Whole Genome Shotgun Sequencing on Nasopharyngeal microbiome in Hong Kong Pre-school Children with Asthma Exacerbations

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Background

Asthma exacerbation (AE) exerts heavy burden on society. Microbiome is an important driver for the development of immune-mediated diseases, but very few studies looked at microbiome during AE. There was limited whole genome shotgun (WGS) sequencing data on respiratory samples. This study aimed to provide a high-resolution view on airway microbiome.

Methods

Eighty-one nasopharyngeal (NP) secretions were collected from three groups of pre-school children, including 24 cases hospitalised for HRV-associated AEs, 21 inpatient controls admitted for upper respiratory tract infections (RTI), and 36 community controls without any RTI within four weeks. Extracted genomic DNA was sent for WGS sequencing by Illumina HiSeq X Ten platform. Host contamination was removed using kneadData with default human reference (GRCh37/hg19). Approximately 1.4-4% reads deemed as non-human were used to generate taxonomy profiles through MetaPhlAn2. Alpha-diversity was calculated as Shannon index, and LDA Effect Size (LEfSe) analysis was conducted to identify the most representative microbes for each group. HUMAnN2 was used for predicting metabolic function.

Results

The mean (SD) age of cases, inpatient controls and community controls was 3.5 (1.0), 3.5 (1.0) and 4.9 (0.7) years respectively. Biodiversity in AE group was significant lower than community controls (median [IQR]: 0 [0-0.749] vs 1.073 [0.787-1.941], P=0.0002) but similar to inpatient RTI group (0.692 [0-0.996], P=0.11). LEfSe analysis revealed *Dolosigranulum pigrum* to be the most characteristic species among community controls (log LDA score 5.57, P=0.0025) while *Actinobacteria* was significantly more abundant in this group (log LDA score 4.59, P=0.0063). In patients with AE, *Proteobacteria* was the only significant phylum (log LDA score 5.11, P=0.0441) while no specific species were identified as representative. Two pathways on inosine-5'-phosphate biosynthesis was identified in various bacteria from both control groups but not AE patients.

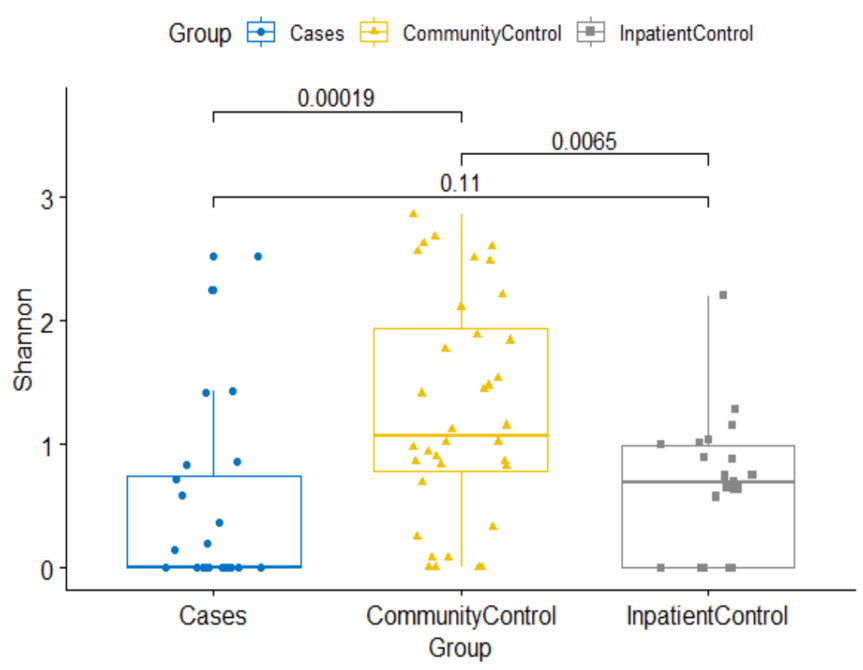

Fig 1. Boxplot of Shannon indexes in case group, community control group and inpatient control group. P-value of paired Mann-Whitney U test was indicated.

