Received: 17 August 2017 | Accepted: 30 March 2018
DOI: 10.1002/ppul.24037

ORIGINAL ARTICLE: RESPIRATORY INFECTIONS

WILEY PEDIATRIC PULMONOLOGY

Microbiome characteristics of induced sputum compared to bronchial fluid and upper airway samples

Shi-qi An[1,2] | Adilia Warris[3,4] | Steve Turner[3]

[1] Division of Molecular Microbiology, University of Dundee, Belfast, UK

[2] Wellcome Wolfson Institute for Experimental Medicine, Queen's University Belfast, Belfast, UK

[3] Department of Child Health, University of Aberdeen, Aberdeen, UK

[4] MRC Centre for Medical Mycology, University of Aberdeen, Aberdeen, UK

Correspondence
Steve Turner, Child Health, Royal Aberdeen Children's Hospital, Aberdeen, UK, AB25 2ZG.
Email: s.w.turner@abdn.ac.uk

Funding information
NHS Grampian Endowments Fund; Wellcome Trust Strategic Award, Grant number: (grant 097377); MRC Centre for Medical Mycology, Grant number: (grant MR/N006364/1)

Abstract

Objective: The study of the community of microorganisms (the microbiota) in the lower airways in children is restricted to opportunistic sampling in children undergoing elective general anaesthetic. Here we tested the hypothesis that induced sputum is a valid alternative to directly sampling the lower airways to study lower airway microbiota.

Methods: Children scheduled for elective operations were recruited. Pre-operatively a sample of induced sputum was obtained. After anaesthesia was induced, a bronchial brushing and swabs of the upper respiratory tract were obtained. Bacterial community analysis was performed by amplification of the V3–V4 16S rRNA gene region.

Results: Twenty children were recruited, mean age 10.7 years. Induced sputum samples were obtained from 12 children, bronchial brushing from 14 and nasal, mouth, and throat samples in 15, 16, and 17 children. The profile of bacterial communities was similar in the mouth, throat, and sputum samples with the nose and bronchial samples being different. Actinobacteria species dominated the nose and mouth, Fusobacteria were the dominant species in the throat and sputum while Proteobacteria species dominated in bronchial samples. Forty-one percent of detected bacteria in bronchial samples were unclassified. Bacterial communities from the mouth, throat, and induced sputum were tightly clustered and were distinct from nose and those found in bronchial communities.

Conclusions: Induced sputum may not be a valid surrogate for microbiome assessment of the lower airways in all individuals. Many bacteria in bronchial samples were not recognized by standard testing, suggesting that our understanding of the lower airway microbiota in children remains rudimentary.

KEYWORDS
bronchial fluid, child, microbiota, sputum

1 | INTRODUCTION

The lower airways were traditionally considered sterile in healthy individuals, and this paradigm was based on observations that standard microbiology culture from samples collected from healthy individuals yielded no pathogens.[1] The advent of polymerase chain reaction (PCR) testing for bacterial DNA has now identified communities of bacteria in lower airway secretions from healthy individuals.[2,3] The relevance of bacteria in airway microbiota to respiratory symptoms is unclear, but there is evidence suggesting that the microbiota may be relevant to respiratory exacerbations[2,4] or even to the aetiology of chronic respiratory conditions such as asthma.[5] Exacerbations of chronic conditions such as cystic fibrosis (CF) and chronic obstructive pulmonary disease are linked to a change[2] (termed "airway dysbiosis")

or reduction[4] in the dominant bacterial species in the microbiota. In young infants, those carrying *Haemophilus influenzae* or *Moraxella catarrhalis* in the hypopharynx were more likely to have asthma symptoms at three years of age.[5]

A major challenge to studying the lower airway microbiota is obtaining samples without contamination from the upper airways,[3] and it is known that the microbiota in the naso- and oropharynx differ from that in the bronchus in adults and children.[6–8] Three papers have used bronchoscopic sampling to compare the upper and lower airway microbiota in children and while there are similarities in the dominant phyla identified in some studies, there are differences in genera identified and also in the clustering of bacterial communities.[6–8] The characteristics of the lower airway microbiome may also vary between individuals depending on their asthma status[6] and using a nasal or oral approach to the lower airways.[8] Two studies in young people with CF have compared the microbiota in induced sputum and oropharyngeal swabs,[9,10] and both concluded that a throat swab sample may be a valid surrogate for sputum for microbiota analysis. Induced sputum is a non-invasive alternative to direct bronchial sampling, and is used in the clinical management of tuberculosis and CF in children and also in research. The study of the lower airway microbiota would be considerably easier if induced sputum was a valid surrogate of bronchial fluid. The aim of the current study was to collect induced sputum and samples from the bronchus, nose, throat, and mouth in children and undertake a comprehensive assessment of upper and lower airway microbiota in children. Our hypothesis was that induced sputum is a valid alternative to directly sampling the lower airways to study lower airway microbiota.

