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The Tracheal Microbiota in Patients with a Tracheostomy Before, During, and After an Acute Respiratory Infection

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Abstract

Examining tracheal microbiota before, during, and after acute respiratory infection (ARI) in patients with a tracheostomy demonstrated large baseline intra-patient microbiota variability and a significant bloom of *Haemophilus* and *Moraxella* on day 1 of ARI symptoms. The tracheal microbiota community composition changed significantly from baseline to 1 month after ARI.

Keywords

longitudinal study; microbiome; acute respiratory infection; tracheal microbiome; tracheostomy

BACKGROUND

Acute respiratory infections (ARI) cause ~5% of all deaths globally [1] and are particularly problematic in children with a tracheostomy requiring home ventilation [2]. Although this

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Conflict of Interest:
The authors declare that they have no competing interests.

high-risk population group is increasing in number [3], there is no consensus about best clinical practices for ARI treatment in this at-risk group, even within the 2016 American Thoracic Society guidelines for the care of children requiring chronic home ventilation [4]. Given the life-threatening potential of ARIs in this population and the lack of clear guidance, many clinicians choose to treat ARIs in these patients with antibiotics based on conventional cultures or to help prevent clinical deterioration [5]. Unfortunately, these current testing and treatment approaches [5] not only do not distinguish between colonizing bacteria and true pathogens, but also do not account for the variation in intra-patient dynamics of the tracheal microbial communities [6]. More effective treatment of ARIs in this population, and possibly in healthy populations as well, will require a more nuanced understanding of ARIs that helps clinicians move beyond their current reductionist approach of classifying ARIs as viral, bacterial, or secondary bacterial [7].

One new conceptual model of pneumonia pathogenesis that addresses this need suggests that pneumonia is an emergent phenomenon caused by bacterial blooms arising from the colonizing microbiota [7]. The objective of this study was to extend this complex adaptive system model [7] to ARIs in patients with a tracheostomy by examining the dynamics of the tracheal microbiota before, during, and after an ARI. We hypothesized that at the beginning of the ARI there would be a “bloom” of at least one genus already present in the airway.

METHODS

The present cohort was a convenience sample of children cared for by the Critical Care, Anesthesia, Perioperative Extension (CAPE) and Home Ventilation Program. Between November 2013 and May 2014, we stored one tracheal aspirate per week per patient. When patients developed symptoms of an ARI, we retrieved the sample from the week prior to infection (day 0, D0) and from the first day of symptoms (D1). Additionally, after ARI onset we collected one sample every week for four weeks (W1 to W4). An ARI was defined as any illness with increased mucus production that required increased oxygen delivery or higher ventilator settings over baseline. We ensured standardized sample collection by observing parents or visiting nurses collecting the first sample in person during a home or clinic visit. The aspirates were stored at 0°C within 15 minutes of collection. The study team retrieved the samples during home visits and subsequently stored them at −80°C. The Institutional Review Board approved this study. See Supplementary Digital Content (SDC) for a description of the detailed methods.

Virology

We used D1 samples and the Luminex xTAG Respiratory Viral Panel FAST v2 multiplex PCR (Toronto, ON, Canada) to test for 18 respiratory viruses.

High-throughput sequencing

We sequenced the V4 region (~250 bp) of the 16S rRNA gene on the Illumina MiSeq sequencing platform. Raw sequence files were processed and clustered into to Operational Taxonomic Units (OTUs) and normalized as previously reported (see SDC for detailed methods) [6].

Statistical analyses

The estimation of alpha-diversity, beta-diversity, and dissimilarity between samples and a complete description of the analytic methods are described in the methods SDC. Briefly, linear mixed-effects (LME) models, as implemented in the lmer4 R package, were applied to both alpha-diversity indices and microbial genera abundances (i.e., sum of OTUs sharing a taxonomic genus) while accounting for non-independence of subjects and time. Beta-diversity Unifrac indices were compared using permutational multivariate analysis of variance (adonis). We performed multiple rounds of analysis that included time and the following co-variables: meteorological seasons, age, gender, feeding route (i.e., oral, gastrostomy tube (G-tube), oral + G-tube, gastro-jejunal tube), ventilator use (i.e., none, when sleeping, or continuous), oxygen requirement, tracheostomy change frequency (i.e., more than once per month or not), prophylactic antibiotics, daily inhaled corticosteroids, and antibiotics during ARI. Our preliminary analyses showed that only feeding route had a significant association with microbial diversity and taxon abundance. Hence, our final, most parsimonious LME and adonis models included one predictor (time) and one co-variable (feeding route). Benjamini-Hochberg FDR multiple test correction was applied.

