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Kohei Hasegawa, *Harvard Medical School*
Rachel Linnemann, *Emory University*
Jonathan M. Mansbach, *Boston Childrens Hospital*
Nadim J. Ajami, *Baylor College of Medicine*
Janice A. Espinola, *Harvard Medical School*
Joseph F. Petrosino, *Baylor College of Medicine*
Pedro A. Piedra, *Baylor College of Medicine*
Michelle D. Stevenson, *University of Louisville*
Ashley F. Sullivan, *Harvard Medical School*
Amy D. Thompson, *Alfred I. duPont Hospital for Children*

*Only first 10 authors above; see publication for full author list.*

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# Nasal Airway Microbiota Profile and Severe Bronchiolitis in Infants: A Case-Control Study

**Kohei Hasegawa, MD, MPH**[1], **Rachel W. Linnemann, MD**[2], **Jonathan M. Mansbach, MD, MPH**[3], **Nadim J. Ajami, PhD**[4], **Janice A. Espinola, MPH**[1], **Joseph F. Petrosino, PhD**[4], **Pedro A. Piedra, MD**[5], **Michelle D. Stevenson, MD, MS**[6], **Ashley F. Sullivan, MS, MPH**[1], **Amy D. Thompson, MD**[7], and **Carlos A. Camargo Jr., MD, DrPH**[1]

[1]Department of Emergency Medicine, Massachusetts General Hospital, Harvard Medical School, Boston, MA

[2]Department of Pediatrics, Emory University School of Medicine, Atlanta, GA

[3]Department of Medicine, Boston Children's Hospital, Boston, MA

[4]Alkek Center for Metagenomics and Microbiome Research, Department of Molecular Virology and Microbiology, Baylor College of Medicine, Houston, TX

[5]Department of Molecular Virology and Microbiology, and Pediatrics, Baylor College of Medicine, Houston, TX

[6]Department of Pediatrics, University of Louisville, Louisville, KY

[7]Alfred I. duPont Hospital for Children, Wilmington, DE

## Abstract

**Background—**Little is known about the relationship of airway microbiota with bronchiolitis in infants. We aimed to identify nasal airway microbiota profiles and to determine their association with the likelihood of bronchiolitis in infants.

**Methods—**A case-control study. As a part of multicenter prospective study, we collected nasal airway samples from 40 infants hospitalized with bronchiolitis. We concurrently enrolled 110 age-matched healthy controls. By applying 16S rRNA gene sequencing and an unbiased clustering approach to these 150 nasal samples, we identified microbiota profiles and determined the association of microbiota profiles with likelihood of bronchiolitis.

**Results—**Overall, the median age was 3 months and 56% were male. Unbiased clustering of airway microbiota identified four distinct profiles: *Moraxella*-dominant profile (37%), *Corynebacterium/Dolosigranulum*-dominant profile (27%), *Staphylococcus*-dominant profile (15%), and mixed profile (20%) Proportion of bronchiolitis was lowest in infants with *Moraxella*-dominant profile (14%) and highest in those with *Staphylococcus*-dominant profile (57%),

Corresponding Author: Dr. Kohei Hasegawa, Department of Emergency Medicine, Massachusetts General Hospital, 125 Nashua Street, Suite 920, Boston, MA 02114., Tel: 617-726-5276, Fax: 617-724-4050, khasegawa1@partners.org.

**Conflict of Interest:** Dr. Mansbach has provided bronchiolitis-related consultation for Regeneron. Drs. Ajami and Petrosino own shares at Diversigen Inc., a microbiome research company. The other authors have no financial relationships relevant to this article to disclose.

corresponding to an OR of 7.80 (95%CI, 2.64–24.9; P<0.001). In the multivariable model, the association between *Staphylococcus*-dominant profile and greater likelihood of bronchiolitis persisted (OR for comparison with *Moraxella*-dominant profile, 5.16; 95%CI, 1.26–22.9; P=0.03). By contrast, *Corynebacterium/Dolosigranulum*-dominant profile group had low proportion of infants with bronchiolitis (17%); the likelihood of bronchiolitis in this group did not significantly differ from those with *Moraxella*-dominant profile in both unadjusted and adjusted analyses.

**Conclusions—**In this case-control study, we identified four distinct nasal airway microbiota profiles in infants. *Moraxella*-dominant and *Corynebacterium/Dolosigranulum*-dominant profiles were associated with low likelihood of bronchiolitis while *Staphylococcus*-dominant profile was associated with high likelihood of bronchiolitis.

