Microbial Composition in the Nasophayrnx of Children with and without Viruses during Asthma Exacerbations.

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Abstract

Rationale: Recent evidence suggests microbial composition of the respiratory tract can influence respiratory health and disease. We sought to further elucidate the role of microbial communities in the upper respiratory tract during acute respiratory viral infections and exacerbations of asthma in a pediatric population.

Methods: We recruited 111 children, ages 4-18 years, during asthma exacerbations (AE) (n=89) or during cold symptoms (CS) without history of asthma (n=22) from the emergency department over 4 years. A nasopharyngeal swab was obtained to provide both RNA for virus identification by viral genome sequencing and DNA for microbial composition by 16S rRNA gene sequencing. LEfSe analysis was performed to determine differences in relative contribution between microbial communities within and between groups.

Results: Subjects with AE and viral infection were found to have more abundant communities of Pseudomonadales (Moraxellaceae) (LDA score 5;p<0.05), while those with AE without virus were found to have more Bifidobacteriacea (LDA 3;p<0.05). Heat maps of bacterial communities clustered by weighted UniFrac suggest microbial composition initially separates between AE and CS cohorts. However, viral detection noticeably alters the microbial composition and further separates each cohort into those with and without infection.

Conclusions: In our pediatric population, the bacterial family Moraxellaceae was most closely related to AE during viral infections. Furthermore, viral infection was associated with compositional shifts of the microbiota within each cohort (AE and CS). Taken together, our data suggests AEs are increased during a viral infection that happens concurrently with an upper-respiratory tract that is composed of specific bacterial communities, such as Moraxellaceae.

Introduction

Through the years, microbiome research has attempted to elucidate the role of microbial composition in relationship to health and disease. One of these focuses is on the relationship between the airway microbiome and asthma. Recent research has shown that noninvasive nasopharyngeal swabs reflect bronchial bacterial composition, highlighting the important link in which inflammation in the upper respiratory tract can influence inflammation in the lower airways.[1] Based on these findings, multiple studies have discovered differences between the nasal microbial composition in asthmatics compared to controls.[2,3] This difference in microbial composition of the nasopharynx in asthmatics is also linked to both the frequency[4,5] and the severity of asthma exacerbations.[5]

Viral infections have been well studied and are one of the known triggers for asthma exacerbations causing lower airway inflammation.[6,7] Early viral infections in neonates have been implicated in the development of asthma.[8] Further investigation has confirmed that early infections in combination with certain microbial composition in pre-school aged children are not only suggestive of long term development of asthma but are also associated with increased likelihood of severe lower respiratory symptoms.[9] This is also reproduced in school aged children where microbiome composition predominated by Moraxella is associated with higher detection of upper respiratory viruses and predicted progression to acute asthma exacerbations.[5]

Our study evaluates the role of the nasal microbiome in children with and without asthma in conjunction with viral infections to determine if there are interactions between viral upper respiratory infections and the nasal microbiome. We hypothesize that there are different important microbial communities when considering children with and without asthma as well as those with and without viral infections.

Methods

Sample Population

- **Asthmatics** (n=89) were identified based on the medical/social history provided by next of kin and/or medical records. **Controls** were reported as having no history of asthma (n=22).
- Children age 4-18 years old who presented to the Emergency Department and were diagnosed with asthma exacerbations or cold symptoms in non asthmatics
- Nasopharyngeal swabs were obtained from each child

Nasopharyngeal Swab Processing

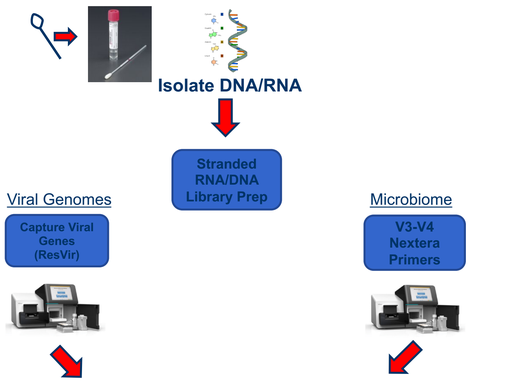
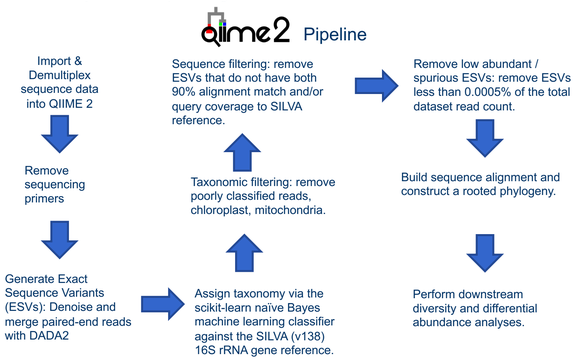