RESULTS

Twenty patients had an ARI during the study period. The median age was 12 years (interquartile range, 4–24 years) and 70% were male. Sixty percent had neuromuscular disorders and the remainder had lung disease, other than cystic fibrosis. Fifteen (75%) received antibiotics for their ARI. Clinical characteristics for the study cohort are presented in supplementary Table SDC1. We collected a total of 92 tracheal samples during the study period. Twenty-eight (23%) tracheal samples were missing due to missed sample collections and two patients dying from their ARI during the study. From the 92 samples, we obtained a total of 1,485,077 sequences ranging from 1,030 to 63,835 sequences per sample (mean=17,070; median=10,076) after quality control analyses and OTU filtering.

Virology

Of the 17 patients with sample available for virology testing on D1, five (29%) had enterovirus/rhinovirus, three (18%) had respiratory syncytial virus, three (18%) had coronavirus, one (6%) had human metapneumovirus, one (6%) had influenza A, and five (29%) had no virus detected. One patient (6%) had viral co-infection.

The taxonomic composition of the tracheal microbiota

The tracheal microbiota across all 20 patients was dominated by the eight genera listed in Table 1. The most abundant genera in the tracheal microbiotas were: *Streptococcus* (21%), *Haemophilus* (10%), *Corynebacterium* (9%), *Neisseria* (9%) and *Moraxella* (8%). The remaining genera each accounted for <3% of the total sequences.

Dynamics of the tracheal microbiota around ARIs

Alpha-diversity varied significantly [P(>F)<0.05] over time in three of four indices (Table 1). Compared with the pre-ARI sample (D0), the mean alpha-diversity was significantly

lower in samples from D1, W2 and W4 after the ARI with W2 to W4 having the lowest alpha-diversity.

Beta-diversity unweighted Unifrac distances (uUd) varied significantly (P=0.045) from D0 to W4, but weighted Unifrac distances (wUd) did not. uUd distances also varied significantly (P<0.05) between D0–W1 samples and D0–W2 samples; all other pairs did not significantly differ.

Among the 8 most abundant bacterial genera, the relative mean proportions of *Haemophilus* and *Moraxella* fluctuated significantly (P<0.05) over time (Table 1). Both genera showed significantly (P<0.05) higher abundances (i.e., *Haemophilus* increased 274% and *Moraxella* 64%) on D1 of ARI compared with the pre-ARI (D0) sample (Table 1).

Patients' microbiota varied over the one month post-ARI as indicated by the PCoA of uUd (see Figure 1, SDC). Intra-patient microbiomes before ARI (D0) and on D1 were more similar to each other than samples from subsequent weeks. Indeed, after the onset of ARI most tracheal microbiomes appeared unique, as suggested by the lack of overlap (colored dots) in the longitudinal PCoA plots.

DISCUSSION

In this study, we investigated the composition and temporal dynamics of microbial communities inhabiting the trachea of children and young adults with a tracheostomy before, during, and after ARI. The results demonstrate lower species richness and evenness during and after ARI as would be expected with a respiratory infection in addition to variation in microbial community composition (as suggested by uUd) over one month, but not significant changes in microbial structure (as suggested by wUd). Moreover, the results confirm that similar to previously observed ecological phenomena [8], two previously present genera (i.e., *Haemophilus* and *Moraxella*) bloom by day 1 of ARI despite the highly variable baseline microbiota of patients with a tracheostomy [7].