### Keywords

bronchiolitis; respiratory infection; microbiota; airway; hospitalization; cluster; *Staphylococcus*; *Moraxella*; *Corynebacterium*; *Dolosigranulum*

## INTRODUCTION

Bronchiolitis is a significant public health problem worldwide.[1–4] In the U.S., bronchiolitis is the second leading cause of emergency department visits among infants[2,4] and the leading cause of hospitalizations, accounting for 18% of all infant hospitalizations.[3] Although almost all infants are exposed to respiratory syncytial virus (RSV) and other causative viruses (e.g., rhinovirus), not all of infants develop bronchiolitis.[5] Additionally, severity of infection varies widely from a minor nuisance to fatal infection. It is less understood why distinct outcomes may occur across a population infected with the same circulating strain of virus.[6]

The recent development of sensitive, culture-independent methods for bacteria identification has revealed the presence of highly functional bacterial communities – the microbiota – that inhabit humans.[7] Most work initially focused on microbiota in the gut and its relationship to gastrointestinal diseases. However, since the airway is a major portal for microbial exposure, application of phylogenetic sequencing techniques to the investigation of airway microbiota and its role in airway disease was an intuitive extension. Emerging evidence has revealed that airway microbiota may influence immune responses[8–11] and innate inflammatory capacities,[12,13] suggesting a role of airway microbiota in the development of acute respiratory infections in children, such as bronchiolitis. However, to the best of our knowledge, no prior study has related airway microbiota to the development of bronchiolitis in infants.

In this context, we conducted a case-control study, using a multicenter prospective cohort of infants hospitalized with bronchiolitis (“severe bronchiolitis”) and healthy age-matched controls, to determine the association between nasal airway microbiota and likelihood of severe bronchiolitis in infants.

# MATERIALS AND METHODS

## Design, Setting and Participants

We conducted a case-control study to examine the nasal microbiota of infants with severe bronchiolitis (cases) and that of healthy infants (controls). As a part of a multicenter prospective cohort study, called the 35th Multicenter Airway Research Collaboration (MARC-35),[14–16] we enrolled 40 infants (age <12 months) hospitalized with an attending physician diagnosis of bronchiolitis at one of three 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 between November 2013 and April 2014. Bronchiolitis was defined by the American Academy of Pediatrics guidelines as an acute respiratory illness with some combination of rhinitis, cough, tachypnea, wheezing, crackles, and retractions.[17] We excluded infants with previous enrollment into MARC-35, those who were transferred to a participating hospital >48 hours after the original hospitalization, those who were consented >24 hours after hospitalization, gestational age <32 weeks, or those with known comorbidity (i.e., cardiopulmonary disease, immunodeficiency, or immunosuppression).

Healthy infants (n=110) were included as the controls in this case-control study. The setting and participants have been reported previously.[18,19] Briefly, using a standardized protocol, we enrolled healthy infants – age-matched within 1.5 months of the cases – from a primary care group practice at Massachusetts General Hospital (Boston, MA) between November 2013 and May 2014. We excluded infants with gestational age <32 weeks, comorbidities, previous lower respiratory infection that resulted in an urgent clinic visit, emergency department visit, or hospitalization, current fever, respiratory illness, or gastrointestinal illness, 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 Emergency Medicine Network Coordinating Center and site investigators were queried about missing data and discrepancies identified by manual data checks.

Trained investigators collected nasal swabs from the anterior nares, using a standardized protocol,[20] within 24 hours of hospitalization (in the cases) or during the clinic visit (in the controls). Both 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. Samples were shipped on dry ice to the laboratory 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.[21,22] 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 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.[23]

Sequencing read pairs were demultiplexed based on the unique molecular barcodes, and reads were merged using USEARCH v7.0.1090,[24] allowing zero 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 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.[25] OTUs were mapped to the SILVA Database[26] 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 (within-sample measures of similarity or dissimilarity [e.g., Shannon index]) and beta-diversity (between-sample measures of similarity or dissimilarity (e.g., [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. We conducted analyses at the genus-level. Because each genus was dominated by one OTU (e.g., the detected *Staphylococcus* genus consisted solely of one OTU), we collapsed all OTUs assigned to the same genus into a single group for reporting. To identify nasal airway microbiota profiles, we performed unbiased clustering by partitioning around medoids[27] with the use of 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. The number of clusters to choose for the data was determined by using the gap statistic.[28]

To determine the association of microbiota profiles with the likelihood of being a severe bronchiolitis case, we fitted two logistic regression models. First, we constructed an unadjusted model that included only microbiota profiles as the independent variable. Second, we fitted a multivariable model adjusting for up to five potential confounders (age, sex, prematurity, breastfeeding status, and history of systemic antibiotic use before enrollment) because of the relatively small number of cases. These variables were chosen based on clinical plausibility and *a priori* knowledge.[5,17,29]

To compare the abundances of the ten most abundant bacteria in the nasal airway microbiota between bronchiolitis cases and healthy controls, we used the linear discriminant analysis effect size method.[30] In this method, vectors resulting from the comparison of abundances (e.g., *Moraxella* relative abundance) between the groups were used as input to the linear discriminant analysis. This method has the advantage over traditional statistical tests (e.g., pairwise tests) that an effect size is produced in addition to a P-value. This enables us to sort the results of multiple testing by the magnitude of the between-group difference, not only by P-values, as the two are not necessarily correlated.[30] Analyses used R version 3.2 with the phyloseq package.[31]

