Association of household siblings with nasal and fecal microbiota in infants

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Abstract

Background—Early-life exposure to older siblings is associated with a lower risk of asthma. To date, no study has addressed the impact of having siblings on both the airway and fecal microbiota during infancy. We aimed to profile the nasal airway and fecal microbiota in infants, and to examine the association between having siblings and microbiota profiles.

Methods—We conducted a cross-sectional study of 105 healthy infants (aged <1 year). By applying 16S rRNA gene sequencing and an unbiased clustering approach to the nasal airway and fecal samples, we identified microbiota profiles and then determined the association between having siblings and microbiome profiles.

Results—Overall, the median age was 3.4 months (IQR, 2.0–4.7 months); 43% had siblings in the household. Unbiased clustering of nasal airway microbiota identified three profiles: *Moraxella*-dominant (43%), *Corynebacterium/Dolosigranulum*-dominant (36%), and mixed (21%). Infants with siblings were more likely to have *Moraxella*-dominant profile than *Corynebacterium/*

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Dolosigranulum-dominant profile (76% vs. 18%) while those without siblings had the opposite pattern (18% vs. 50%) (multivariable-adjusted P<0.001). Fecal microbiota consisted of three profiles: *Bifidobacterium*-dominant (39%), *Escherichia*-dominant (31%), and *Enterobacter*-dominant (30%). Infants with siblings were more likely to have *Bifidobacterium*-dominant profile than *Escherichia*-dominant profile (49% vs. 24%) while those without siblings had the opposite pattern (32% vs. 37%) (multivariable-adjusted P=0.04).

Conclusions—In this cross-sectional study, we found that infants with siblings were more likely to have *Moraxella*-dominant nasal microbiota profile and *Bifidobacterium*-dominant fecal microbiota profile. Our findings should facilitate further investigation of the interplay between early-life environmental exposures, the microbiome, and childhood asthma.

Keywords

airway microbiota; fecal microbiota; sibling; infants; asthma

BACKGROUND

In recent decades, epidemiologic studies have reported an inverse relationship between exposure to microbially-rich environments in infancy and risk of developing allergic diseases.[1–4] For example, the literature has documented that early-life exposure to older siblings and farm animals is protective against the development of childhood asthma.[1,2,5,6] This finding was furthered by culture-independent high-throughput sequencing approaches revealing that the human microbiota – highly functional bacterial communities that inhabit humans – influences the rate and pattern of maturation of immune function in early life.[7,8] These data suggest that the microbiota may mediate the link between environmental factors and development of childhood asthma.[7]

To date, most microbiome research has focused on the gut, and, with regard to above, the possible link between the presence of older siblings and specific fecal microbiota.[9–11] However, the airway is a major portal for microbial exposure and studies have revealed that the airway microbiota influences immune responses in patients with asthma.[7] Further, the airway microbiota may be modulated by host-microbe interactions in the gut and systemic dissemination of metabolites produced by the gut microbiota.[12] To the best of our knowledge, no study has addressed the impact of household siblings on both airway and fecal microbiota simultaneously in infants. To address this knowledge gap, we profiled the nasal airway and fecal microbiota in infants, and examined the association between having siblings in the household and microbiota profiles.

METHODS

Design, Setting, and Participants

We conducted a cross-sectional study to examine the nasal and fecal microbiota of healthy infants. The setting and participants have been reported previously.[13] Briefly, we enrolled 120 infants from a primary care group practice at Massachusetts General Hospital (Boston, MA) from November 2013 through May 2014. Inclusion criteria were infants aged <1 year and gestational age >32 weeks. Exclusion criteria included comorbidities (i.e., heart-lung

disease, immunodeficiency, immunosuppression, or chronic gastrointestinal disorder), previous lower respiratory infection, diarrheal illness in the past week, treatment with antibiotics in the past week, and current acute respiratory infection or gastrointestinal illness.[14] The local institutional review board approved the study. Informed consent was obtained from the parents or guardians.

Data Collection

We conducted a structured interview and chart review that assessed participants' demographic characteristics, family history, prenatal and past medical history, and home environmental characteristic including presence of siblings in the household. Trained investigators collected nasal swabs from the anterior nares (i.e., not from nasal turbinates or nasopharynx), using a standardized protocol,[15] during the clinic visit. Both anterior nares were swabbed with a single nylon, pediatric FLOQSwab (Copan, Brescia, Italy). The swab was then added to 2 mL of viral transport medium and frozen at −80°C. Fecal samples were collected, using a standardized protocol,[16] within 24 hours of the clinic visit. Diapers containing feces were refrigerated or stored in a cooler immediately after collection. The fecal samples were then added to sterile feces collection containers (Sarstedt, Nümbrecht, Germany) and immediately stored at −80°C. Nasal and fecal samples were shipped on dry ice to the Alkek Center for Metagenomics and Microbiome Research at Baylor College of Medicine, where we characterized the microbiota using 16S rRNA gene sequencing.