The traditional reductionist approach of categorizing ARIs as either viral or bacterial may be too simplistic a clinical framework for ARIs in individuals with a tracheostomy, and possibly all people with ARIs [7]. On D1 of ARI, the majority of the current prospective cohort had a virus detected and a "bloom" of already present genera (i.e., *Haemophilus* and *Moraxella*). The tracheal finding of *Haemophilus* and *Moraxella* blooming are consistent with findings from previous studies utilizing nasopharyngeal samples to examine acute respiratory illness outcomes [9]. And although viral-bacterial interactions during ARIs have been described [10], the present results extend previous research by suggesting that these ARIs were not infections due to acquisition of a new bacterial pathogen as Koch's postulates suggest, but rather a bloom of colonizing genera in the context of a viral infection. Conceptualizing ARIs as "blooms" may be more complex to operationalize clinically than the current reductionist approach, but may eventually provide opportunities for novel, targeted treatment methods. Although beyond the scope of these data, ARIs may be best understood as an emergent phenomenon [7] that 1) is driven by a complex interplay among the

infecting virus, microbiome, and host response [9] and 2) results in a continuum of ARI severity anchored by pneumonia.

The next step is to better understand the pathobiology of ARI in this high-risk population with variable underlying microbiota in order to develop novel targets for ARI treatment and to provide guidance about when to use antimicrobials and which bacteria to treat. Until this time of improved ARI understanding and clinical guidance, many clinicians will continue to overuse and misuse antimicrobials for ARIs in children with a tracheostomy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Mean alpha-diversity indices, beta-diversity indices, and mean relative proportions of dominant genera (>3%) in decreasing order of abundance in a cohort of 20 patients with a tracheostomy

	Total	D0	D1	W1	W2	W3	W4	F	DF	P(>F)
Alpha-diversity index										
ACE	76.7	87.4	65.9	86.3	71.3	77.0	71.5	0.6	78.7	0.435
Shannon	2.1	2.3	2.0*	2.2	1.9*	2.1	2.0*	4.4	75.9	**0.042**
Fisher	9.3	10.9	8.8*	10.2	7.9*	9.1	8.2*	4.1	77.1	**0.045**
PD	6.6	7.5	6.2*	7.2	6.2*	6.5	6.2*	4.1	77.1	**0.044**
Beta-diversity										
Unifrac-unw								1.4	2	**0.045**
Unifrac-w								0.7	2	0.401
Genus										
Streptococcus	20.8	21.8	19.2	25.3	17.7	16.3	24.8	0.5	90.0	0.477
Haemophilus	10.1	5.4	14.8*	4.5	7.9	5.6	22.5*	5.0	67.8	**0.035**
Corynebacterium	8.9	6	4.5	9.4	20.8	5.8	6.7	0.5	44.1	0.484
Neisseria	8.5	6.6	6.7	8.8	8.4	10.6	9.9	1.8	80.1	0.183
Moraxella	7.8	6.9	11.3*	5.9	8.6	13.8*	6.6	4.6	119.2	**0.046**
Stenotrophomonas	4.1	2.7	4.7	3.7	3.7	5.9	4	0.2	77.1	0.667
Prevotella	3.9	3.1	3.7	7.2	4.1	3.2	2	0.05	83.1	0.833
Pseudomonas	3.4	2.4	2.6	3.2	3.7	5.2	3.3	3.9	81.2	0.063

Linear mixed-effects (LME) models results are shown for alpha-diversity indices and taxa proportions, while permutational multivariate analysis of variance (adonis) results are shown for beta-diversity indices. Significance of LME models was estimated using ANOVA type II or III with Satterthwaite approximation. For each test we report the relevant F statistic (F), degrees of freedom (DF) and significance (P(>F)). Significant associations are indicated in bold. If overall LME tests were significant we also compared the pre-ARI group against each post-ARI group and those pairwise comparisons resulting significant are marked with an * (= P≤0.05).

Abbreviations: ARI, acute respiratory infection. D0, day 0 or pre-ARI; D1, day 1 of ARI; W1, week 1 post-ARI, W2–4, week 2–4 post-ARI.