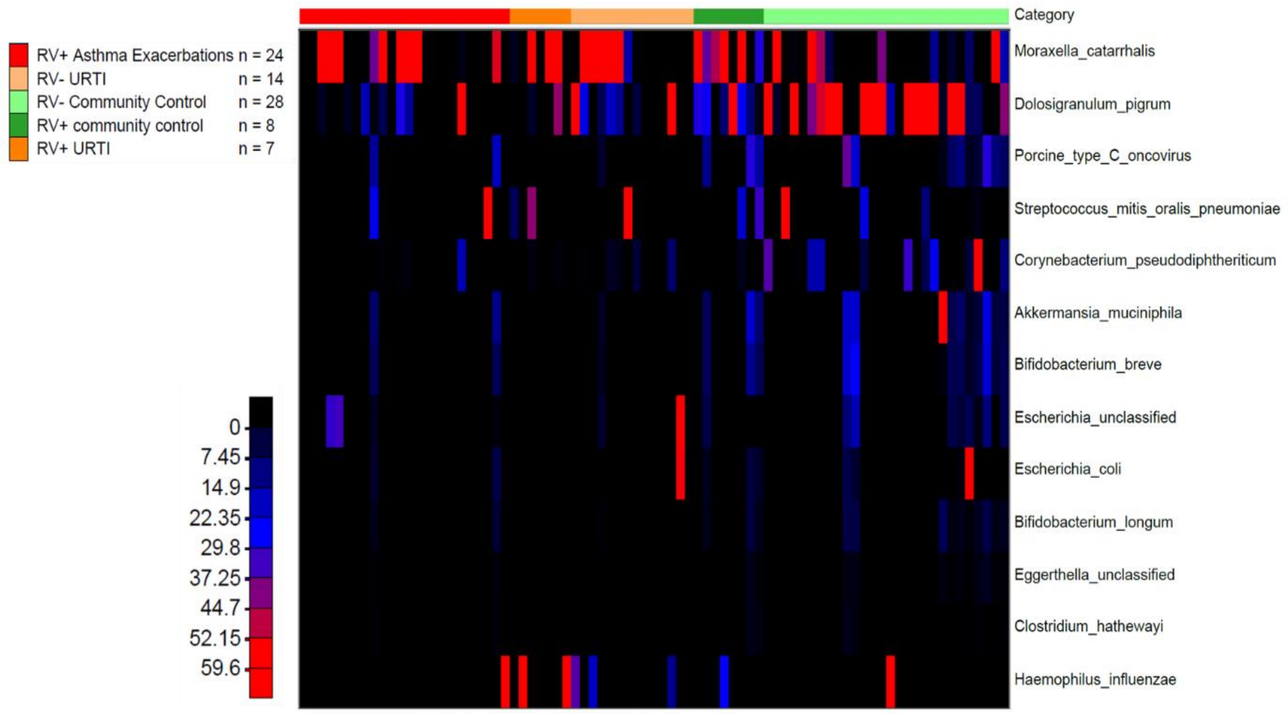

Fig 2. Heat map of relative abundance of top 13 eukaryotic species from total 81 samples. Asthma exacerbations correspond to case group.

