The Fecal Microbiota Profile and Bronchiolitis in Infants

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abstract

BACKGROUND: Little is known about the association of gut microbiota, a potentially modifiable factor, with bronchiolitis in infants. We aimed to determine the association of fecal microbiota with bronchiolitis in infants.

METHODS: We conducted a case–control study. As a part of multicenter prospective study, we collected stool samples from 40 infants hospitalized with bronchiolitis. We concurrently enrolled 115 age-matched healthy controls. By applying 16S rRNA gene sequencing and an unbiased clustering approach to these 155 fecal samples, we identified microbiota profiles and determined the association of microbiota profiles with likelihood of bronchiolitis.

RESULTS: Overall, the median age was 3 months, 55% were male, and 54% were non-Hispanic white. Unbiased clustering of fecal microbiota identified 4 distinct profiles: *Escherichia*-dominant profile (30%), *Bifidobacterium*-dominant profile (21%), *Enterobacter/Veillonella*-dominant profile (22%), and *Bacteroides*-dominant profile (28%). The proportion of bronchiolitis was lowest in infants with the *Enterobacter/Veillonella*-dominant profile (15%) and highest in the *Bacteroides*-dominant profile (44%), corresponding to an odds ratio of 4.59 (95% confidence interval, 1.58–15.5; $P = .008$). In the multivariable model, the significant association between the *Bacteroides*-dominant profile and a greater likelihood of bronchiolitis persisted (odds ratio for comparison with the *Enterobacter/Veillonella*-dominant profile, 4.24; 95% confidence interval, 1.56–12.0; $P = .005$). In contrast, the likelihood of bronchiolitis in infants with the *Escherichia*-dominant or *Bifidobacterium*-dominant profile was not significantly different compared with those with the *Enterobacter/Veillonella*-dominant profile.

CONCLUSIONS: In this case–control study, we identified 4 distinct fecal microbiota profiles in infants. The *Bacteroides*-dominant profile was associated with a higher likelihood of bronchiolitis.

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WHAT'S KNOWN ON THIS SUBJECT: Recent studies have demonstrated a link between gut microbiota and respiratory diseases, such as asthma. However, little is known about the association of gut microbiota, a potentially modifiable factor, with bronchiolitis in infants.

WHAT THIS STUDY ADDS: In this case–control study of infants hospitalized with bronchiolitis and healthy age-matched controls, we identified 4 distinct fecal microbiota profiles in their fecal samples. We found that the *Bacteroides*-dominant microbiota profile was associated with a higher likelihood of bronchiolitis.

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Bronchiolitis is a major public health problem for children in the United States and worldwide.[1–3] Indeed, bronchiolitis is the leading cause of hospitalizations in US infants, accounting for 18% of all infant hospitalizations.[3] Although causative viral pathogens (eg, respiratory syncytial virus [RSV]) are ubiquitous, not all infants develop bronchiolitis.[4] Likewise, severity of infection ranges from a minor nuisance to fatal bronchiolitis. The reasons for these differences remain largely unclear.[5]

The recent advent of culture-independent techniques revealed the presence of highly functional communities of microbes inhabiting the human body (ie, the human microbiota) that contribute to host immune development and homeostasis.[6] Within the human body, the intestinal tract is the most densely colonized surface, with bacterial loads of $\sim 10^{14}$ bacteria.[7] Disruption of balance in the gut microbiota and microbiota-derived regulatory T cell response are linked with inflammatory diseases in the local environment (eg, inflammatory bowel disease).[8,9] Recent studies also demonstrate that the gut microbiota modulates the immune function in distant mucosal locations, such as the respiratory tract,[7] and thereby remotely contributes to pathogenesis of asthma and cystic fibrosis.[10–12] Despite the evidence suggesting the existence of a "common mucosal response" in host immune development,[13,14] to the best of our knowledge no studies have investigated the relationship of gut microbiota, a potentially modifiable factor, with bronchiolitis in infants.

In this context, we conducted a case–control study of a multicenter prospective cohort of infants hospitalized with bronchiolitis and healthy matched controls to determine the association of fecal microbiota and bronchiolitis in infants.