2 | METHODS

2.1 | Study design

Children aged 5-16 years and scheduled for elective ear nose and throat operations at Royal Aberdeen Children's Hospital between January and June 2015 were invited to take part. All children were scheduled for tonsillectomy and some also had grommets inserted. These operations require endotracheal intubation, which allows sampling of the lower airways without contamination by the upper airways. Children younger than 5 years were excluded since they were unlikely to provide a sample of induced sputum. Other exclusion criteria included receipt of antibiotics within the last month and CF. In the hours before the operation (when the child was fasted), children took part in an assessment which included completion of a questionnaire, height, and weight measurement, spirometry (in accordance with international guidelines[11]) and induction of sputum using 4% and if required 5% saline as previously described.[12] An adequate quality sputum sample was defined as the presence of a white sputum plug on visual inspection; samples were not sufficiently large enough to allow for cell count analysis. Immediately after anaesthesia was induced and the endotracheal tube in situ, a sterile 2.7 mm interdental brush (Dento Care Professional, London UK) was used to collect a sample of nasal secretions and a separate interdental brush was gently brushed on the buccal surface of the mouth to collect oral secretions. A standard bacterial swab (Transwab, Medical Wire Equipment, Corsham, UK) was used to obtain a sample of pharyngeal secretions under direct inspection. Finally, a bronchoscopy cytology brush (10 mm disposable cytology brush, BC 202D-2010, Olympus, Southend-on-Sea, Essex, UK) was passed down the endotracheal tube until resistance was met and then rotated and withdrawn to obtain "blind" bronchial samples. Blood was taken for serum total IgE assay. The study was approved by the North of Scotland Research Ethics Committee (13/NS/0144), written parental consent was obtained and verbal assent was obtained from all participants.

2.2 | DNA extraction and sequencing

The bacterial community analysis was carried out as described previously[13] with some modification as detailed below. Briefly, samples taken from the nose, mouth, throat, sputum, and bronchial brushing were frozen at −80°C prior to analysis. Samples were then transported from Aberdeen to Dundee for processing. Upon arrival the sample were thawed and suspended in phosphate buffer saline where genomic DNA was extracted and purified using the DNA/RNA All Prep kit[14] (Qiagen, Hilden, Germany) and stored according to manufacturer's instructions. Standard protocol, 16S Metagenomic Sequencing Library Preparation Guide (Illumina), was followed to prepare sequencing libraries targeting the variable V3 and V4 regions of the 16S rRNA gene and paired-end sequencing was performed on the MiSeq System (Illumina). We followed sampling and controls procedures described by the Earlham Institute (http://www.earlham.ac.uk/sites/default/files/docs/Genomic%20Services/Sample%20Guidelines%20Aug17.pdf) and Illumina (https://support.illumina.com/documents/documentation/chemistry_documentation/16s/16s-metagenomic-library-prep-guide-15044223-b.pdf) which are designed to minimize the risk for contamination. Quality controls and sequencing were performed at Earlham Institute (Norwich, UK).

2.3 | Data and statistical analysis

Bioinformatics analyses on raw data was performed using the Biomedical Genomics Workbench version 4.0 (Qiagen) equipped with the Microbial Genomics Module version 2.0 (Qiagen) plugin. Sequences were imported and processed for optional merge paired reads, adapter trimming, fixed length trimming, and then the sequences were filtered based on the number of reads to obtain sequences that are comparable in length and coverage for clustering. Quality and chimera filtering were performed using the recommended programme parameters (for complete details see www.qiagenbioinformatics.com). Samples with low coverage were removed from further analysis. Operational Taxonomic Units (OTU) clustering and taxonomic assignment were done using Greengenes v13_5 (97%) as reference. New OTUs were indicated when similarity percentage was lower than 80% with minimum occurrence of five reads. Low abundant OTUs were discarded from further analyses (minimum combined abundance was set at 10). Summary of processed sequence data is described in Supplementary

