	0.0	−0.2 to 0.2	.9
December	−0.1	−0.3 to 0.2	.5

Data are derived from linear regression analysis. Coefficient of adjusted analysis was used for all variables. Reference variables were age of 2 months and January.

TABLE E5. Adjusted analysis of the relative abundance of Streptococcaceae

	Adjusted analysis		
Streptococcaceae	**Coefficient**	**95% CI**	***P* value**
Age (mo)			
3	0.1	−7.2 to 7.5	1.0
4	0.0	−7.5 to 7.5	1.0
5	0.3	−6.9 to 7.6	.9
6	0.1	−7.5 to 7.7	1.0
7	−2.5	−9.9 to 4.9	.5
8	0.5	−6.9 to 8.0	.9
9	2.2	−5.5 to 9.8	.6
10	−3.4	−10.8 to 4.1	.4
11	−3.5	−11.0 to 4.0	.4
12	−4.6	−12.2 to 3.1	.2
Month of year			
February	2.9	−4.9 to 10.7	.5
March	0.5	−7.1 to 8.0	.9
April	4.9	−2.8 to 12.5	.2
May	1.9	−5.7 to 9.4	.6
June	1.3	−6.4 to 9.1	.7
July	2.0	−5.9 to 9.8	.6
August	−0.2	−8.0 to 7.5	1.0
September	0.8	−7.0 to 8.5	.8
October	7.2	−0.3 to 14.8	.1
November	5.6	−1.9 to 13.1	.1
December	−0.6	−8.3 to 7.0	.9

Data are derived from linear regression analysis. Coefficient of adjusted analysis was used for all variables. Reference variables were age of 2 months and January.

TABLE E6. Adjusted analysis of the relative abundance of Moraxellaceae

	Adjusted analysis		
Moraxellaceae	**Coefficient**	**95% CI**	***P* value**
Age (mo)			
3	3.5	−6.2 to 13.2	.5
4	7.5	−2.4 to 17.4	.1
5	16.2	6.6 to 25.8	<.05
6	14.4	4.5 to 24.4	<.05
7	13.5	3.7 to 23.3	<.05
8	12.5	2.7 to 22.4	<.05
9	7.0	−3.1 to 17.1	.2
10	17.0	7.2 to 26.9	<.05
11	14.2	4.3 to 24.2	<.05
12	17.6	7.5 to 27.7	<.05
Month of year			
February	4.0	−6.3 to 14.3	.4
March	2.9	−7.1 to 12.9	.6
April	−4.4	−14.4 to 5.7	.4
May	0.0	−9.9 to 9.9	1.0
June	1.8	−8.4 to 12.0	.7
July	−6.2	−16.6 to 4.1	.2
August	−7.0	−17.3 to 3.3	.2
September	−1.7	−12.0 to 8.5	.7
October	−11.3	−21.2 to −1.4	<.05
November	−3.4	−13.2 to 6.5	.5
December	3.6	−6.5 to 13.7	.5

Data are derived from linear regression analysis. Coefficient of adjusted analysis was used for all variables. Reference variables were age of 2 months and January.

TABLE E7. Adjusted analysis of the relative abundance of Staphylococcaceae

	Adjusted analysis		
Staphylococcaceae	**Coefficient**	**95% CI**	***P* value**
Age (mo)			
3	−3.5	−8.5 to 1.5	.2
4	−6.9	−12.0 to −1.8	<.05
5	−8.9	−13.9 to −4.0	<.05
6	−10.1	−15.2 to −4.9	<.05
7	−12.3	−17.3 to −7.2	<.05
8	−13.4	−18.5 to −8.3	<.05
9	−14.4	−19.6 to −9.2	<.05
10	−13.5	−18.6 to −8.4	<.05
11	−12.9	−18.1 to −7.8	<.05
12	−13.5	−18.7 to −8.3	<.05
Month of year			
February	−2.3	−7.6 to 3.0	.4
March	−3.7	−8.9 to 1.5	.2
April	−0.1	−5.3 to 5.1	1.0
May	0.4	−4.7 to 5.5	.9
June	−0.9	−6.1 to 4.4	.7
July	0.9	−4.5 to 6.2	.8
August	−0.1	−5.4 to 5.2	1.0
September	−5.0	−10.3 to 0.3	.1
October	2.8	−2.3 to 7.9	.3
November	1.8	−3.3 to 6.9	.5
December	2.1	−3.2 to 7.3	.4

Data are derived from linear regression analysis. Coefficient of adjusted analysis was used for all variables. Reference variables were age of 2 months and January.