METHODS

Study Design, Setting, and Participants

We conducted a case–control study to examine the fecal microbiota of infants hospitalized with bronchiolitis (cases) and that of healthy infants (controls). As part of a multicenter prospective cohort study, the 35th Multicenter Airway Research Collaboration,[15] we enrolled 40 infants age <12 months hospitalized with an attending physician diagnosis of bronchiolitis at 1 of 3 US hospitals (Alfred I. duPont Hospital for Children, Wilmington, DE; Boston Children's Hospital, Boston, MA; and Kosair Children's Hospital, Louisville, KY) during a bronchiolitis season from November 2013 through April 2014. Bronchiolitis was defined by American Academy of Pediatrics guidelines as an acute respiratory illness with some combination of rhinitis, cough, tachypnea, wheezing, crackles, and retractions.[4] We excluded infants with previous enrollment into the 35th Multicenter Airway Research Collaboration, those who were transferred to a participating hospital >48 hours after the original hospitalization, those whose parents gave consent >24 hours after hospitalization, or those with known cardiopulmonary disease, immunodeficiency, immunosuppression, or gestational age $\leq$32 weeks.

Healthy infants (n = 115) were enrolled as the controls in this case–control study. The setting and participants have been reported previously.[16] Briefly, using a standardized protocol, we enrolled healthy infants (age-matched within 1.5 months of cases) from a primary care group practice at Massachusetts General Hospital (Boston, MA) from November 2013 through May 2014. We excluded infants with current fever, respiratory illness, or gastrointestinal illness,[17] or antibiotic treatment in the preceding 7 days. The institutional review board at each of the participating hospitals approved the study. Written informed consent was obtained from the parent or guardian.

Data and Sample Collection

Site investigators conducted a structured interview and chart review that assessed patients' demographic characteristics, family history, prenatal and past medical history, home environmental characteristics, and hospital course (in the cases only). All data were reviewed at the Study Coordinating Center, and site investigators were queried about missing data and discrepancies identified by manual data checks.

Fecal samples were collected via a standardized protocol at the time of hospitalization (in the cases) or at home before the clinic visit (in the controls). First, diapers containing feces were refrigerated or stored in a cooler by hospital staff or parents immediately after collection. The fecal samples were then placed in sterile Sarstedt feces collection containers (Sarstedt, Nümbrecht, Germany) and immediately stored at −80°C. Frozen samples were shipped on dry ice to Baylor College of Medicine, where we characterized the microbiota via 16S rRNA gene sequencing.

16s rRNA Gene Sequencing

We adapted 16S rRNA gene sequencing methods from the methods developed for the National Institutes of Health Human Microbiome Project.[18,19] Briefly, bacterial genomic DNA was extracted with a Mo BIO PowerMag DNA Isolation Kit (Mo Bio Laboratories, Carlsbad, CA). The 16S rDNA V4 region was amplified by polymerase chain reaction (PCR) and sequenced in the MiSeq platform (Illumina, San Diego, CA) via the 2- × 250-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.[20]

Sequencing read pairs were demultiplexed based on the unique molecular barcodes, and reads were merged in USEARCH v7.0.1090,[21] allowing 0 mismatches and a minimum overlap of 50 bases. Merged reads were trimmed at the first base with a Q5 quality score. In addition, a quality filter was applied to the resulting merged reads, and reads containing >0.05 expected errors were discarded. Rarefaction curves of bacterial operational taxonomic units (OTUs) were constructed with 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 resequenced to ensure that the majority of bacterial taxa were encompassed in our analyses. Details of the quality control may be found in the Supplemental Information.

The 16S rRNA gene sequences were clustered into OTUs at a similarity cutoff value of 97% via the UPARSE algorithm.[22] OTUs were mapped to the SILVA Database[23] containing only the 16S V4 region to determine taxonomies. We recovered abundances 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 2 steps for downstream analyses of α-diversity (eg, Shannon index) and β-diversity (eg, Bray–Curtis distance).

Statistical Analyses

We calculated the relative abundance of each OTU for each fecal sample. We conducted analyses at the genus level; because our sequences were dominated by 1 OTU per genus, we collapsed all OTUs assigned to the same genus into a single group for reporting.[24] To identify fecal microbiota profiles, we performed unbiased clustering by partitioning around medoids[25] by using Bray–Curtis distances. Each cluster is defined by a point designated as the center, the "medoid," and minimizes the distance between samples in a cluster. We determined the number of clusters to choose for the data by using the gap statistic.[26]

To examine the association of microbiota profiles with the likelihood of being a bronchiolitis case, we constructed 2 logistic regression models. First, we fitted an unadjusted model that included only microbiota profiles as the independent variable. Second, we constructed an adjusted model controlling for ≤5 potential confounders (ie, age, gender, prematurity, mode of birth, and history of systemic antibiotic use before enrollment), given the small number of bronchiolitis cases. These variables were chosen based on clinical plausibility and a priori knowledge.[4,5,27] We did not control for breastfeeding status because it was considered an ancestor variable of the association of interest (ie, the relationship between breastfeeding and likelihood of bronchiolitis may be mediated by gut microbiota), and adjustment of an ancestor variable would bias the inference toward the null.