Table S1. MUSCLE was used for OTUs alignment in order to reconstruct a maximum likelihood phylogeny with neighbor joining as construction method and Jukes Cantor as nucleotide substitution model. We compared community structures and diversity across patient cohorts to determine if inter-cohort differences in structure were seen. To achieve this a rarefaction sampling analysis was carried out using a standard methodology.[15] In order to minimize the risk for contamination, we sought to identify a high (ie, conservative) number of sequences required to characterize bacteria from the samples. In the absence of a standard international stringency cut off the default settings in the workbench software (Qiagen) were applied; the default settings filters out many reads and generates fewer unassigned reads and is therefore highly stringent. These adjustments were assessed by analyzing a previously characterized mock community to confirm that the data handling was consistent with other studies (sequencing files of the project: ERP021973, https://www.ebi.ac.uk/metagenomics/projects/ERP021973/samples/ERS1588932). Data generated in the study was assessed using the Qiagen software and also applied to EBI Metagenomics analysis pipeline V3.0 to verify results (acknowledging that fewer reads would remain in the analysis with Qiagen). The findings in the controls were taken into account during the analysis. Alpha diversity was calculated using number of OTUs. Beta diversity was obtained using D_0.5 UniFrac and represented as Principal Coordinate Analysis (PCoA). Robustness analysis was performed using PERMANOVA with UniFrac distances. All sequencing data have been deposited in NCBI Sequence Read Archive (SRA) database under the follow BioProject ID: PRJNA388557

3 | RESULTS

3.1 | Study subjects

Twenty patients were recruited of which eight were boys, seven with diagnosed asthma and the mean (standard deviation) age was 10.7 (2.8) years. Descriptives of the children recruited are given in Table 1. Induced sputum was obtained from 12 children and samples of fluid from bronchus, throat mouth, and nose were obtained in all children. Three control samples were collected.

3.2 | Sample quality control

A total of 11 750 879 PCR reads were were analysed, and 606 227 high quality reads were obtained. The resulting OTU table contained 1053 OTUs, where 532 OTUs were assigned based on the Greengenes v13_5 (clustered at 97% similarity), and 1011 OTUs were novel (see Supplementary Table S1). These processing criteria allowed data from 74 samples from patients for further analyses. Of these 15 were from the nose, 16 from mouth, 17 from throat, 12 from sputum, and 14 from bronchial samples (Supplementary Table S2). Samples from all five sites were available in six children. The median read counts for the five sites were as follows: bronchus 4438; mouth 4400; throat 4868; nose 8927; and sputum 11250. The results from the mock community analysis using Qiagen and EBI Metagenomics analysis pipelines were highly consistent. However Qiagen software assigned phyla for all samples whereas the EBI Metagenomics did not assign phyla in 15% of samples (see Supplementary Table S3).

3.3 | Bacterial abundance

The relative bacterial abundance in each sample cohort is presented in Figure 1. The overall profile of bacterial communities was largely similar in the mouth, throat, and sputum samples with the nose and bronchial being different. All sample cohorts contained five major bacterial phyla: Actinobacteria, Bacteroidetes, Firmicutes, Fusobacteria, and Proteobacteria. The three most abundant phyla of bacteria observed by analysis in nose, mouth, throat, bronchial, and sputum were Actinobacteria, Fusobacteria, and Bacteroidetes (seen in Figure 1). Actinobacteria dominated in the nose (73% of all total species) and mouth (37%) samples, Fusobacteria dominated in throat (31%) and sputum (38%) samples and Proteobacteria were dominant in bronchial samples (34%). Both nasal and bronchial samples had more unclassified OTUs, (12% and 41% respectively), than other samples. The relative abundance of each individual sample is shown in Figure 2. Supplementary Figure S1 presents the phylogenetic tree of the microbial community in all analysed samples. In two of the control samples there was no identifiable bacteria DNA signal however some DNA signal was detected in the one of the three control and this was taken into account during analysis (See Figure 2).

3.4 | Comparison between complete community structures within samples from different sites

The rarefaction analysis suggested that 2000 sequences would be sufficient for characterizing the majority of bacteria present in these communities. The numbers of reads were lowest in bacterial communities from mouth and bronchus (Figure 3; Supplementary Table S2). A comparison of individual bacterial communities, using PERMANOVA principal coordinate analysis (PCoA), showed a distinct clustering by sample cohorts based roughly on where the sample was taken from. Bacterial communities in samples from the mouth, throat, and induced sputum were tightly clustered together (padj = 1) and were distinct from those of the nose and bronchus (padj <0.001), which independently clustered (Figure 4; Table 2); these clustering results were seen when children with asthma and without asthma were considered separately (Supplementary Table S4), Table S5 demonstrates that clustering of bacterial communities was not evident between sites among subgroups stratified by asthma/not asthma or atopy/not atopy.