## RESULTS

### Study Population

At the four participating hospitals, we enrolled a total of 40 infants with severe bronchiolitis (cases) and 110 age-matched healthy infants (controls). Overall, the median age was 3 months (IQR, 2–5 months), 56% were male, and 53% were non-Hispanic white. Among the cases of severe bronchiolitis with virus detection, RSV was detected in 65% and rhinovirus in 23% (RSV/RV co-infection in 13%). Patient characteristics differed between cases and controls (Table 1). For example, infants with severe bronchiolitis were, compared to healthy controls, more likely to have parental history of asthma, maternal smoking during pregnancy, history of premature birth, previous breathing problems before the enrollment, sibling at home, smoking exposure at home, and corticosteroid use prior to the enrollment while they were less likely to be breastfed (all $P<0.05$).

### Nasal Airway Microbiota Sequence and Profiles

We analyzed nasal samples from all of the enrolled infants by 16S rRNA gene sequencing, and obtained 1,521,977 high-quality merged sequences of which 1,495,217 (98%) were

mapped to the database. All 150 samples had sufficient sequence depth to obtain high degree of sequence coverage (rarefaction cutoff, 1,066 reads per sample) and were used for the analysis. The sequencing identified 24 phyla and 292 genera. The nasal airway microbiota was dominated by three genera – *Moraxella* (32%), *Corynebacterium* (16%), and *Staphylococcus* (15%) – followed by *Dolosigranulum* (8%) and *Streptococcus* (7%).

Partitioning around medoids clustering of nasal airway microbiota identified four distinct microbiota profiles: 1) *Moraxella*-dominant profile (37%), 2) *Corynebacterium/Dolosigranulum*-dominant profile (27%), 3) *Staphylococcus*-dominant profile (15%), and 4) mixed profile (20%) (Figure 1). The first three profiles were dominated either by *Moraxella*, *Corynebacterium/Dolosigranulum* (co-dominated), or *Staphylococcus* genus. In contrast, the mixed profile had the highest bacterial richness (P<0.001) and Shannon index (P<0.001) with highest relative abundance of *Streptococcus* (Benjamini-Hochberg adjusted P=0.003; Table 2). The multidimensional scaling plots also demonstrated that the infants cluster together according to their airway microbiota profile (Figure 2).

Some of the patient characteristics differed across the four microbiota profiles (Table 3). For example, compared to infants with a *Moraxella*-dominant profile, those with a *Staphylococcus*-dominant profile were younger, but more likely to have used corticosteroids prior to the enrollment (both P<0.05). In contrast, infants with a *Moraxella*-dominant or *Corynebacterium/Dolosigranulum*-dominant profile had a non-significantly higher rate of breastfeeding compared to those with a *Staphylococcus*-dominant profile (P=0.07).

## Microbiota Profiles and Likelihood of Bronchiolitis

The proportion of infants with severe bronchiolitis differed across the microbiota profile groups – lowest in the *Moraxella*-dominant profile (14%) and highest in the *Staphylococcus*-dominant profile (57%; Table 3), corresponding to an OR of 7.80 (95%CI, 2.64–24.9; P<0.001; Table 3). The *Corynebacterium/Dolosigranulum*-dominant profile group also had a low proportion of infants with severe bronchiolitis (17%). In the multivariable model adjusting for age, sex, prematurity, breastfeeding, and history of systemic antibiotic use prior to the enrollment, the association of *Staphylococcus*-dominant profile with a higher likelihood of severe bronchiolitis persisted (OR for comparison with *Moraxella*-dominant profile, 5.16; 95%CI, 1.26–22.9; P=0.03; Table 4). Likewise, infants with a mixed profile also had a higher likelihood of severe bronchiolitis (adjusted OR, 4.77; 95%CI, 1.46–16.8; P=0.01). In a sensitivity analysis adjusting for a different set of covariates (age, sex, parental history of asthma, maternal smoking during pregnancy, mode of birth, and prematurity), the results did not change materially – e.g., infants with a *Staphylococcus*-dominant profile had a higher likelihood of severe bronchiolitis (OR, 9.27; 95%CI, 2.38–40.7; P=0.002). By contrast, the likelihood of bronchiolitis in infants with a *Corynebacterium/Dolosigranulum*-dominant profile did not significantly differ from those with a *Moraxella*-dominant profile in both unadjusted and adjusted analyses. In another sensitivity analysis adjusting for virus pathogens (sole RSV infection, sole rhinovirus infection, RSV/RV co-infection, others), the results did not change materially – e.g., infants with a *Staphylococcus*-dominant profile had a higher likelihood of severe bronchiolitis (OR, 9.60; 95%CI, 2.17–52.0; P=0.004).

The use of linear discriminant effect size method revealed that *Staphylococcus* genus was positively associated with the likelihood of severe bronchiolitis while *Moraxella, Corynebacterium, Dolosigranulum, Streptococcus,* and *Tumebacillus* genera were negatively associated with the likelihood of severe bronchiolitis (all $P<0.05$; Figure 3).