Figure 1. Methods. DNA was isolated using the Zymo Microbiomics kit. Amplification of 16S rRNA regions V3-V4 was completed by PCR using primers designed to adhere to conserved regions of the gene and overhanging Illumina Nextera® linker adapter sequences. A second eight-cycle PCR step was completed to add one of 96 unique Nextera® XT indexes to each sample. After each of the PCR steps, PCR products were purified with 0.8 volume of AMPure® XP beads (Beckman Coulter, Pasadena, CA, USA) to remove unused primers. For each sample, the successful amplification of the desired 16S rRNA region was confirmed by electrophoresis using an Agilent Technologies 2100 bioanalyzer (Agilent, Santa Clara, CA, USA). Sequencing was conducted on the Illumina® MiSeq platform using version 3 sequencing chemistry and 2 × 300-bp read lengths.



Results

Table I. Demographics.

	Cohort (n=111)	Asthma (n=89)	Control (n=22)
Sex (%M)	58.6	61.8	45.5
Age (years),mean [SD]	10.23 [4.263]	10.26 [4.171]	10.07 [4.228]
Race			
Caucasian	24.3	21.4	36.4
African American	77.5	80.9	63.6
Native American	1.8	2.2	0
Ethnicity			
Hispanic	1.8	1.1	4.5
Virus (% positive)	72.0	74.1	74.2

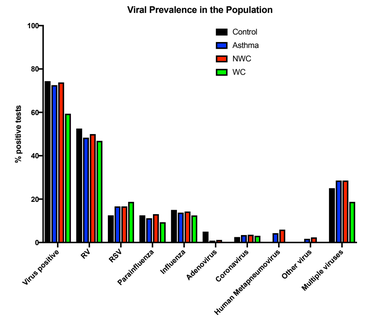


Figure 2. Prevalence of Viral infections within the population.

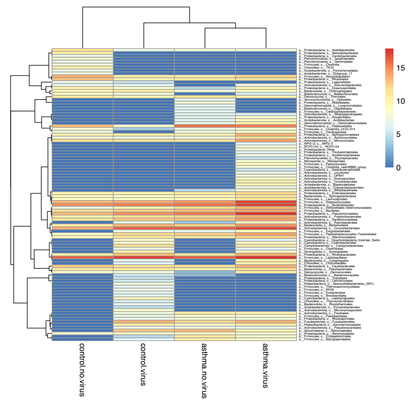


Figure 3. Heatmap of microbial composition of nasal passages of children with asthma and controls with and without virus. Asthmatic individuals with virus tend to have more Moraxella, while control subjects with virus are more likely to have Carnobacteriaciae (lactobacillus).

Results

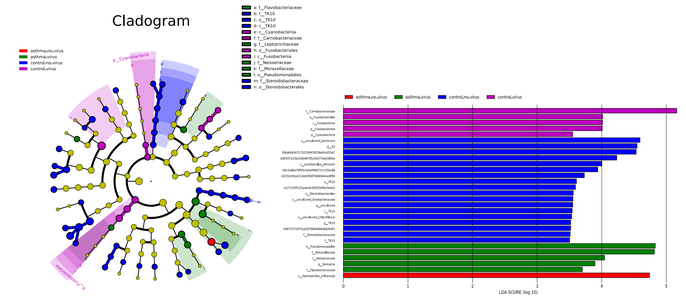


Figure 3. Lefse analysis comparing microbial composition of nasopharyngeal passages of children with asthma and controls with and without virus. Green shaded areas are asthmatic individuals with viral infection; Red dots are asthma without viral infection; Lavender shaded areas are control individuals with virus; Blue/purple shaded regions are control individuals without virus (LDA 3.5 or better).

Conclusions

- Microbial composition is different in children with asthma exacerbations and non-asthmatic with cold symptoms.
  - Viral infections in both cohorts, asthma exacerbations and non asthmatics with cold symptoms, resulted in microbiota shifts
- Children with asthma exacerbations with viral infections had more abundant communities of Pseudomonadales (Moraxellaceae) compared to asthma exacerbations without viral infections which showed higher communities of Haemophilus Influenzae.
- Our data suggests AEs are increased during a viral infection that happens concurrently with an upper-respiratory tract that is composed of specific bacterial communities, such as Moraxellaceae.

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