The bacterial communities in the five sites sampled did not differ between the children with and without asthma; there was evidence of some differences in bacterial abundance and diversity between groups (see Supplementary Figure S2). A comparison of the abundant phyla of bacteria identified in bronchial samples taken from asthma and non-asthma patients showed the profiles were similar, but the abundance of a signal for phyla was different (but did not reach significance). In bronchial samples from asthma patients, the abundant phyla identified

TABLE 1 Details of the children recruited

	All children (*n* = 20 unless stated)	Children with asthma (*n* = 7 unless stated)	Children without asthma (*n* = 13 unless stated)
Mean age (SD), y	10.7 (2.8)	12.1 (2.2)	9.9 (2.9)
Proportion male (number)	40% (8)	43% (3)	39% (5)
Mean % FEV_1/FVC ratio (SD)	103% (8) *n* = 19	101% (9.5)	104% (8) *n* = 12
Median plasma IgE (SEM), kU/l	118 (36) *n* = 19	193 (48) *n* = 6	82 (49)
Mean BMI centile (SD)	70% (28)	64% (28)	73% (28)
Proportion where the sample met the quality control criteria for analysis (*n*)			
Bronchial fluid	70% (14)	86% (6)	62% (8)
Induced sputum	60% (12)	86% (6)	46% (6)
Throat swab	85% (17)	100% (7)	77% (10)
Mouth swab	80% (16)	100% (7)	69% (9)
Nose swab	75% (15)	100% (7)	62% (8)
Asthma treatment		Three SABA only	
		Two ICS plus SABA	
		Three ICS, LABA plus SABA	

ICS, inhaled corticosteroids; LABA, long acting beta agonist; SABA, short acting beta agonist.

was Proteobacteria while those samples taken from non-asthma patients showed a greater abundance for Fusobacteria.

4 | DISCUSSION

This study was designed to determine whether induced sputum is a valid alternative to directly sampling of the lower airways to study airway microbiota in children. The main finding was that the characteristics of the microbiota in induced sputum and in bronchial samples were different. A second finding was that a minority of bacterial DNA in bronchial samples was unclassified. In two individuals (P12 and P16, see Figure 2) the proportion of phyla identified from the bronchial sample was similar to the sputum sample but for the remainder of participants there were clear differences in the proportions of different phyla from bronchial and sputum samples. Together these findings suggest that induced sputum is not a valid surrogate for direct bronchial sampling to study the lower airway microbiota in all children, but that there is still a pressing need to better understand the lower airway microbiota in children. Opportunistic bronchial sampling under clinically indicated general anaesthetic should remain the gold standard for studying lower airway microbiota in children.

To our knowledge there are only three studies which have compared the microbiota of upper and lower airway secretions in healthy children or children with asthma.[6–8] A study where the majority of recruits were adults showed that the bacterial community in the oropharynx and bronchus were similar in healthy individuals.[6] One solely paediatric study found very little overlap between the microbiota of the oropharynx and lower airways[7] whille a second paediatric study found similar predominant phyla in upper and lower airway samples but different genera.[8] Both paediatric studies found significant differences in the clustering of bacterial communities in the upper and lower airways.[7,8] The dominant phyla in bronchial samples is not consistent between studies but there is consistency for upper airway samples. For example, the microbiota of the upper airways is predominated by bacteria from the Firmicutes (eg, *Staphylcoccus* and *Streptococcus*) and the Actinobacteria phyla (eg, *Corynebacteria*) in our study and three others.[6–8] In contrast and in bronchial samples, bacterial species within the *Proteobacteria phylum* (including *Moraxella* and *Haemophilus*) predominated in our study and one other[7] but Firmicutes dominated in a third study.[8] Differences in lower airway microbiome between studies may be due to different sampling methods[8] and treatment with current or recent antibiotics.[7,8] Our results, where we find no differences between the microbiome of induced sputum and throat swab, are also consistent with previous studies which suggested that a throat swab may be a useful surrogate of induced sputum in young children with CF.[9,10] As our study did not include children with CF we cannot comment on the validity of induced sputum as an index of lower airway infection in this specific clinical setting. The consistency of our results with previous studies assures us that our findings are valid, despite the relatively small sample size.

Our study findings were based on samples taken from a group of children who were well. Recent work has given insight into the relationship between airway microbiota in the upper airways of children with acute infection, with results which differ from the present study. For example, there is evidence that in the context of acute upper and lower respiratory tract infection, different bacterial species predominate in the nasopharynx (eg, Proteobacteria and Firmicutes which contrast with Actinobacteria and Fusobacteria in our study).[15] The characteristics of the nasopharyngeal microbiota also change during the course of acute otitis media infection.[16]

Our study design minimized the risk of false positive results arising from contamination and from samples containing low DNA yields.