Next, to compare the abundances of bacteria within fecal microbiota between bronchiolitis cases and healthy controls, we used the linear discriminant analysis effect size method.[28] In this method, vectors resulting from the comparison of abundances (eg, *Bacteroides* relative abundance) between the groups are used as inputs to the linear discriminant analysis. This method has the advantage over traditional statistical tests (eg, pairwise tests) that an effect size is produced in addition to a *P* value. This advantage enables us to sort the results of multiple testing by the magnitude of the between-group difference, not only by *P* values, because the 2 are not necessarily correlated.[28] Analyses used R version 3.2 with the phyloseq package.[29]

RESULTS

Study Population

At the 4 participating hospitals, we enrolled a total of 40 infants hospitalized with bronchiolitis (cases) and 115 age-matched healthy infants (controls). Overall, the median age was 3 months (IQR, 2–5 months), 55% were male, and 54% were non-Hispanic white. Of cases of bronchiolitis, RSV was detected in 65% and rhinovirus in 23%. Subject characteristics differed between cases and controls (Table 1). For example, compared with healthy controls, infants with bronchiolitis were more likely to have a parental history of asthma, maternal antibiotic use during pregnancy, a history of premature birth, a sibling at home, and corticosteroid use before the enrollment, but they were less likely to be breastfed (all *P*s < .05).

Fecal Microbiota Sequence and Profiles

We analyzed fecal samples from all enrolled infants by 16S rRNA gene sequencing and obtained 484 669 high-quality merged sequences, of which 456 888 (94%) were mapped to the database. All 155 samples had sufficient sequence depth to obtain a high degree of sequence coverage (rarefaction cutoff, 1470 reads per sample) and were used for the analysis. The fecal microbiota was composed primarily of 4 genera, *Escherichia* (22%), *Bifidobacterium* (19%), *Enterobacter* (15%), and *Bacteroides* (13%), followed by *Veillonella* (5%).

Partitioning around medoid clustering of fecal microbiota identified 4 distinct microbiota

profiles (Fig 1): *Escherichia*-dominant profile (30%), *Bifidobacterium*-dominant profile (21%), *Enterobacter/Veillonella*-dominant profile (22%), and *Bacteroides*-dominant profile (28%). The first 2 profiles were dominated by either the *Escherichia* or *Bifidobacterium* genus, and the third profile was codominated by *Enterobacter* and *Veillonella* genera (Table 2). The fourth profile had the highest relative abundance of *Bacteroides,* with highest bacterial richness ($P < .001$) and α-diversity index (Shannon index, $P < .001$). The nonmetric multidimensional scaling of fecal microbiota also revealed that the subjects clustered together according to their microbiota profile (Fig 2). Some of the patient characteristics differed across the 4 microbiota profiles (Supplemental Table 4). For instance, compared with infants with an *Enterobacter/Veillonella*-dominant profile, those with a *Bacteroides*-dominant profile were older and more likely to have maternal smoking history during pregnancy and history of vaginal birth (both $P < .05$).

TABLE 1 Characteristics of Infants With Bronchiolitis (Cases) and Healthy Infants (Controls)