16s rRNA Gene Sequencing

16S rRNA gene sequencing methods were adapted from the methods developed for the NIH Human Microbiome Project.[17,18] Briefly, bacterial genomic DNA was extracted using MO BIO PowerMag DNA Isolation Kit (Mo Bio Laboratories; Carlsbad, CA). The 16S rDNA V4 region was amplified by PCR and sequenced in the MiSeq platform (Illumina; SanDiego, CA) using the 2x250 bp paired-end protocol yielding pair-end reads that overlap almost completely. The primers used for amplification contain adapters for MiSeq sequencing and single-end barcodes allowing pooling and direct sequencing of PCR products.[19]

Sequencing read pairs were demultiplexed based on the unique molecular barcodes, and reads were merged using USEARCH v7.0.1090,[20] allowing zero mismatches and a minimum overlap of 50 bases. Merged reads were trimmed at the first base with a Q5 quality score. Additionally, a quality filter was applied to the resulting merged reads and reads containing above 0.05 expected errors were discarded. Rarefaction curves of bacterial operational taxonomic units (OTUs) were constructed using sequence data for each sample to ensure coverage of the bacterial diversity present. Samples with suboptimal amounts of sequencings reads (<80% of the taxa are represented) were re-sequenced to ensure that the majority of bacterial taxa were encompassed in our analyses.

16S rRNA gene sequences were clustered into OTUs at a similarity cutoff value of 97% using the UPARSE algorithm.[21] OTUs were mapped to the SILVA Database[22] containing only the 16S V4 region to determine taxonomies. Abundances were recovered by mapping the demultiplexed reads to the UPARSE OTUs. A custom script constructed a rarefied OTU

table from the output files generated in the previous two steps for downstream analyses of alpha-diversity (e.g., Shannon index) and beta-diversity (e.g., Bray-Curtis distance).

Quality Control

The processes involving microbial DNA extraction, 16S rRNA gene amplification, and amplicon sequencing included a set of controls that enabled us to evaluate the potential introduction of contamination or off-target amplification. Non-template controls (extraction chemistries) were included in the microbial DNA extraction process and the resulting material was subsequently used for PCR amplification. Additionally, at the step of amplification, another set of non-template controls (PCR-mix) was included to evaluate the potential introduction of contamination at this step. Similarly, a positive control comprised of known and previously characterized microbial DNA was included at this step to evaluate the efficiency of the amplification process. Before samples (unknowns) were pooled together, sequencing controls were evaluated and the rejection criteria were the presence of amplicons in any of the non-template controls or the absence of amplicons in the positive control. In the present study, no amplicons were observed in the non-template controls and a negligible amount of raw reads were recovered after sequencing.

Statistical Analyses

We calculated the relative abundance of each OTU for each nasal airway sample and fecal sample. As each genus was dominated by one OTU, we collapsed all OTUs assigned to the same genus into a single group for reporting. To identify nasal airway and fecal microbiota profiles, we performed unbiased clustering by the partitioning around medoids method [23] with the use of Bray-Curtis distances. Each cluster was defined by a point designated as the center (the "medoid") and minimizes the distance between samples in a cluster. The number of clusters to choose for the data was determined by using the gap statistic.[24] We also examined the within-subject association between nasal airway microbiota profile and fecal microbiota profile (i.e., the marginal frequencies of two nominal outcomes) by marginal homogeneity test.

To examine the association between the presence of siblings and the microbiota profiles, we constructed multinomial regression model for nasal and fecal microbiota, separately. We adjusted for up to six potential confounders (age, sex, maternal antibiotic use during pregnancy, maternal smoking, breast-feeding status, and history of systemic [i.e., oral, intravenous, intramuscular] antibiotic use in infancy) while being mindful of the relatively small number of subjects within microbiota profiles. These variables were chosen based on clinical plausibility and *a priori* knowledge.[9–11] In the sensitivity analysis, we repeated the multivariable model with the number of siblings as ordinal variable. We also performed unadjusted analyses stratified by mode of delivery in all study participants as well as stratified analyses by feeding status in infants aged <6 months. Analyses used R version 3.2 with the *phyloseq* package.[25]

RESULTS

The analytic cohort comprised 105 infants with both nasal and fecal samples. Overall, the median age was 3.4 months (IQR, 2.0–4.7 months), 56% were male, and 52% were non-Hispanic white. With regard to the primary exposure, 43% of infants had siblings in the household (Table 1).

We analyzed nasal airway samples from 105 infants by 16S rRNA gene sequencing. All 105 yielded high quality sequence data (rarefaction cutoff, 1,066 reads per sample). The sequencing identified 21 phyla and 306 genera. The nasal airway microbiota was dominated by three genera – *Moraxella* (37%), *Corynebacterium* (17%), and *Staphylococcus* (9%) – followed by *Dolosigranulum* (8%) and *Streptococcus* (8%). Partitioning around medoids clustering of nasal airway microbiota identified three distinct microbiota profiles: 1) *Moraxella*-dominant (43%), 2) *Corynebacterium/Dolosigranulum*-dominant (36%), and 3) mixed (21%) (Figure 1a). The first two profiles were dominated either by *Moraxella* or *Corynebacterium/Dolosigranulum* (co-dominant) genus. By contrast, the mixed profile had the highest relative abundance of *Staphylococcus, Streptococcus,* and *Tumebacillus* (Benjamini-Hochberg adjusted P<0.05; Table 2). The nonmetric multidimensional scaling (NMDS) plots also demonstrated that the infants cluster together according to their nasal airway microbiota profile (Figure 2a).