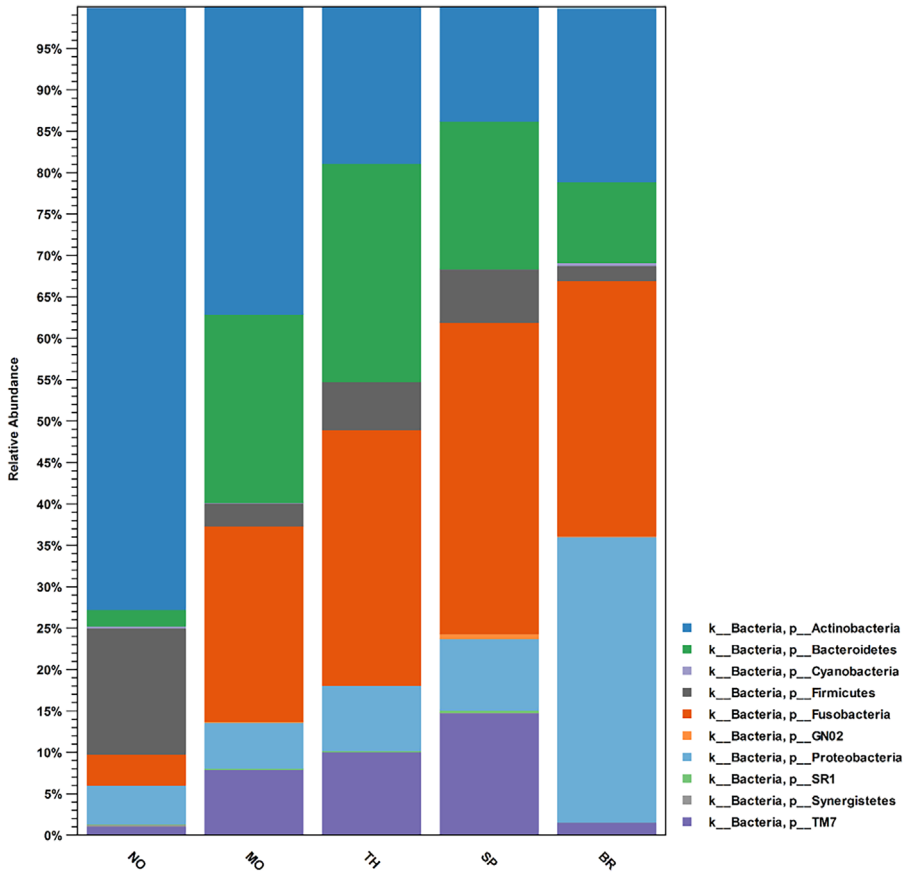

FIGURE 1 Relative abundances of bacterial phyla identified as operational taxonomic units (OTUs) from the sequence reads generated from airway samples taken from children. The bar chart illustrates the taxonomic composition of each cohort of samples from a particular site. A detailed summary of the bacteria in each sample is described in Figure 2

There is no consensus on the number of control samples which should be collected for microbiome studies, and two of the three controls we collected contained no bacterial DNA and a third contained only bacteria with a very different profile of phyla compared to the samples from study subjects. We set out to have a high threshold for reads (>2000) and this filtered out samples with low DNA yields. Another source of potential contamination comes from reagents used to analyze the samples and this risk was minimized by taking standard precautions and by including control samples in the analysis. While we cannot exclude the possibility that some contamination may have occurred, our robust methodology, and the consistency of our findings with previous studies assure us that any contamination has not substantially affected our findings.

Finding a small proportion of unassigned bacterial DNA in the nose and bronchial samples was unexpected and worthy of further investigation. The presence of "unclassified bacteria" has been described in nasopharyngeal samples from children,[7] but not in bronchial samples. We have carefully reviewed the unassigned sequences and removed human DNA sequences, and we therefore believe that there are a number of bacteria in the respiratory tract which are not identified by standard microbiota methodologies currently used. We analyzed data from a mock community (a "positive control") and this demonstrated that the Qiagen software used for analysis of our samples was able to detect bacteria in similar proportions to another software "pipeline." The Qiagen software identified all phyla whereas the alternative software could not assign 15% of bacterial DNA and this may be explained by the higher stringency set for the Qiagen software. There is no standard for stringency but these results suggest that although the higher stringency reduces the number of reads included in the analysis, the results are highly comparable to other methods, and possibly less affected by potential contaminants. Our data are publicly available and colleagues are welcome to apply different stringencies to our data.