Characteristics	Infants With Bronchiolitis (Cases), *n* = 40	Healthy Infants (Controls), *n* = 115	*P*
Demographics			
Age, mo, mean (SD)	4 (3)	4 (2)	.76
Male gender	18 (55)	64 (56)	.99
Race or ethnicity			.04
Non-Hispanic white	23 (57)	61 (53)	
Non-Hispanic black	6 (15)	11 (10)	
Hispanic	10 (25)	19 (17)	
Other	1 (2)	24 (21)	
Parental history of asthma	16 (40)	21 (18)	.01
Prenatal history			
Maternal smoking during pregnancy	8 (20)	3 (3)	.001
Maternal antibiotic use during pregnancy	11 (29)	13 (11)	.02
Maternal antibiotic use during labor	12 (34)	35 (30)	.82
Past medical history and home environmental characteristics			
Mode of birth			.13
Vaginal birth	31 (78)	72 (63)	
Cesarean delivery	9 (22)	43 (37)	
Prematurity (32–37 wk)	12 (30)	11 (10)	.004
Previous breathing problems before enrollment[a]	8 (21)	0 (0)	<.001
History of eczema	8 (21)	17 (15)	.56
Ever attended day care	9 (23)	14 (12)	.16
Sibling at home	34 (87)	47 (41)	<.001
Smoking exposure at home	8 (21)	4 (3)	.002
Mostly breastfed for the first 3 mo of age	16 (52)	89 (77)	.009
Systemic antibiotic use before enrollment	8 (21)	13 (11)	.24
Systemic corticosteroid use before enrollment	9 (23)	0 (0)	<.001
Hospitalization course			
Hospital length-of-stay, d, median (IQR)	3 (2–4)	—	—
Admission to ICU	8 (20)	—	—
Use of mechanical ventilation[b]	5 (16)	—	—

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

[a] Defined as an infant having a cough that wakes him or her at night or causes emesis, or when the child has wheezing or shortness of breath without cough.

[b] Defined as use of continuous positive airway pressure or intubation during inpatient stay, regardless of location at any time during the index hospitalization.

Microbiota Profiles and Bronchiolitis

The proportion of infants with severe bronchiolitis differed across the 4 microbiota profile groups: lowest in the *Enterobacter/Veillonella*-dominant profile (15%) and highest in the *Bacteroides*-dominant profile (44%; Table 2), corresponding to an odds ratio (OR) of 4.59 (95% confidence interval [CI], 1.58–15.5; $P = .008$). In the multivariable model adjusting for age, gender, prematurity, mode of birth, and history of systemic antibiotic use, the association between the *Bacteroides*-dominant profile and a greater likelihood of severe bronchiolitis case remained significant (OR for comparison with the *Enterobacter/Veillonella*-dominant profile, 3.89; 95% CI, 1.19–14.6; $P = .03$; Table 3). In a sensitivity analysis adjusting for a different set of covariates (age, gender, parental history of asthma, maternal antibiotic use during pregnancy, and systemic corticosteroid use before enrollment), the results did not change materially: Infants with a *Bacteroides*-dominant profile had a greater likelihood of bronchiolitis (OR, 4.12; 95% CI, 1.28–15.2; $P = .02$).

In contrast, the likelihood of bronchiolitis in infants with an *Escherichia*-dominant or *Bifidobacterium*-dominant profile was not significantly different compared with those with an *Enterobacter/Veillonella*-dominant profile in both unadjusted and adjusted analyses. Similarly, the use of linear discriminant effect size method demonstrated that *Veillonella* genus was negatively associated with likelihood of bronchiolitis, whereas *Bacteroides* genus was positively associated with likelihood (both Benjamini–Hochberg adjusted *P*s < .05; Fig 3).

DISCUSSION

In this case–control study of 40 infants with bronchiolitis and 115 healthy age-matched controls, we identified 4 distinct fecal microbiota profiles. We found that, compared with infants with an *Enterobacter/Veillonella*-dominant profile, those with a *Bacteroides*-dominant

TABLE 2 Richness, α-Diversity, Relative Abundance, and Case–Control Status by Fecal Microbiota Profile