Nasal airway microbiota profiles differed between infants with siblings and those without. For instance, infants with siblings were more likely to have a *Moraxella*-dominant profile than a *Corynebacterium/Dolosigranulum*-dominant profile (76% vs. 18%; Table 1) while those without siblings had the opposite pattern (18% vs. 50%), corresponding to a relative risk ratio (RRR) of 11.6 (95%CI, 4.12–32.6; P<0.001; Table 3). This significant finding persisted in the multivariable model, (adjusted RRR, 18.9; 95%CI, 5.30–67.3; P<0.001) and in the sensitivity analysis modeling the number of siblings as ordinal variable (adjusted RRR per each incremental sibling, 6.05; 95%CI, 2.26–16.2; P<0.001). Furthermore, in the stratified analyses by mode of delivery (Table 4) and by feeding status (Table 5), the results did not change materially across the strata. In addition to the differences in nasal microbiota profiles, infants with siblings had a significantly lower bacterial richness (P<0.001) and alpha-diversity index (Shannon index, P<0.001) compared to those without (Table 1).

All 105 fecal samples yielded high quality reads (rarefaction cutoff, 1,470 reads per sample). The sequencing identified 7 phyla and 108 genera. The fecal microbiota was composed primarily of three genera – *Escherichia* (23%), *Bifidobacterium* (20%), *Enterobacter* (17%) – followed by *Bacteroides* (9%) and *Veillonella* (6%). Partitioning around medoids clustering of fecal microbiota identified three distinct microbiota profiles: 1) *Escherichia*-dominant (31%), 2) *Bifidobacterium*-dominant (39%), and 3) *Enterobacter*-dominant (30%) (Figure 1b). The *Bifidobacterium*-dominant profile had the highest bacterial richness (P=0.005) and Shannon index (P=0.02; Table 6). The NMDS plots also demonstrated that the infants cluster together according to their fecal microbiota profile (Figure 2b). Additionally, there was a borderline significant association between nasal airway microbiota profiles and fecal microbiota profiles within subjects (P=0.07 by marginal homogeneity test; Table 7). Fecal microbiota profiles differed between infants with siblings and those without.

For example, infants with siblings were more likely to have a *Bifidobacterium*-dominant profile than an *Escherichia*-dominant profile (49% vs. 24%; Table 1) while those without siblings had the opposite pattern (32% vs. 37%), corresponding to an adjusted RRR of 2.90 (95%CI, 1.03–8.20; P=0.04; Table 3). In the sensitivity analysis, infants with a higher number of siblings had a non-significantly higher RRR to have a *Bifidobacterium*-dominant profile (adjusted RRR per each incremental sibling, 1.98; 95%CI, 0.94–4.15; P=0.07). In the stratified analyses by mode of delivery (Table 4) and by feeding status (Table 5), while the statistical power is limited owing to the small sample sizes, the direction of association (i.e., the point estimates of RRR) did not change across the strata.

DISCUSSION

In this study of 105 community-based healthy infants, we identified three nasal airway microbiota profiles and three fecal microbiota profiles. We also observed, both before and after adjusting for potential confounders, associations between having siblings in the household and two microbiota profiles: *Moraxella*-dominant nasal microbiota profile and *Bifidobacterium*-dominant fecal microbiota profile. Additionally, there was a non-significant association between nasal airway microbiota profiles and fecal microbiota profiles within the subjects.

Within the sparse literature on airway microbiota in infants, our findings are in agreement with those in the Childhood Asthma Study.[26] By applying 16S rRNA gene sequencing on the nasopharyngeal samples from 234 Australian infants at high-risk of atopy, the authors reported that co-habiting with siblings was associated with higher abundances of *Moraxella* genus in the nasopharynx. This finding is of particular interest because a recent cohort study of 60 Dutch infants found that early (1.5 to 6 months) colonization by *Moraxella* in the upper airway is associated with a lower frequency of respiratory infection in early childhood,[27] which is a major risk factor of incident asthma.[28] Although the underlying mechanism remains to be elucidated, these findings collectively suggest that cohabiting with siblings and *Moraxella*-dominant airway microbiota profile in early childhood, via fewer lower respiratory infections thereby protecting against airway damage and remodeling during the critical window of lung development, lead to a lower risk of incident asthma. However, Bisgaard et al., by applying a culture-dependent approach on the hypopharyngeal aspirate from 312 healthy neonates in Denmark, found that colonization of the airways with *M. catarrhalis* (as well as *S. pneumoniae* or *H. influenzae*) was associated with a higher risk of incident asthma by age 5 years.[29] These data, including the apparent discrepancies, underscore the importance of high-quality longitudinal studies examining the complex interplay between early-life environmental exposures, the airway microbiome, and asthma pathogenesis.

Our data also demonstrated that infants with siblings had a significantly lower bacterial richness and alpha-diversity index in the nasal airway. Although there has been only one study using a culture-independent approach that have examined the relationship between cohabiting siblings in the household and upper airway microbiota (the Childhood Asthma Study[26]), this previous study did not examine bacterial richness or alpha-diversity. Our novel finding is intriguing because it is generally considered that a higher bacterial richness and

diversity is favorable. Yet, recent studies have demonstrated that a higher bacterial diversity may be associated with diseases.[16,30,31] For instance, Huang *et al.* reported that patients with asthma had a higher bacterial richness and diversity in their airway when compared to healthy controls.[30] These data collectively suggest that the structure of microbiota (and likely the function of microbiota), rather than bacterial richness or diversity in the airway niche, plays an important role in the development of respiratory diseases.