## DISCUSSION

In this case-control study of 40 infants hospitalized for bronchiolitis and 110 healthy age-matched controls, we identified four distinct nasal airway microbiota profiles. Infants with a *Moraxella*-dominant profile or *Corynebacterium/Dolosigranulum*-dominant profile had a low likelihood of severe bronchiolitis while those with a *Staphylococcus*-dominant profile or mixed profile had a high likelihood. To the best of our knowledge, this is the first study to have examined the relationship of airway microbiota with likelihood of bronchiolitis in infants. Our data corroborate and build on previous reports linking bacteria composition in the airway to acute respiratory infection outcomes,[32–37] a finding of both clinical and research significance.

Previous studies on acute respiratory infection in children have demonstrated inconsistent associations of upper airway bacteria with the incidence of infection. For instance, Kloepfer *et al.*, by applying quantitative PCR technique to nasal samples of U.S. school-age children in the RhinoGen cohort, found that *Moraxella catarrhalis* together with rhinovirus infection contributes to an increased risk of acute respiratory infection.[34] Similarly, Vissing *et al.*, by applying a culture-dependent technique to hypopharyngeal aspirates of infants from the Copenhagen Prospective Studies on Asthma in Childhood2000 ($COPSAC_{2000}$) cohort in Denmark, reported that 1-month-old infants with colonization of *Haemophilus influenzae, M. catarrhalis,* or *Streptococcus pneumoniae* had an increased risk of subsequent development of bronchiolitis.[36] By contrast, in a subsequent analysis of hypopharyngeal culture during acute respiratory illness episodes in the $COPSAC_{2000}$ cohort, Carlson *et al.,* found no association between bacteria (*H. influenzae, M. catarrhalis,* or *S. pneumoniae*) and duration of wheezing episodes in children aged <3 years.[35] Potential explanations for the inconsistency across studies include differences in study design, setting, patient populations, laboratory techniques for microbial identification (e.g., culture, bacterial PCR), or any combinations of these factors. In contrast, the validity of our findings is buttressed by the use of 16S rRNA gene sequencing of the nasal airway microbiota of infants enrolled in multicenter investigation with extensive clinical characterization.

Although the observed microbiota-bronchiolitis association may challenge the conventional virus-centric view of bronchiolitis, the nature of this association requires further elucidation. For example, our data may suggest a causal pathway linking the presence of certain bacteria composition (*Staphylococcus*-dominant airway microbiota) during early infancy to enhancement of viral pathogenesis (risk microbiota[38]). Indeed, studies have demonstrated that *Staphylococcus aureus* enhances replication and infectivity of respiratory viruses (e.g., rhinovirus,[39] influenza virus[7]). Alternatively, the *Staphylococcus*-dominant microbiota may be solely a marker of infants who have an increased susceptibility to severe bronchiolitis. Additionally, reverse causation is also possible – i.e., viral respiratory infection might

rapidly alter the airway microenvironment and thereby result in overgrowth of *Staphylococcus* locally.[40] These possibilities are not mutually exclusive.

Another potential mechanism is a depletion of airway microbiota that protects against development of severe bronchiolitis (resilience microbiota[38]). Indeed, our data demonstrated that *Moraxella, Corynebacterium,* and *Dolosigranulum* genera are not only associated with a lower likelihood of severe bronchiolitis but also sparse in infants with a *Staphylococcus*-dominant profile and those with a mixed profile. *Corynebacterium*, a gram-positive clade of the Actinobacteria, is not only a common colonizer of human skin and mucosa but also a member of the core microbiota detected in human breast milk.[41] *Dolosigranulum* has not been well characterized; the first genome was mapped by whole genome sequence recently.[42] *Dolosigranulum* is a gram-positive lactic acid bacteria, which are generally considered to a member of healthy microbiota in humans.[43] Consistent with our findings, recent studies from Netherlands[33,44] and Australia [32] also reported the relation between these bacteria and respiratory health in infants. For example, Biesbroeck *et al.,* by applying 16S rRNA gene sequencing to the nasopharyngeal samples collected from healthy Dutch infants, found that early (1.5 to 6 months) colonization by *Moraxella, Corynebacterium,* or *Dolosigranulum* genus was associated with a lower frequency in parent-reported upper respiratory infections.[33] Similarly, another post-hoc analysis of a randomized controlled trial by the same research group demonstrated that breastfeeding was associated with a higher abundance of *Corynebacterium* and *Dolosigranulum* but a lower abundance of *Staphylococcus,* and that *Dolosigranulum* abundance was inversely associated with the frequency of upper respiratory infections during early infancy.[44] Our data, in conjunction with these studies, collectively suggest that *Moraxella*-dominant and *Corynebacterium/Dolosigranulum*-dominant profiles may be beneficial for respiratory health – i.e., resilience microbiota – in infants.