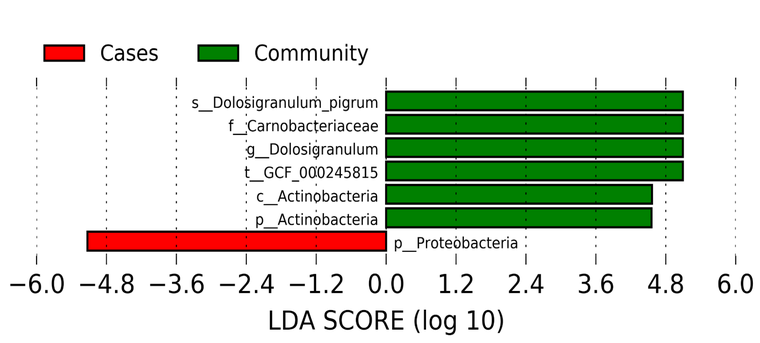

Fig. 3. Histogram of discriminative microbes between cases group (red) and community control group (green) with logged linear discriminative analysis (LDA) effect size score >2 and p <0.05 for Kruskal-Wallis test using clinical features as grouping variable and HRV infection status as biological hypothesis, the absolute value of LEfSe scores are in proportion to the portion of difference that could be contributed to the corresponding taxon.

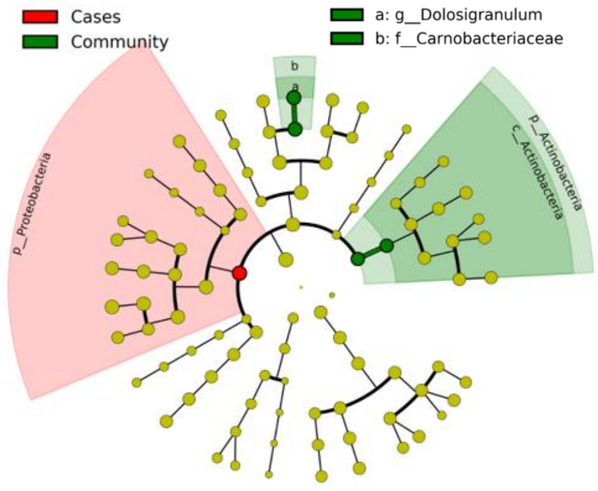

Fig. 4. Cladogram of discriminating taxa from Fig. 3. Yellow spots are taxa of no statistical significance. Size of circles correspond to relative abundance of the taxon.

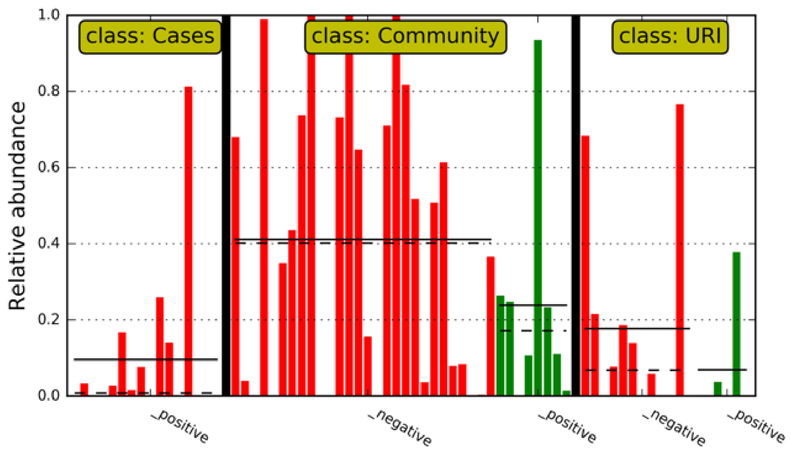

Fig. 5. Histogram of *Dolosigranulum pigrum* relative abundance in cases group, inpatient URTI group and community control group. Red bars indicate samples positive for HRV infection, green bars represent those negative for HRV status. Solid and dashed lines indicate mean and median, respectively.

Conclusion

Pre-school children with AE had lower diversity in their NP microbiome in which *Proteobacteria* is predominant. Our results suggest that *Dolosigranulum pigrum* may protect against AE. Further longitudinal studies are needed to investigate the dynamic differences of airway microbiome between stable asthma patients and those with AE.

Funding: Research Committee's One-off Fund for Research (3132910) of CUHK and Hong Kong Institute of Allergy Research Grant 2018