Previous studies on fecal microbiota in infants have demonstrated inconsistent associations between the presence of siblings and microbial composition – e.g., having siblings is associated with increased abundances of *Bifidobacterium* (similar to our finding),[11,32] *Faecalibacterium*,[9] and *Bacteroides,*[33] and decreased abundances of *Peptostreptococcaceae.*[10] Potential explanations for this inconsistency include differences in study population, setting, and laboratory techniques for microbial identification (e.g., culture,[34] bacterial PCR[32,33]), or any combinations of these factors. Nevertheless, our data build on prior research linking the presence of siblings to the human microbiota, and extend them by examining both airway and fecal microbiota using the 16S rRNA gene sequencing approach in U.S. infants.

The mechanisms linking the presence of siblings in the household to unique microbiota profiles in infants is likely multifactorial. The original explanation was the exposure to microbes from older siblings through direct contact or intermediate reservoirs in the home environment (e.g., house dust and maternal microbiota).[6] Alternatively, the presence of siblings may contribute, via *in-utero* immunological programming ("birth order effect"[33]), to perturbation of microbiota in infants. These two possibilities are not mutually exclusive.

Our study has potential limitations. First, the cross-sectional analysis was unable to examine the association between the microbiota and the development of childhood asthma. To address this important question, the children are being followed longitudinally, with airway and fecal sampling at multiple time-points. Second, airway microbiota was examined in nasal airway samples since lower airway sampling in asymptomatic infants is both technically and ethically challenging. Nevertheless, prior research has reported a strong correlation between upper and lower airway microbiology in children.[35] In addition, one may surmise that the anterior nasal swab sampling is susceptible to contamination with skin microbiota compared to "deeper" sampling methods (e.g., nasopharyngeal aspirates), our samples demonstrated that *Moraxella* genus was the most abundant genera and that *Staphylococcus* genus accounted for only 9% of microbiota. The observed relative abundance of *Staphylococcus* was also consistent with the recent study of Australian infants – *Staphylococcus* accounted for 10% of their nasopharyngeal microbiota.[26] Therefore, it is unlikely that potential contamination with skin microbiota would have substantially affected our results. Third, 16S rRNA gene sequencing precluded us from assessing the functional capacity of microbiota. We hope to address this important issue in future work using metagenomic and metatranscriptomic approaches. Fourth, our inferences might be biased by unmeasured confounders (e.g., use of complementary food in older infants). In addition, because of the relatively small number of subjects within microbiota profiles, we were unable to adjust for all sets of potential confounders that were measured in the study. However, the significant associations persisted after controlling for clinically and

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biologically important covariates. Finally, this single-year study recruited infants from one hospital, which may limit our generalizability to other settings.

Conclusions

In summary, in this cross-sectional study of community-based healthy infants, we identified three nasal airway microbiota profiles and three fecal microbiota profiles. We also found that infants living with siblings are more likely to have a *Moraxella*-dominant nasal microbiota profile and a *Bifidobacterium*-dominant fecal microbiota profile. Our findings should facilitate further epidemiologic and mechanistic investigation of the interplay between early-life environmental exposures, the microbiome, and asthma pathogenesis, which, in turn, could offer a new avenue (e.g., microbiome modification) for the primary prevention of childhood asthma.