There are a number of settings where upper airway samples have been shown to be valid surrogates of lower airway samples for the clinical diagnosis of infective and non-infective conditions. Viral aetiology in bronchiolitis (a lower respiratory tract infection) can be established by nasopharyngeal aspirate[17] and the nasal mucosa is used for diagnosis of primary ciliary dyskinesia[18] and (in some situations) CF.[19] In the research setting, sampling from the upper airways has been validated as a surrogate for lower airway sampling.[20,21] However, we demonstrated that the microbiota of the nose was distinct to mouth and bronchus in most individuals and our results indicate that the microbiota of the upper airway is probably not a valid surrogate of lower airway microbiota in children. Previous papers have suggested that with regards to lower airway samples, upper airway samples are "imperfect but reliable"[7] and "both similar and different"[8] and overall, our work

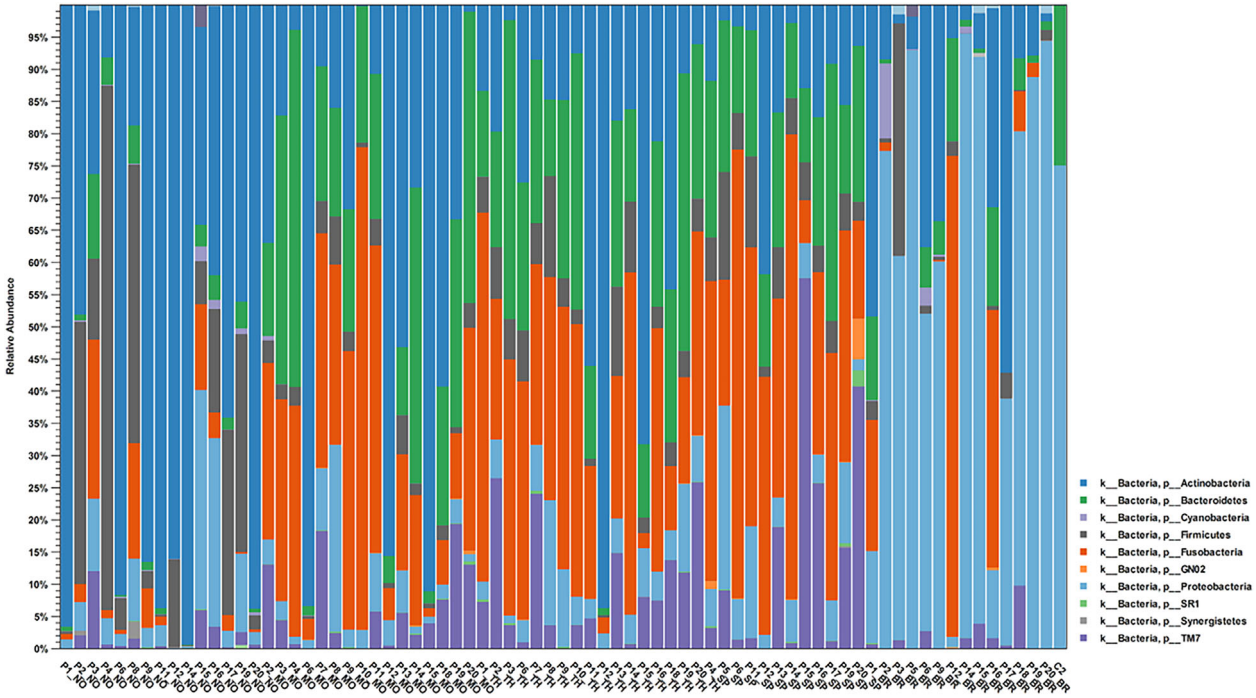

FIGURE 2 Operational Taxonomic Units based relative sequence abundance of bacterial phyla based on 16S rRNA of all 75 samples (ie, 74 patient samples plus the control with detectable bacterial DNA). A detailed summary of the bacteria in each sample is described. On the horizontal axis, the number following P is the patient number and NO, nose; MO, mouth; TH, throat; SP, Sputum; BR, bronchial sample. C2 = the control sample where bacterial DNA was detected. N/A = not applicable

finds more evidence for the upper airways samples being "imperfect and different" to lower airway samples and not "reliable and similar."

The novelty of our study is that we demonstrate how the microbiota of induced sputum differs from bronchial samples and very closely matches the microbiota of mouth and throat, most likely due to contamination of lower airway secretions as they pass through the oropharynx, including sputum which is commonly mixed with expectorant. Our findings are consistent with a study of 78 children (mean age