	Escherichia-Dominant Profile, *n* = 46 (30%)	*Bifidobacterium*-Dominant Profile, *n* = 32 (21%)	*Enterobacter/Veillonella*-Dominant Profile, *n* = 34 (22%)	*Bacteroides*-Dominant Profile, *n* = 43 (28%)	*P*
Richness					
Number of genera, median (IQR)	13 (10–17)	15 (11–17)	11 (9–14)	20 (15–25)	<.001
α-Diversity, median (IQR)					
Shannon index	1.86 (1.20–2.46)	1.96 (1.62–2.39)	1.69 (1.35–2.20)	2.51 (2.22–2.96)	<.001
Relative abundance of 10 most abundant genera, mean (SD)					
Escherichia	0.53 (0.22)	0.12 (0.12)	0.03 (0.08)	0.13 (0.14)	.003[a]
Bifidobacterium	0.12 (0.12)	0.50 (0.19)	0.07 (0.09)	0.12 (0.12)	.003[a]
Enterobacter	0.04 (0.10)	0.06 (0.11)	0.49 (0.26)	0.08 (0.11)	.003[a]
Bacteroides	0.03 (0.07)	0.04 (0.07)	0.04 (0.12)	0.37 (0.23)	.003[a]
Veillonella	0.03 (0.05)	0.02 (0.05)	0.15 (0.19)	0.02 (0.05)	.003[a]
Lachnospiraceae *incertae sedis*	0.07 (0.12)	0.02 (0.04)	0.04 (0.10)	0.07 (0.09)	.85[a]
Streptococcus	0.02 (0.04)	0.05 (0.11)	0.03 (0.06)	0.01 (0.01)	.49[a]
Clostridium sensu stricto 1	0.04 (0.08)	0.00 (0.01)	0.03 (0.05)	0.01 (0.02)	.20[a]
Enterococcus	0.02 (0.04)	0.02 (0.04)	0.02 (0.05)	0.01 (0.02)	.96[a]
Akkermansia	0.00 (0.02)	0.03 (0.09)	0.03 (0.11)	0.01 (0.08)	.99[a]
Case–control status					.01
Bronchiolitis	10 (22%)	6 (19%)	5 (15%)	19 (44%)	
Healthy control	36 (78%)	26 (81%)	29 (85%)	24 (56%)	

[a] Benjamini–Hochberg adjusted *P* value accounting for multiple comparisons.

profile had a higher likelihood of bronchiolitis. In contrast, the likelihood of bronchiolitis in infants with an *Escherichia*-dominant or *Bifidobacterium*-dominant profile was not significantly different. To our knowledge, this is the first study that has investigated the association of fecal microbiota with the risk of bronchiolitis in infants. Our study also highlights the importance of integrating discovery-driven (ie, the identification of microbiota profiles) and hypothesis-driven (ie, the determination of association between the microbiota profiles and bronchiolitis) approaches.

Studies of prebiotic and probiotic supplements, despite their heterogeneity in study populations, treatment regimens, and outcomes,[30] have demonstrated the promise of modulating gut microbiota and potentially reducing the morbidity of viral acute respiratory infections (ARIs).[31–33] For example, a randomized controlled trial of 94 preterm infants reported that supplementation of prebiotics and probiotics (*Lactobacillus rhamnosus*) led to a lower incidence of rhinovirus ARIs.[31] Another clinical trial of 326 healthy children also reported that