TABLE E8. Adjusted analysis of the relative abundance of Pasteurellaceae

	Adjusted analysis		
Pasteurellaceae	**Coefficient**	**95% CI**	***P* value**
Age (mo)			
3	0.8	−4.1 to 5.7	.7
4	0.2	−4.8 to 5.2	.9
5	0.0	−4.9 to 4.8	1.0
6	3.1	−1.9 to 8.2	.2
7	1.5	−3.5 to 6.4	.6
8	3.2	−1.7 to 8.2	.2
9	7.1	2.0 to 12.2	<.05
10	3.8	−1.1 to 8.8	.1
11	5.0	−0.1 to 10.0	.1
12	4.1	−1.0 to 9.2	.1
Month of year			
February	−5.0	−10.2 to 0.2	.1
March	−4.3	−9.4 to 0.7	.1
April	−5.5	−10.6 to −0.4	<.05
May	−7.8	−12.8 to −2.8	<.05
June	−5.0	−10.2 to 0.1	.1
July	−7.8	−13.0 to −2.6	<.05
August	−6.0	−11.1 to −0.8	<.05
September	−3.0	−8.1 to 2.2	.3
October	−2.4	−7.4 to 2.6	.4
November	−3.3	−8.3 to 1.7	.2
December	−2.7	−7.8 to 2.4	.3

Data are derived from linear regression analysis. Coefficient of adjusted analysis was used for all variables. Reference variables were age of 2 months and January.

TABLE E9. Adjusted analysis of the relative abundance of Corynebacteriaceae

	Adjusted Analysis		
Corynebacteriaceae	**Coefficient**	**95% CI**	***P* value**
Age (mo)			
3	−3.0	−6.3 to 0.2	.1
4	−4.1	−7.4 to −0.8	<.05
5	−6.1	−9.3 to −2.9	<.05
6	−6.4	−9.9 to −3.0	<.05
7	−6.4	−9.7 to −3.1	<.05
8	−8.3	−11.6 to −5.0	<.05
9	−9.6	−13.0 to −6.3	<.05
10	−9.8	−13.1 to −6.5	<.05
11	−9.4	−12.7 to −6.0	<.05
12	−10.0	−13.4 to −6.6	<.05
Month of year			
February	0.1	−3.3 to 3.5	1.0
March	0.8	−2.6 to 4.1	.7
April	2.0	−1.4 to 5.3	.3
May	1.4	−2.0 to 4.7	.4
June	2.8	−0.6 to 6.2	.1
July	0.7	−2.8 to 4.1	.7
August	4.3	0.9 to 7.7	<.05
September	4.5	1.1 to 8.0	<.05
October	0.7	−2.6 to 4.0	.7
November	−0.3	−3.6 to 3.0	.9
December	−0.1	−3.5 to 3.2	.9

Data are derived from linear regression analysis. Coefficient of adjusted analysis was used for all variables. Reference variables were age of 2 months and January.

TABLE E10. Adjusted analysis of the relative abundance of other bacterial families

	Adjusted analysis		
Other bacterial families	**Coefficient**	**95% CI**	***P* value**
Age (mo)			
3	2.3	−6.7 to 11.3	.6
4	3.3	−5.9 to 12.5	.5
5	−1.4	−10.4 to 7.5	.8
6	−1.0	−10.3 to 8.3	.8
7	6.6	−2.6 to 15.7	.2
8	5.4	−3.8 to 14.6	.2
9	7.9	−1.5 to 17.3	.1
10	6.0	−3.2 to 15.1	.2
11	6.4	−2.8 to 15.7	.2
12	6.5	−2.9 to 15.9	.2
Month of year			
February	0.3	−9.3 to 9.9	1.0
March	4.0	−5.3 to 13.4	.4
April	3.0	−6.4 to 12.4	.5
May	4.0	−5.3 to 13.2	.4
June	−0.5	−10.0 to 9.0	.9
July	10.6	0.9 to 20.2	<.05
August	8.9	−0.7 to 18.4	.1
September	4.3	−5.2 to 13.9	.4
October	2.9	−6.3 to 12.2	.5
November	−0.3	−9.5 to 8.9	.9
December	−2.0	−11.4 to 7.4	.7

Data are derived from linear regression analysis. Coefficient of adjusted analysis was used for all variables. Reference variables were age of 2 months and January.