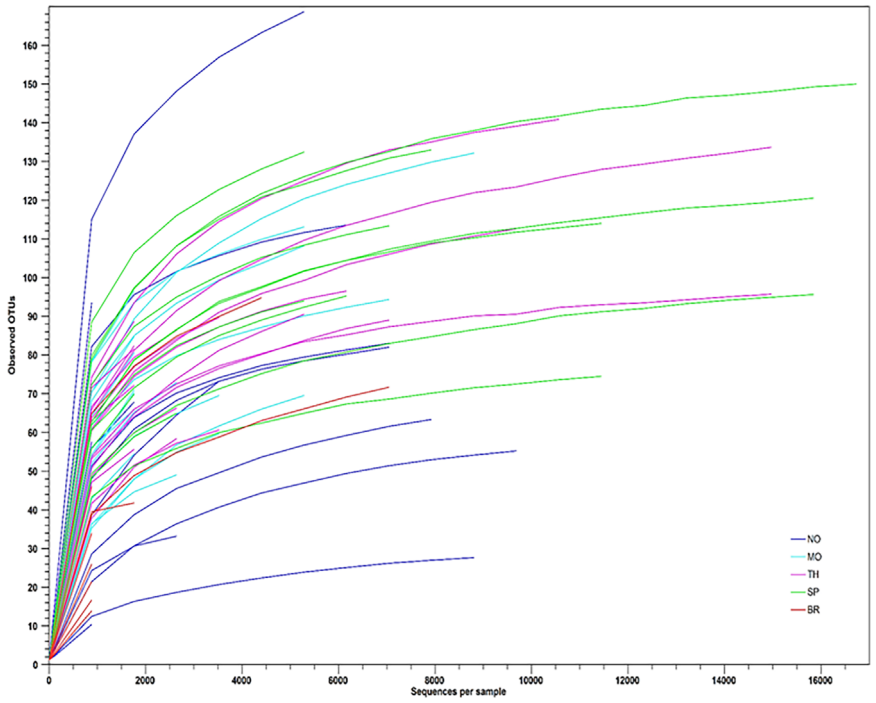

FIGURE 3 Alpha diversity rarefaction curves of samples based on total number of observed Operational Taxonomic Units. Color denotes different sample cohort ("nose" (NO), "mouth" (MO), "throat" (TH), "sputum" (SP), and "bronchial" (BR) samples)

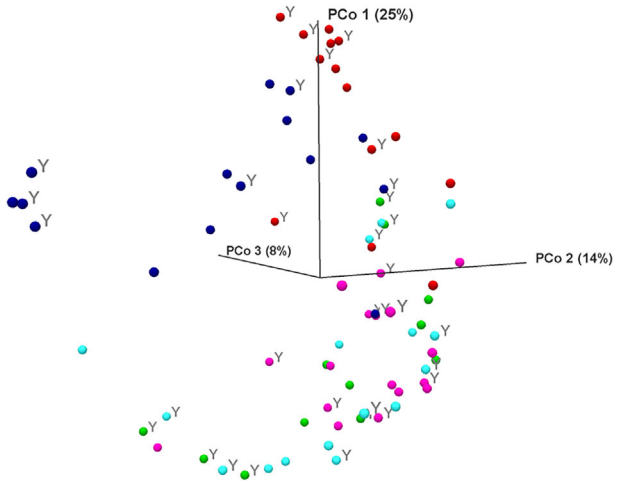

FIGURE 4 Diversity analysis demonstrating differences in the bacterial phyla community between samples taken from airway samples taken from asthmatic and non-asthmatic children. Principal coordinate (PCo) analysis of all samples based on D_0.5 UniFrac distance. Color denotes different sample cohort ("nose" (NO), "mouth" (MO), "throat" (TH), "sputum" (SP), and "bronchial" (BR) samples). Y = sample from child with asthma

2 years, almost half having received recent antibiotic treatment) which also describes differences in the microbiota between nose and mouth and between mouth and bronchial fluid.[7] Consistent with our findings, a study of adults and children (mean age 11 years) reports Actinobacteria being the predominant phyla in the nose but being almost absent from the oropharynx and bronchus. A different pattern is shown for Proteobacteria which are increasing from nose through to oropharynx and bronchus.[6] The apparent presence of different dominant bacterial communities in the upper and lower airways raises the question "where do bacteria in the lower airways originate?"

In children the lower airways are in direct communication with and adjacent to the oropharynx but there are several mechanisms that may lead to the establishment of different bacterial communities above and below the vocal cords. Perhaps most obviously, a child's mouth is regularly exposed to food and inedible items introduced to the oral cavity (including cutlery and fingers) whereas the lower airways are exposed only to inhaled exposures. Micro aspiration due to laryngopharyngeal reflux is thought to occur on a regular basis[22] and exposure to acid and other gastric contents (including bacteria) may affect the microbiota of the lower airways but not the oropharynx. There are differences in the innate defences of the upper and lower airways which may also explain differences in their respective microbiota, for example, lactoferrin concentrations are twice as high in the lower airways relative to upper airways[23] whereas nitric oxide concentrations (known to have antimicrobial properties[24]) are typically one hundred times higher in the upper airways.