Acknowledgments

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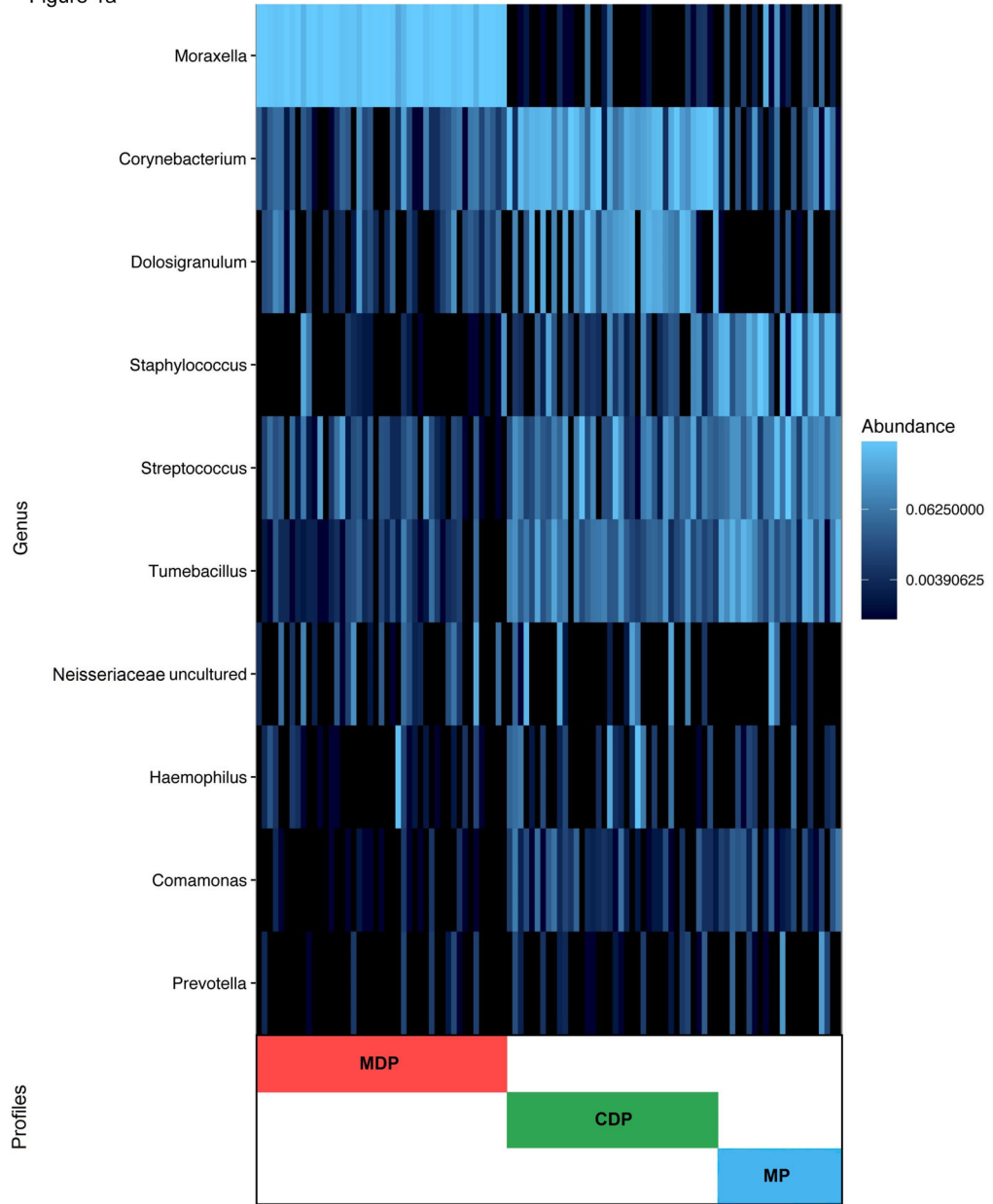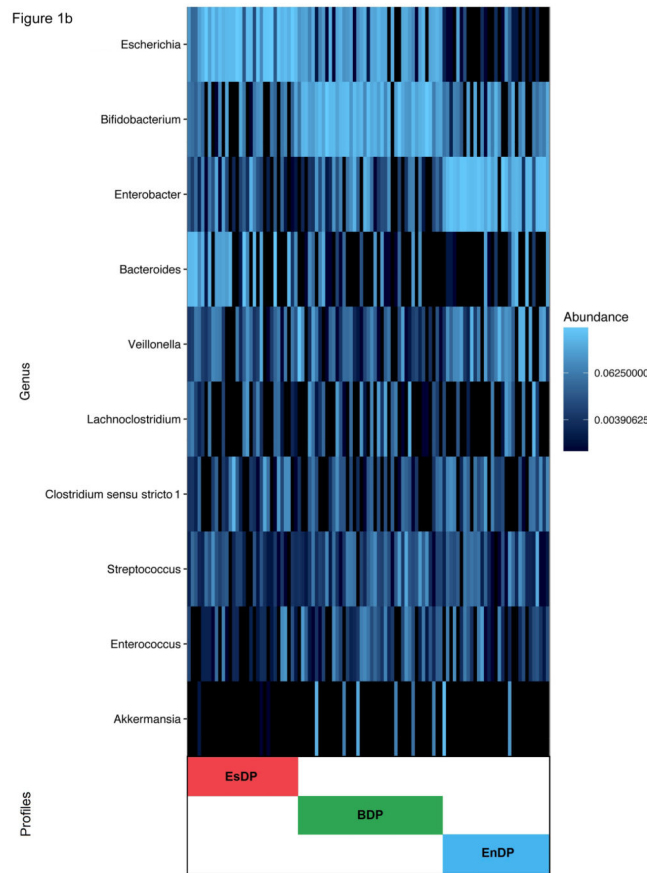Figure 1a

Figure 1. Clustering and Composition in Nasal Airway and Fecal Microbiota in 105 Infants
All nasal airway and fecal microbiota profiles of 105 infants were clustered using the partitioning around medoids clustering method with the use of Bray-Curtis distance. The optimal number of clusters was identified by using the gap statistic. To obtain further information about the bacterial composition of samples within microbiota profiles, the ten most abundant genera present in an adjacent heatmap were displayed. **A)** Nasal airway microbiota. Colored bars indicate three microbiota profiles: *Moraxella*-dominant (red), *Corynebacterium/Dolosigranulum*-dominant (green), and mixed (blue). MDP = *Moraxella*-dominant profile; CDP= *Corynebacterium/Dolosigranulum*-dominant profile; MP = mixed profile. **B)** Fecal microbiota. Colored bars indicate three microbiota profiles: *Escherichia*-dominant (red), *Bifidobacterium* -dominant (green), and *Enterobacter*-dominant (blue). EsDP = *Escherichia*-dominant profile; BDP = *Bifidobacterium*-dominant profile; EnDP = *Enterobacter*-dominant profile.