## Potential Limitations

This study has several potential limitations. First, although bronchiolitis is a disease of lower airways, our data are based on nasal airway samples. However, lower airway sampling is both ethically and technically challenging in infants. Nonetheless, studies have reported a strong correlation between upper and lower airway microbiology in children.[45,46] Second, with the present study design, we were unable to assess the association between airway microbiota and the succession of childhood respiratory illnesses (e.g., bronchiolitis, recurrent wheeze, incident asthma). To address this important question, the study populations are currently being followed longitudinally up to age 6 years, with airway sampling at multiple time-points. Third, 16S rRNA gene sequencing precluded us from evaluating the microbiota at the species-level or their functional capacity. We hope to address this important issue in future work using metagenomic approaches. Fourth, because of the relatively small number of severe bronchiolitis cases, we were unable to control for all sets of potential confounders, including site effect. Yet, the significant associations persisted even after adjusting for many clinically important covariates. Fourth, one may surmise that the sample size of the study is relatively small. However, because of the large effect sizes observed in the study, the associations between the airway microbiota profiles and the likelihood of bronchiolitis were significant. Lastly, the study cases were composed of infants

hospitalized for bronchiolitis. Thus, our findings might not be generalizable to those with milder illness (i.e., bronchiolitis requiring ambulatory care without hospitalization). Yet, given the greater severity contrast, this case selection approach was efficient to determine the association of interest. Furthermore, our inferences remain directly relevant to 130,000 U.S. children hospitalized for bronchiolitis each year – a vulnerable population with high morbidity and healthcare utilization.[3]

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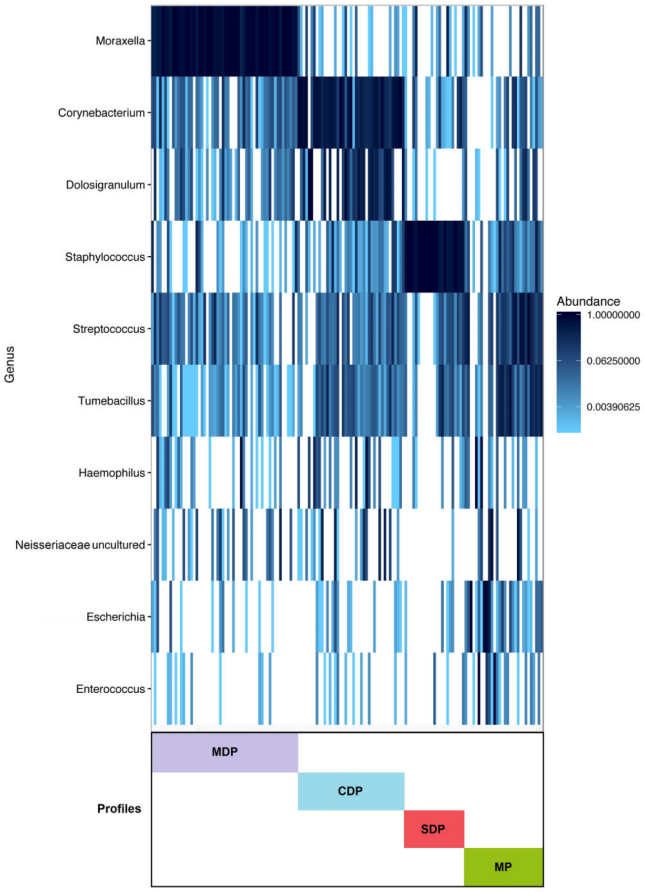


**Figure 1. Clustering and Composition in Nasal Airway Microbiota in 150 Infants**

All nasal airway microbiota profiles of 40 infants with severe bronchiolitis (cases) and 110 age-matched healthy infants (controls) were clustered using partitioning around medoids clustering method with the use of Bray-Curtis distance. Colored bars indicate four microbiota profiles: *Moraxella*-dominant profile (purple), *Corynebacterium/ Dolosigranulum*-dominant profile (blue), *Staphylococcus*-dominant profile (red), and mixed profile (green). 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. The taxonomy depicted is at the genus-level because our sequences were dominated by one OTU per genus.

MDP = *Moraxella*-dominant profile; CDP= *Corynebacterium/Dolosigranulum*-dominant profile; SDP = *Staphylococcus*-dominant profile; and MP = mixed profile.