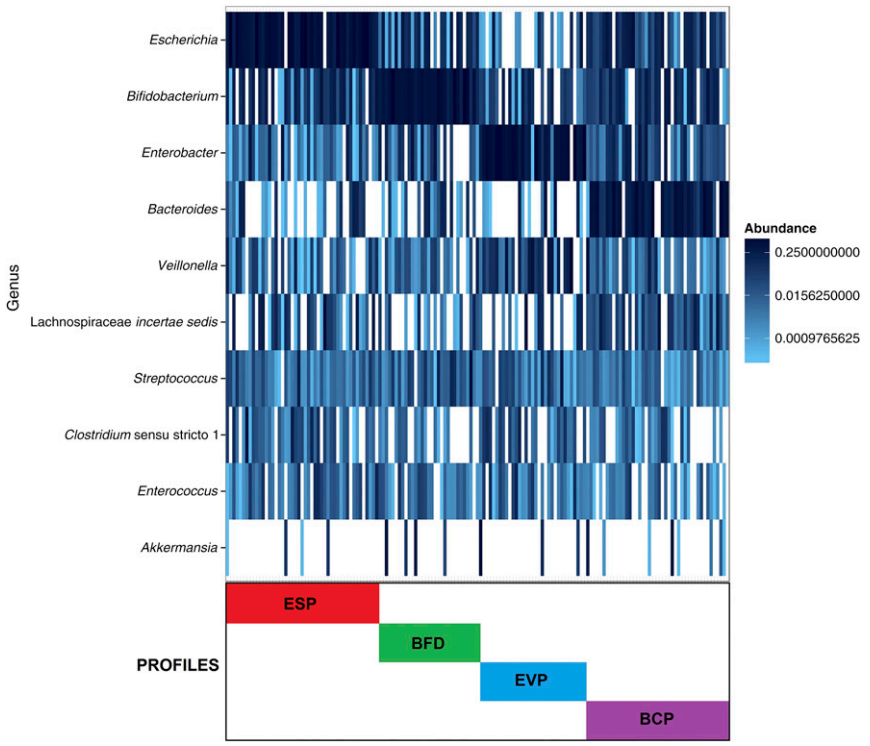

FIGURE 1
Clustering and composition of fecal microbiota in 155 infants. All fecal microbiota profiles of cases and controls were clustered via partitioning around medoids clustering method with Bray–Curtis distance. Colored bars indicate 4 microbiota profiles: *Escherichia*-dominant profile (ESP; red), *Bifidobacterium*-dominant profile (BFD; green), *Enterobacter/Veillonella*-dominant profile (EVP; blue), and *Bacteroides*-dominant profile (BCP; purple). The optimal number of clusters was identified by use of the gap statistic. To obtain additional information about the bacterial composition of samples within microbiota profiles, the 10 most abundant genera present in an adjacent heatmap were displayed. The taxonomy depicted is on the genus level because our sequences were dominated by 1 OTU per genus.

the use of *Lactobacillus acidophilus* and *Bifidobacterium animalis* reduced ARI symptoms.[32] However, none of these trials has investigated the gut microbiota itself. Although data are scarce, murine studies have deciphered the relationship of gut microbiota with host response against viral ARIs (eg, influenza, RSV).[34–36] For instance, Ichinohe et al,[34] using an antibiotic-treated mouse model, reported that a disruption of gut microbiota (ie, dysbiosis) impairs the generation of virus-specific CD4 and CD8 T cells and antibody responses after influenza virus infection, suggesting the need for an intact commensal bacterial community in the establishment of immune response against viral ARIs. Our study corroborates these earlier findings and extends them by demonstrating the association of *Bacteroides*-dominant fecal microbiota profiles with bronchiolitis in infants.

Our observations, in conjunction with the earlier studies, suggest a causal pathway linking the gut microbiota in early infancy to the respiratory tract immune response against viral infection. That is, the *Bacteroides*-dominant gut microbiota in early infancy attenuates the development of immune function in the respiratory tract and thereby leads to an increased susceptibility to bronchiolitis. Indeed, Sjögren et al,[37] examining a prospective cohort of 64 infants in Sweden, reported that a high abundance of *Bacteroides fragilis* in fecal samples during the first month of age was associated with lower levels of Toll-like receptor 4 expression and lipopolysaccharide-induced production of inflammatory cytokines in the peripheral blood mononuclear cells. These data fit into the larger concept of a "common mucosal response,"[13,14] that is, antigen presentation at 1 mucosal site (eg, gut), via systemic