Figure 2a
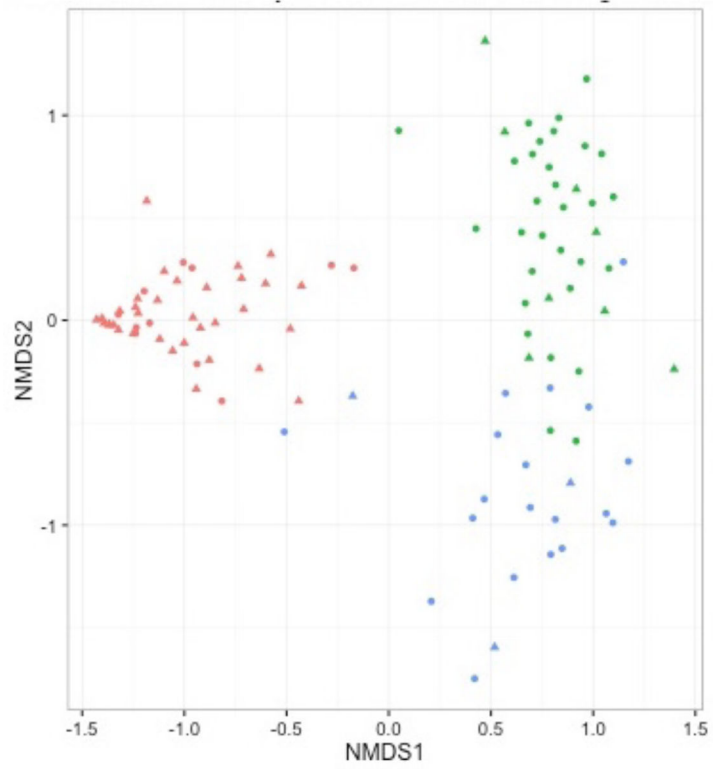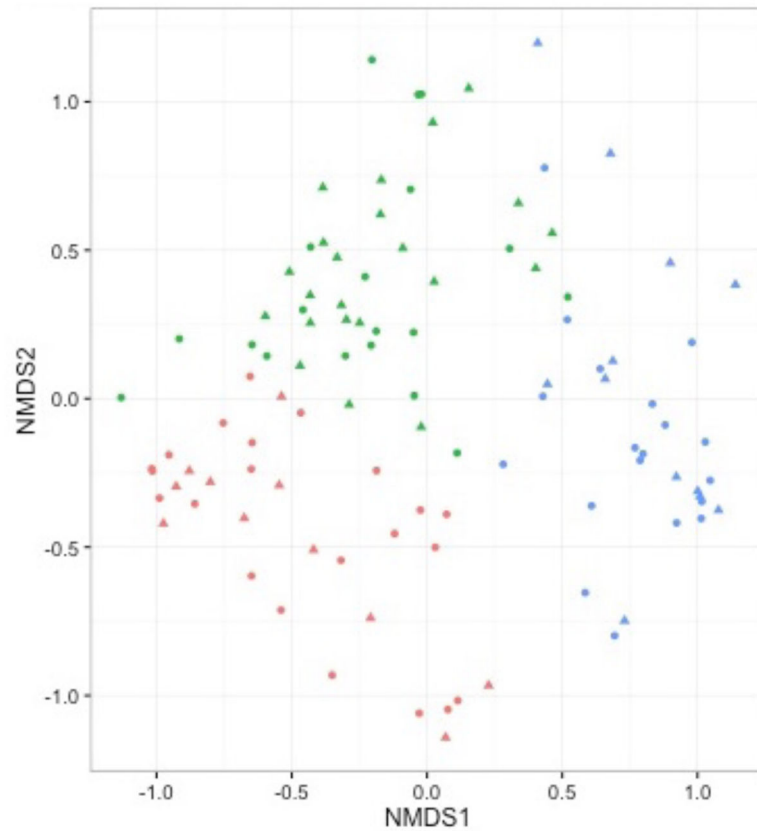
Figure 2b

Figure 2. Nonmetric Multidimensional Scaling Plots on Nasal Airway and Fecal Microbiota
To show the differences in nasal airway and fecal microbiota among 105 infants, nonmetric
multidimensional scaling (NMDS) plots based on the Bray-Curtis distance between all
infants were generated. Each *dot* represents the overall bacterial community in each subject.
Shapes indicate the status of siblings at household: infants with sibling (triangle) and those
without (circle). **A)** Nasal airway microbiota. Colors indicate three microbiota profiles:
Moraxella-dominant (red), *Corynebacterium/Dolosigranulum*-dominant (green), and mixed
(blue). **B)** Fecal microbiota. Colors indicate three microbiota profiles: *Escherichia*-dominant
(red), *Bifidobacterium*-dominant (green), and *Enterobacter*-dominant (blue).

Table 1

Infant and Microbiota Characteristics, According to the Presence of Siblings in the Household

Characteristics	Infants with siblings n=45 (43%)	Infants without siblings n=60 (57%)	P-value
Subject characteristics			
Demographics			
Age (mo)			0.45
<2	8 (18)	17 (28)	
2–5.9	27 (60)	31 (52)	
6–12	10 (22)	12 (20)	
Male sex	31 (69)	28 (47)	0.04
Race/ethnicity			0.01
Non-Hispanic white	20 (44)	35 (58)	
Non-Hispanic black	7 (16)	3 (5)	
Hispanic	4 (9)	14 (23)	
Other	14 (31)	8 (13)	
Parental history of asthma	9 (20)	11 (18)	0.99
Prenatal history			
Maternal antibiotic use during pregnancy	7 (16)	6 (10)	0.58
Maternal antibiotic use during labor	14 (31)	17 (28)	0.93
Maternal smoking during pregnancy	3 (7)	0 (0)	0.15
Past medical history			
Mode of birth			0.47
Vaginal birth	26 (58)	40 (67)	
C-section	19 (42)	20 (33)	
Prematurity (32–37 weeks)	5 (11)	5 (8)	0.89
History of eczema	7 (16)	9 (15)	0.99
Previous breathing problems before enrollment	0 (0)	0 (0)	_*
Systemic antibiotic use before enrollment	6 (13)	6 (10)	0.83
Systemic corticosteroid use before enrollment	0 (0)	0 (0)	_*
Home environmental characteristics			
Smoking exposure at home	3 (7)	0 (0)	0.15
Mostly breastfed for the first 3 months of age	32 (71)	49 (82)	0.30
Ever attended daycare	10 (22)	4 (7)	0.04
Number of siblings in the household			_*
0	0 (0)	60 (100)	
1	36 (80)	0 (0)	
2	5 (11)	0 (0)	
3	3 (7)	0 (0)	
4	1 (2)	0 (0)	
Microbiota characteristics			
Nasal microbiota			