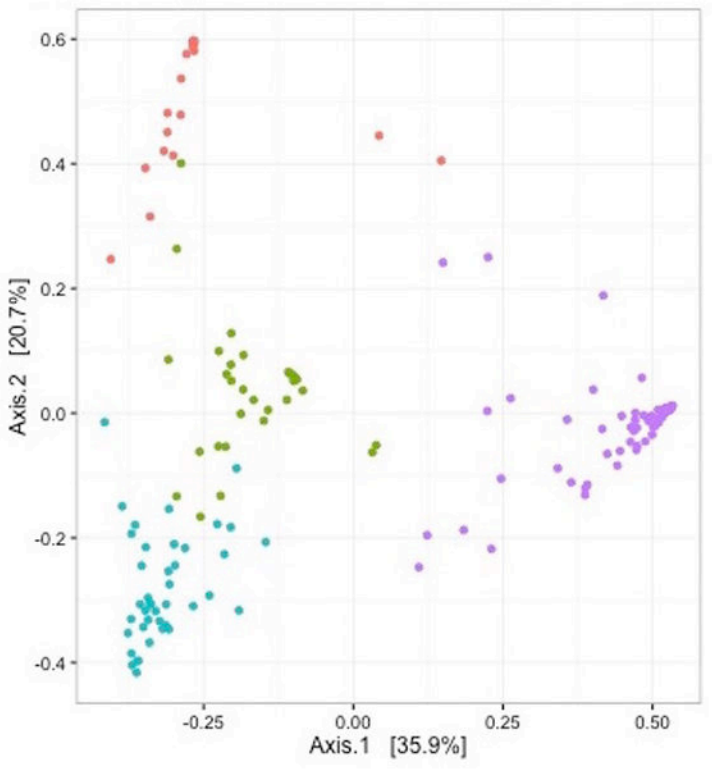


**Figure 2. Multidimensional Scaling Plots on Nasal Airway Microbiota**

To show the differences in nasal airway microbiota among 150 infants, multidimensional scaling plots based on the Bray-Curtis distance between all infants were generated. Each *dot* represents the overall bacterial community in each subject. Colors indicate four microbiota profiles: *Moraxella*-dominant profile (purple), *Corynebacterium/Dolosigranulum*-dominant profile (blue), *Staphylococcus*-dominant profile (red), and mixed profile (green). Infants cluster together according to their airway microbiota profile.

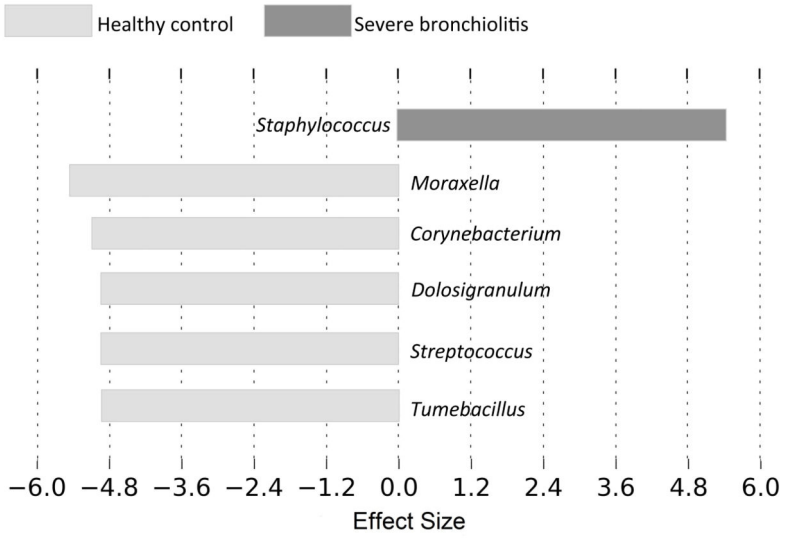


**Figure 3. Effect Sizes of Genera that were Significantly Associated with Likelihood of Being a Case (Severe Bronchiolitis) or Healthy Control**

The linear discriminant effect size method was used to compare the abundances of all detected bacteria among cases and controls, computing an effect size for each comparison. Results shown here are significant by Kruskal-Wallis test ($P<0.05$) and represent large differences between groups (absolute effect size >2.0). Positive values (right) correspond to the effect sizes representative of severe bronchiolitis (cases) while negative values (left) correspond to the effect sizes healthy infants (controls). *Staphylococcus* genus was found to be overrepresented in infants with severe bronchiolitis while *Moraxella, Corynebacterium, Dolosigranulum, Streptococcus,* and *Tumebacillus* genera were overrepresented in healthy infants.

## Table 1

Characteristics of Infants with Severe Bronchiolitis (Cases) and Healthy Infants (Controls)