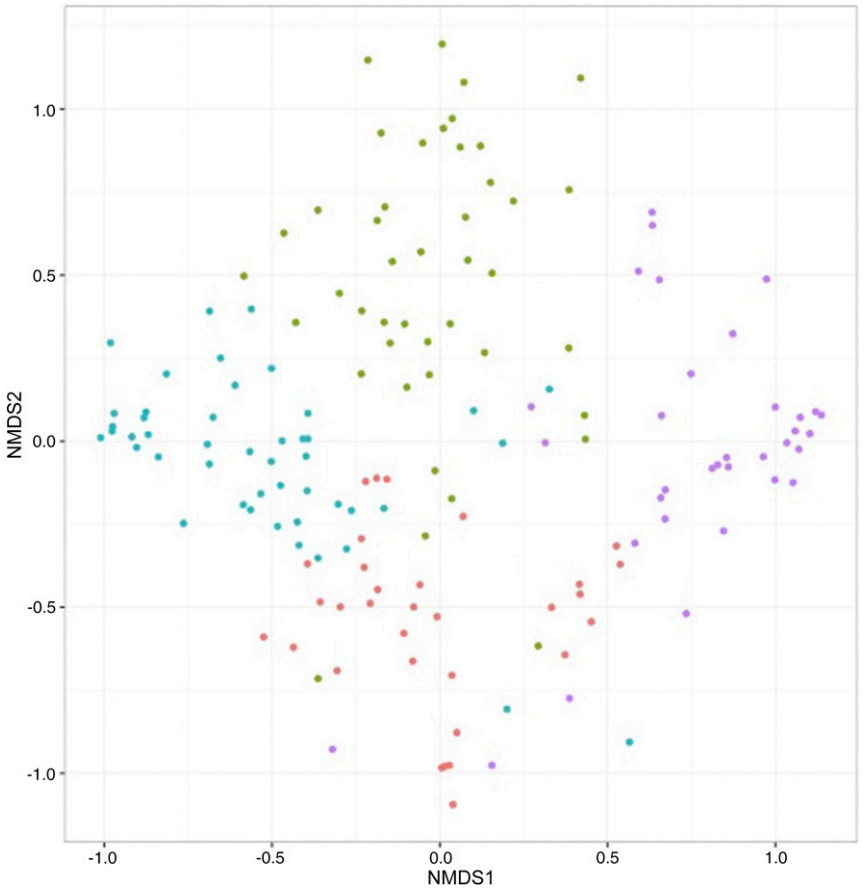

FIGURE 2
Nonmetric multidimensional scaling (NMDS) ordination of fecal microbiota. The Bray–Curtis distance between all cases and controls was calculated and used to generate nonmetric multidimensional scaling plots. Each dot in the figure represents the microbiota profile of a single subject in a low-dimensional space. Colored dots indicate 4 microbiota profiles: *Escherichia*-dominant profile (red), *Bifidobacterium*-dominant profile (green), *Enterobacter/Veillonella*-dominant profile (blue), and *Bacteroides*-dominant profile (purple). The subjects cluster together according to their microbiota profiles.

immune responsiveness, shapes immune function at distant mucosal sites (eg, respiratory tract). Alternatively, the *Bacteroides*-dominant fecal microbiota might be simply a marker of infants who have a higher propensity for viral ARI, including bronchiolitis. It is also possible that viral ARI alters the gut microenvironment, leading to overgrowth of *Bacteroides* locally (ie, reverse causation[38]). Furthermore, any combinations of these mechanisms are also possible.

Interestingly, infants with a *Bacteroides*-dominant profile had the highest bacterial richness and diversity. Although it is generally considered that higher diversity is protective against morbidity, recent studies have demonstrated that higher bacterial diversity may be associated with higher disease morbidity. For example, Huang et al[39] found that, compared with healthy controls, patients with asthma had a higher bacterial richness and diversity in their airway. This finding was concordant with an independent study of a European corticosteroid-using population of patients with asthma.[40] Although the underlying mechanism remains to be elucidated, these data may suggest that a depletion of gut microbiota that protects against

TABLE 3 Unadjusted and Multivariable Associations Between Fecal Microbiota Profiles and Likelihood of Bronchiolitis

	Unadjusted Model		Adjusted Model		Sensitivity Analysis	
Variables	OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*
Microbiome profile						
Escherichia-dominant profile	1.61 (0.51–5.66)	.43	1.63 (0.49–5.97)	.44	1.64 (0.49–6.07)	.44
Bifidobacterium-dominant profile	1.24 (0.36–5.14)	.66	1.28 (0.33–5.13)	.72	1.12 (0.26–4.79)	.88
Enterobacter/Veillonella-dominant profile	Reference		Reference		Reference	
Bacteroides-dominant profile	4.59 (1.58–15.5)	.008	3.89 (1.19–14.6)	.03	4.12 (1.28–15.2)	.02
Covariates						
Age, mo (per incremental month)	—	—	0.90 (0.75–1.07)	.24	0.89 (0.74–1.05)	.19
Female gender	—	—	1.22 (0.55–2.78)	.63	0.93 (0.40–2.19)	.87
Prematurity	—	—	4.24 (1.56–12.0)	.005	—	—
Cesarean delivery	—	—	0.63 (0.23–1.63)	.35	—	—
Systemic antibiotic use before enrollment	—	—	1.68 (0.55–4.95)	.35	2.12 (0.66–6.53)	.19
Parental history of asthma	—	—	—	—	3.27 (1.35–7.99)	.009
Maternal antibiotic use during pregnancy	—	—	—	—	3.11 (1.23–8.56)	.27

development of bronchiolitis (resilience microbiota[6]), rather than microbial richness or diversity, plays a role in the development of bronchiolitis. Despite the complexity, identification of the association between the *Bacteroides*-dominant microbiota profile and bronchiolitis is an important finding. Our data underscore the importance of understanding microbiome–host interactions by defining the responsible mechanisms, such as systemic dissemination of metabolites produced by the gut microbiota promoting the growth of certain bacteria or acting directly as immunomodulatory molecules in the respiratory tract.[7]