Characteristics	Infants with siblings n=45 (43%)	Infants without siblings n=60 (57%)	P-value
Number of genera, median (IQR)	11 (8–22)	23 (16–30)	<0.001
Shannon index, median (IQR)	0.63 (0.22–1.03)	1.30 (0.92–1.76)	<0.001
Microbiota profiles			
Moraxella-dominant profile	34 (76)	11 (18)	<0.001 [†]
Corynebacterium/Dolosigranulum-dominant profile	8 (18)	30 (50)	-
Mixed profile	3 (7)	19 (32)	0.48 [†]
Fecal microbiota			
Number of genera, median (IQR)	16 (11–21)	14 (10–18)	0.051
Shannon index, median (IQR)	1.40 (1.08–1.87)	1.30 (0.98–1.70)	0.35
Microbiota profiles			
Escherichia-dominant profile	11 (24)	22 (37)	-
Bifidobacterium-dominant profile	22 (49)	19 (32)	0.08 [‡]
Enterobacter-dominant profile	12 (27)	19 (32)	0.65 [‡]

Data are no. (%) of infants unless otherwise indicated. Percentages may not equal 100 because of missingness or rounding IQR, interquartile range

[*] Not computed

[†] Computed by unadjusted multinomial regression model with *Corynebacterium/Dolosigranulum*-dominant profile as reference group

[‡] Computed by unadjusted multinomial regression model with *Escherichia*-dominant profile as reference group

Table 2

Richness, Alpha-diversity and Relative Abundance by Nasal Airway Microbiota Profile

	Moraxella-dominant profile n=45 (43%)	*Corynebacterium/ Dolosigranulum*-dominant profile n=38 (36%)	Mixed profile n=22 (21%)	P-value
Richness, median (IQR)				
Number of genera	11 (7–17)	23 (17–33)	27 (15–29)	<0.001
Alpha-diversity, median (IQR)				
Shannon index	0.51 (0.18–0.84)	1.32 (1.04–1.70)	1.70 (1.01–2.01)	<0.001
Relative abundance of 10 most common genera, mean (SD)				
Moraxella	0.85 (0.17)	0.00 (0.01)	0.03 (0.09)	0.003 [*]
Corynebacterium	0.03 (0.05)	0.41 (0.28)	0.05 (0.08)	0.003 [*]
Dolosigranulum	0.02 (0.04)	0.20 (0.21)	0.01 (0.02)	0.003 [*]
Staphylococcus	0.01 (0.06)	0.03 (0.06)	0.36 (0.32)	0.003 [*]
Streptococcus	0.03 (0.06)	0.09 (0.15)	0.17 (0.23)	0.02 [*]
Tumebacillus	0.01 (0.02)	0.06 (0.08)	0.17 (0.17)	0.003 [*]
Neisseriaceae uncultured	0.02 (0.06)	0.04 (0.11)	0.03 (0.12)	0.98 [*]
Haemophilus	0.02 (0.10)	0.04 (0.13)	0.00 (0.01)	0.98 [*]
Comamonas	0.00 (0.00)	0.01 (0.02)	0.01 (0.02)	0.01 [*]
Prevotella	0.00 (0.00)	0.00 (0.01)	0.02 (0.06)	0.15 [*]

Abbreviations: IQR, interquartile range; SD, standard deviation

[*] Benjamini-Hochberg adjusted P-value accounting for multiple comparisons

Table 3

Associations between Having Siblings in Household and Microbiota Profiles

Microbiota and models	RRR (95% CI)	P-value
Nasal microbiota		
Unadjusted model		
Moraxella-dominant profile	11.6 (4.12–32.6)	<0.001
Corynebacterium/Dolosigranulum-dominant profile	Reference	-
Mixed profile	0.59 (0.14–2.51)	0.48
Adjusted model [*]		
Moraxella-dominant profile	18.9 (5.30–67.3)	<0.001
Corynebacterium/Dolosigranulum-dominant profile	Reference	-
Mixed profile	0.62 (0.14–2.85)	0.54
Sensitivity analysis [*†]		
Moraxella-dominant profile	6.05 (2.26–16.2)	<0.001
Corynebacterium/Dolosigranulum-dominant profile	Reference	-
Mixed profile	0.44 (0.11–1.75)	0.24
Fecal microbiota		
Unadjusted model		
Escherichia-dominant profile	Reference	-
Bifidobacterium-dominant profile	2.32 (0.90–5.98)	0.08
Enterobacter-dominant profile	1.26 (0.45–3.51)	0.65
Adjusted model [‡]		
Escherichia-dominant profile	Reference	-
Bifidobacterium-dominant profile	2.90 (1.03–8.20)	0.04
Enterobacter-dominant profile	1.50 (0.44–5.12)	0.51
Sensitivity analysis [†‡]		
Escherichia-dominant profile	Reference	-
Bifidobacterium-dominant profile	1.98 (0.94–4.15)	0.07
Enterobacter-dominant profile	1.24 (0.51–2.99)	0.64

RRR, relative risk ratio; CI, confidence interval

[*] Adjusted for age, sex, maternal antibiotic use during pregnancy, maternal smoking, and history of systemic antibiotic use in infancy