| Characteristics | Infants with bronchiolitis n=40 | Healthy control infants n=110 | P-value |
|---|---|---|---|
| Demographics | | | |
| Age (mo), mean (SD) | 3.6 (2.5) | 3.9 (2.4) | 0.63 |
| Male sex | 22 (55) | 62 (56) | 0.99 |
| Race/ethnicity | | | |
| Non-Hispanic white | 23 (57) | 56 (51) | 0.051 |
| Non-Hispanic black | 6 (15) | 11 (10) | |
| Hispanic | 10 (25) | 20 (18) | |
| Other | 1 (2) | 23 (21) | |
| Parental history of asthma | 16 (40) | 21 (19) | 0.02 |
| Prenatal history | | | |
| Maternal smoking during pregnancy | 8 (20) | 3 (3) | 0.001 |
| Maternal antibiotic use during pregnancy | 11 (29) | 15 (14) | 0.06 |
| Maternal antibiotic use during labor | 12 (34) | 33 (30) | 0.79 |
| Past medical history and home environmental characteristics | | | |
| Mode of birth | | | 0.16 |
| Vaginal birth | 31 (78) | 70 (64) | |
| C-section | 9 (22) | 40 (36) | |
| Prematurity (32–37 weeks) | 12 (30) | 11 (10) | 0.006 |
| Previous breathing problems before enrollment * | 8 (21) | 0 (0) | <0.001 |
| History of eczema | 8 (21) | 17 (15) | 0.63 |
| Ever attended daycare | 9 (23) | 16 (15) | 0.33 |
| Sibling at home | 34 (87) | 47 (43) | <0.001 |
| Smoking exposure at home | 8 (21) | 3 (3) | 0.001 |
| Mostly breastfed for the first 3 months of age | 16 (52) | 84 (76) | 0.02 |
| Systemic antibiotic use before enrollment | 8 (21) | 14 (13) | 0.36 |
| Systemic corticosteroid use before enrollment | 9 (23) | 0 (0) | <0.001 |
| Hospitalization course | | | |
| Hospital length-of-stay (day), median (IQR) | 3 (2–4) | - | - |
| Admission to intensive care unit | 8 (20) | - | - |
| Use of mechanical ventilation † | 5 (16) | - | - |

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

Abbreviations: IQR, interquartile range; SD, standard deviation

* Defined as an infant having cough that wakes him/her at night and/or causes emesis, or when the child has wheezing or shortness of breath without cough

† Defined as use of continuous positive airway pressure and/or intubation during inpatient stay, regardless of location at any time during the index hospitalization

**Table 2**

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

| | ***Moraxella*-dominant profile n=56 (37%)** | ***Corynebacterium/Dolosigranulum*-dominant profile n=41 (27%)** | ***Staphylococcus*-dominant profile n=23 (15%)** | **Mixed profile n=30 (20%)** | **P-value** |
|---|---|---|---|---|---|
| **Richness** | | | | | |
| Number of genera, median (IQR) | 12 (7–9) | 18 (13–25) | 9 (3–20) | 27 (14–49) | <0.001 |
| **Alpha-diversity**, median (IQR) | | | | | |
| Shannon index | 0.58 (0.22–0.96) | 1.15 (0.83–1.60) | 0.21 (0.02–1.14) | 1.78 (0.67–2.43) | <0.001 |
| **Relative abundance of 10 most common genera**, mean (SD) | | | | | |
| *Moraxella* | 0.81 (0.20) | 0.01 (0.02) | 0.03 (0.10) | 0.02 (0.05) | 0.003 * |
| *Corynebacterium* | 0.04 (0.06) | 0.48 (0.28) | 0.04 (0.08) | 0.03 (0.05) | 0.003 * |
| *Staphylococcus* | 0.02 (0.06) | 0.03 (0.06) | 0.81 (0.21) | 0.05 (0.10) | 0.003 * |
| *Dolosigranulum* | 0.03 (0.05) | 0.25 (0.27) | 0.00 (0.00) | 0.03 (0.07) | 0.003 * |
| *Streptococcus* | 0.04 (0.07) | 0.04 (0.06) | 0.03 (0.04) | 0.20 (0.25) | 0.003 * |
| *Tumebacillus* | 0.01 (0.02) | 0.04 (0.06) | 0.04 (0.07) | 0.14 (0.16) | 0.003 * |
| *Haemophilus* | 0.02 (0.09) | 0.04 (0.12) | 0.00 (0.01) | 0.06 (0.22) | 0.88 * |
| *Neisseriaceae ncultured* | 0.02 (0.05) | 0.05 (0.12) | 0.00 (0.00) | 0.06 (0.16) | 0.75 * |
| *Escherichia* | 0.00 (0.01) | 0.00 (0.01) | 0.00 (0.00) | 0.12 (0.29) | 0.01 * |
| *Enterococcus* | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.01) | 0.06 (0.21) | 0.29 * |

Abbreviations: IQR, interquartile range; SD, standard deviation

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

**Table 3**

Characteristics and Case-control Status of Infants according to Nasal Airway Microbiota Profiles