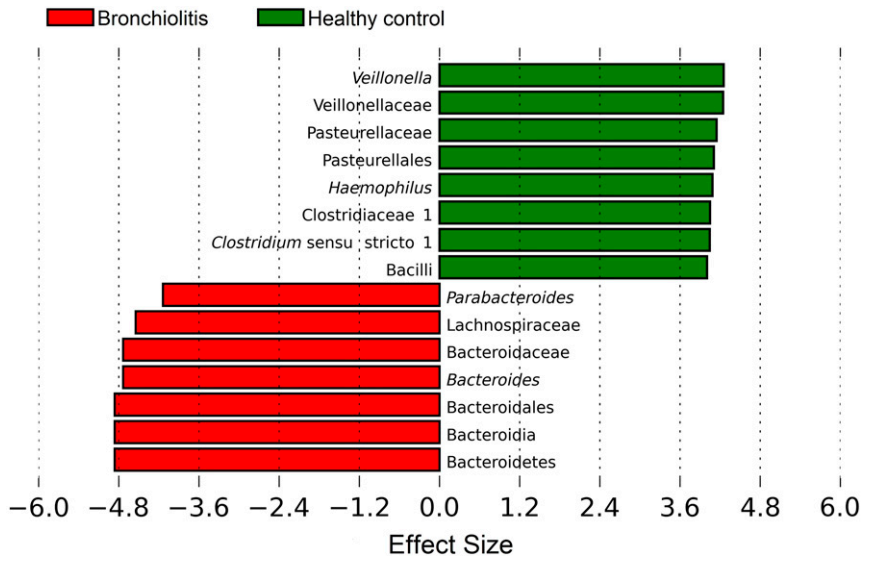

FIGURE 3
Effect sizes of genera that were significantly associated with likelihood of being a case (bronchiolitis) or healthy control. The linear discriminant effect size method was used to compare the abundances of all detected bacteria between cases and controls, computing an effect size for each comparison. Results shown here are significant by Kruskal–Wallis test (Benjamini–Hochberg adjusted $P < .05$) and represent large differences between groups (absolute effect size >3.6). Positive values (right) correspond to the effect sizes representative of healthy infants (controls), and negative values (left) correspond to the effect sizes infants with bronchiolitis (cases). *Veillonella* genus was found to be overrepresented in healthy infants, whereas *Bacteroides* genus was overrepresented in infants with bronchiolitis.

Several potential limitations of our study should be taken into account. First, the study cases consisted of infants hospitalized with bronchiolitis; therefore, our inference might not be extrapolated to those with milder illness (eg, bronchiolitis not necessitating hospitalization). However, our case selection approach, with its greater severity contrast, probably improved the efficiency of investigating the association of interest. Second, the study design precluded investigation of the dynamics and succession of the gut microbiota in relation to respiratory health in early childhood. To address this important question, we are following the study populations longitudinally up to age 6 years, with fecal sampling at multiple time points. Third, with the use of 16S rRNA gene sequence, we were unable to elucidate the differences in bacterial composition at the species level or their functional capacity. These important topics will be the focus of our future investigations. Fourth, as with any observational

study, the association between fecal microbiota and bronchiolitis does not necessarily prove causality and might be explained, at least partly, by unmeasured confounders. Additionally, the small number of bronchiolitis cases prevented us from adjusting for all sets of potential confounders. However, the significant association persisted even after we controlled for clinically important covariates. Finally, participating sites were academic centers in the urban areas. Although these results may not be generalizable to infants in rural areas, our study participants consisted of racially and ethnically diverse samples.

CONCLUSIONS

In this case–control study of infants with bronchiolitis and healthy age-matched controls, we identified 4 distinct fecal microbiota profiles in their fecal samples. We also found that, compared with infants with the *Enterobacter/Veillonella*-dominant profile, those with the *Bacteroides*-dominant profile had a higher likelihood of bronchiolitis. Although causal inferences remain premature, the identification of a *Bacteroides*-dominant microbiota profile in early infancy as the primary culprit in the association between the gut microbiota and host immune response against viral ARIs is an important finding. Our data should facilitate epidemiologic, mechanistic, and interventional investigations to disentangle the complex web of the gut microbiome, respiratory viruses, host immune response, and bronchiolitis pathogenesis in children.

ABBREVIATIONS

ARI: acute respiratory infection
CI: confidence interval
IQR: interquartile range
OR: odds ratio
OTU: operational taxonomic units
PCR: polymerase chain reaction
RSV: respiratory syncytial virus

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