[†] Modeling the number of siblings as ordinal variable

[‡] Adjusted for age, sex, maternal antibiotic use during pregnancy, maternal smoking, breast-feeding status, and history of systemic antibiotic use in infancy

Table 4

Unadjusted Associations between Having Siblings in Household and Microbiota Profiles by Mode of Delivery[*]

Groups and microbiota profiles	RRR (95% CI)	P-value
Nasal microbiota		
Vaginal delivery (n=66)		
Moraxella-dominant profile	12.7 (3.31–48.5)	<0.001
Corynebacterium/Dolosigranulum-dominant profile	Reference	-
Mixed profile	0.25 (0.03–2.41)	0.23
C-section (n=39)		
Moraxella-dominant profile	10.3 (2.00–52.7)	0.005
Corynebacterium/Dolosigranulum-dominant profile	Reference	-
Mixed profile	1.83 (0.22–15.3)	0.58
Fecal microbiota		
Vaginal delivery (n=66)		
Escherichia-dominant profile	Reference	-
Bifidobacterium-dominant profile	1.59 (0.49–5.14)	0.44
Enterobacter-dominant profile	1.09 (0.31–3.88)	0.89
C-section (n=39)		
Escherichia-dominant profile	Reference	-
Bifidobacterium-dominant profile	4.28 (0.80–22.9)	0.09
Enterobacter-dominant profile	1.67 (0.28–9.82)	0.57

RRR, relative risk ratio; CI, confidence interval

[*] Multivariable-adjustment was not performed given the decreased sample sizes

Table 5

Unadjusted Associations between Having Siblings in Household and Microbiota Profiles in 83 Infants Aged <6 Months by Feeding Status

Groups and microbiota profiles	RRR (95% CI)	P-value
Nasal microbiota		
Breast-feeding (n=61)		
Moraxella-dominant profile	21.0 (4.58–96.3)	<0.001
Corynebacterium/Dolosigranulum-dominant profile	Reference	-
Mixed profile	1.27 (0.18–8.79)	0.81
Formula-feeding (n=22)		
Moraxella-dominant profile	5.79 (4.06–8.24)	<0.001
Corynebacterium/Dolosigranulum-dominant profile	Reference	-
Mixed profile	7.39 (0.61–89.5)	0.20
Fecal microbiota		
Breast-feeding (n=61)		
Escherichia-dominant profile	Reference	-
Bifidobacterium-dominant profile	2.17 (0.57–8.19)	0.25
Enterobacter-dominant profile	1.60 (0.41–6.21)	0.50
Formula-feeding (n=22)		
Escherichia-dominant profile	Reference	-
Bifidobacterium-dominant profile	2.00 (0.18–22.01)	0.57
Enterobacter-dominant profile	1.25 (0.12–13.2)	0.85

RRR, relative risk ratio; CI, confidence interval

*
Multivariable-adjustment was not performed given the decreased sample sizes

Table 6

Richness, Alpha-diversity, and Relative Abundance by Fecal Microbiota Profile

	Escherichia-dominant profile n=33 (31%)	*Bifidobacterium*-dominant profile n=41 (39%)	*Enterobacter*-dominant profile n=31 (30%)	P-value
Richness, median (IQR)				
Number of genera	13 (10–20)	17 (12–21)	11 (9–15)	0.005
Alpha-diversity, median (IQR)				
Shannon index	1.16 (0.82–1.72)	1.62 (1.18–1.90)	1.26 (0.97–1.61)	0.02
Relative abundance of 10 most common genera, mean (SD)				
Escherichia	0.46 (0.29)	0.20 (0.15)	0.01 (0.05)	0.003 [*]
Bifidobacterium	0.06 (0.08)	0.38 (0.21)	0.10 (0.12)	0.003 [*]
Enterobacter	0.04 (0.09)	0.06 (0.10)	0.47 (0.24)	0.003 [*]
Bacteroides	0.21 (0.25)	0.03 (0.07)	0.05 (0.13)	0.003 [*]
Veillonella	0.06 (0.14)	0.03 (0.05)	0.12 (0.14)	0.11 [*]
Lachnoclostridium	0.02 (0.04)	0.04 (0.08)	0.03 (0.09)	0.99 [*]
Clostridium sensu stricto 1	0.04 (0.10)	0.01 (0.02)	0.04 (0.05)	0.89 [*]
Streptococcus	0.01 (0.02)	0.04 (0.06)	0.03 (0.07)	0.89 [*]
Enterococcus	0.01 (0.03)	0.03 (0.04)	0.02 (0.04)	0.99 [*]
Akkermansia	0.00 (0.00)	0.02 (0.08)	0.03 (0.11)	0.99 [*]

Abbreviations: IQR, interquartile range; SD, standard deviation

[*] Benjamini-Hochberg adjusted P-value accounting for multiple comparisons

Table 7

Within-subject Association Between Nasal Airway Microbiota Profiles and Fecal Microbiota Profiles [*]

Fecal microbiota profile (n)	Nasal airway microbiota profile (n)		
	Moraxella-dominant profile	*Corynebacterium/Dolosigranulum*-dominant profile	Mixed profile	Sum
Escherichia-dominant profile	10	13	10	33
Bifidobacterium-dominant profile	24	11	6	41
Enterobacter-dominant profile	11	14	6	31
Sum	45	38	22	105

[*]
Marginal homogeneity test, P=0.07