| | ***Moraxella*** **-dominant profile n=56 (37%)** | ***Corynebacterium/Dolosigranulum*** **-dominant profile n=41 (27%)** | ***Staphylococcus*** **-dominant profile n=23 (15%)** | **Mixed profile n=30 (20%)** | **P-value** |
|---|---|---|---|---|---|
| **Characteristics** | | | | | |
| Demographics | | | | | |
| Age (mo), mean (SD) | 4.8 (2.6) | 3.3 (1.9) | 2.3 (1.6) | 3.8 (2.6) | <0.001 |
| Male sex | 36 (64) | 21 (51) | 13 (57) | 14 (47) | 0.39 |
| Race/ethnicity | | | | | 0.44 |
| Non-Hispanic white | 31 (55) | 22 (54) | 10 (43) | 16 (53) | |
| Non-Hispanic black | 7 (12) | 2 (5) | 2 (9) | 6 (20) | |
| Hispanic | 9 (16) | 8 (20) | 8 (35) | 5 (17) | |
| Other | 9 (16) | 9 (22) | 3 (13) | 3 (10) | |
| Parental history of asthma | 17 (30) | 8 (20) | 5 (22) | 7 (23) | 0.64 |
| Prenatal history | | | | | |
| Maternal smoking during pregnancy | 4 (7) | 1 (2) | 4 (17) | 2 (7) | 0.18 |
| Maternal antibiotic use during pregnancy | 11 (20) | 4 (10) | 7 (32) | 4 (13) | 0.16 |
| Maternal antibiotic use during labor | 15 (27) | 11 (27) | 8 (36) | 11 (41) | 0.54 |
| Past medical history and home environmental characteristics | | | | | |
| Mode of birth | | | | | 0.31 |
| Vaginal birth | 33 (59) | 28 (68) | 18 (78) | 22 (73) | |
| C-section | 23 (41) | 13 (32) | 5 (22) | 8 (27) | |
| Prematurity (32–37 weeks) | 7 (12) | 7 (17) | 7 (30) | 2 (7) | 0.10 |
| Previous breathing problems before enrollment * | 1 (2) | 3 (7) | 2 (9) | 2 (7) | 0.50 |
| History of eczema | 7 (12) | 8 (20) | 5 (22) | 5 (17) | 0.71 |
| Ever attended daycare | 15 (27) | 5 (12) | 2 (9) | 3 (10) | 0.09 |
| Sibling at home | 42 (75) | 14 (34) | 12 (52) | 13 (45) | 0.001 |
| Smoking exposure at home | 4 (7) | 1 (2) | 4 (17) | 2 (7) | 0.18 |
| Mostly breastfed for the first 3 months of age | 44 (80) | 26 (65) | 9 (50) | 21 (75) | 0.07 |
| Systemic antibiotic use before enrollment | 8 (14) | 5 (12) | 4 (17) | 5 (17) | 0.92 |
| Systemic corticosteroid use before enrollment | 2 (4) | 0 (0) | 3 (13) | 4 (14) | 0.04 |

| | *Moraxella* -dominant profile n=56 (37%) | *Corynebacterium/Dolosigranulum* -dominant profile n=41 (27%) | *Staphylococcus* -dominant profile n=23 (15%) | Mixed profile n=30 (20%) | P-value |
|---|---|---|---|---|---|
| Hospitalization course | | | | | |
| Hospital length-of-stay (day), median (IQR) | 3 (1–3) | 4 (2–5) | 3 (2–4) | 2 (2–4) | 0.58 |
| Admission to intensive care unit | 1 (17) | 1 (17) | 2 (20) | 1 (11) | 0.96 |
| Use of mechanical ventilation† | 1 (12) | 1 (14) | 3 (23) | 3 (25) | 0.88 |
| **Case-control status** | | | | | <0.001 |
| Severe bronchiolitis | 8 (14) | 7 (17) | 13 (57) | 12 (40) | |
| Healthy control | 48 (86) | 34 (83) | 10 (43) | 18 (60) | |

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

Abbreviations: IQR, interquartile range; SD, standard deviation

*Defined as an infant having cough that wakes him/her at night and/or causes emesis, or when the child has wheezing or shortness of breath without cough

†Defined as use of continuous positive airway pressure and/or intubation during inpatient stay, regardless of location at any time during the index hospitalization

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## Table 4

Unadjusted and Multivariable Associations between Nasal Airway Microbiota Profiles and Likelihood of Severe Bronchiolitis

| | Unadjusted model | | Adjusted model | |
|---|---|---|---|---|
| **Variables** | **OR (95% CI)** | **P-value** | **OR (95% CI)** | **P-value** |
| Microbiome profile | | | | |
| *Moraxella*-dominant profile | Reference | - | Reference | - |
| *Corynebacterium/Dolosigranulum*-dominant profile | 1.24 (0.40–3.76) | 0.71 | 1.21 (0.31–4.54) | 0.78 |
| *Staphylococcus*-dominant profile | 7.80 (2.64–24.9) | <0.001 | 5.16 (1.26–22.9) | 0.03 |
| Mixed profile | 4.00 (1.43–11.8) | 0.009 | 4.77 (1.46–16.8) | 0.01 |
| Covariates | | | | |
| Age (per each incremental month) | - | | 1.10 (0.90–1.34) | 0.34 |
| Female sex | - | | 1.08 (0.44–2.71) | 0.86 |
| Prematurity | - | | 2.69 (0.82–8.63) | 0.10 |
| Breastfed for the first 3 months of age | - | | 0.41 (0.15–1.08) | 0.07 |
| Systemic antibiotic use before enrollment | - | | 1.79 (0.56–5.41) | 0.31 |

Abbreviations: CI, confidence interval; OR, odds ratio