Our study was not designed to relate microbiota to clinical phenotypic data collected, for example, asthma or atopy. However, in light of recent studies examining these parameters in children with asthma[6,7] we made the best use of the data available, even with the limited number we had at our disposal. We examined the possible relationship between the bacteria present in children with and without asthma or atopy, we found no statistically significant differences between the bacteria when the samples were examined as a whole group or as specific sample region cohorts (nose, mouth, throat, bronchial, and sputum samples), Supplementary Figure S2 and Table S3. A descriptive analysis of the bronchial samples collected found Protoebacteria to be the dominant species and this is consistent with Hilty et al.[6]

There are some limitations to our study. First, the number of participants was relatively small. Second, induced sputum was not obtained in all participants and not all samples met our quality control criteria for bacterial DNA analysis and this resulted in missing data for some individuals. Third, as has been reported in other sputum microbiome studies,[6,7] we were not able to perform cell counts on the sputum pellet to determine the presence of neutrophils and absence of squamous epithelial cells. Finally, we did not prospectively calculate the DNA yield from samples although the consistency of our results with the small number of published studies,[6,7] the consistency of results using both the Qiagen and EBI Metagenomics pipelines and

TABLE 2 PERMANOVA analysis results of testing differences in beta-diversity among different sample sites

Sample site 1	Sample site 2	Number of individuals with comparable data	Number of samples included in the analysis (site 1 & site 2)	*P* value (unadjusted)	*P* value (adjusted for multiple testing)
Nose	Mouth	11	15 & 16	0.00001	0.00015
Nose	Throat	13	15 & 17	0.00001	0.00015
Mouth	Throat	14	16 & 17	0.22171	1
Nose	Sputum	10	15 & 11	0.00001	0.00015
Mouth	Sputum	10	16 & 11	0.17806	1
Throat	Sputum	10	17 & 11	0.27851	1
Nose	Bronchus	12	15 & 14	0.00002	0.0003
Mouth	Bronchus	9	16 & 14	0.00001	0.00015
Throat	Bronchus	12	17 & 14	0.00001	0.00015
Sputum	Bronchus	10	11 & 14	0.00003	0.00045

Paired samples were not available in all individuals.

the comparable results from mock communities assure is that low DNA yield has not altered the results.

In summary, we report that neither induced sputum nor swabs from nose or mouth give an accurate indication of the bronchial microbiota in all children. For very obvious practical and ethical reasons, upper airway sampling will be necessary to study airway microbiome in many research setting and our findings adds to the evidence describing the limitations of this pragmatic approach. Our study was not designed to compare differences in the lower airway microbiota of children with and without asthma, and future research is required to supplement the little we do know about this potentially important subject.

ACKNOWLEDGMENTS

The authors would like to thank the study participants and their parents. The study was supported by an NHS-Grampian Endowments. AW is supported by the Wellcome Trust Strategic Award (grant 097377) and the MRC Centre for Medical Mycology (grant MR/N006364/1) at the University of Aberdeen.

CONFLICTS OF INTEREST

None of the authors has any conflicts of interests.

ORCID

Steve Turner http://orcid.org/0000-0001-8393-5060

REFERENCES

1. Kahn FW, Jones JM. Diagnosing bacterial respiratory infection by bronchoalveolar lavage. *J Infect Dis*. 1987;155:862–869.
2. Marsland BJ, Yadava K, Nicod LP. The airway microbiome and disease. *Chest*. 2013;144: 632–637.
3. Rogers GB, Shaw D, Marsh RL, Carroll MP, Serisier DJ, Bruce KD. Respiratory microbiota: addressing clinical questions, informing clinical practice. *Thorax*. 2015;70:74–81.
4. Klepac-Ceraj V, Lemon KP, Martin TR, et al. Relationship between cystic fibrosis respiratory tract bacterial communities and age, genotype, antibiotics and Pseudomonas aeruginosa. *Environ Microbiol*. 2010;12:1293–1303.
5. Bisgaard H, Hermansen MN, Bonnelykke K, et al. Association of bacteria and viruses with wheezy episodes in young children: prospective birth cohort study. *BMJ*. 2010;341:4978.
6. Hilty M, Burke C, Pedro H, et al. Disordered microbial communities in asthmatic airways. *PLoS ONE*. 2010;5:8578.
7. Marsh RL, Kaestli M, Chang AB, et al. The microbiota in bronchoalveolar lavage from young children with chronic lung disease includes taxa present in both the oropharynx and nasopharynx. *Microbiome*. 2016;4:37.
8. Kloepfer KM, Deschamp AR, Ross SE, et al. In children, the microbiota of the nasopharynx and bronchoalveolar lavage fluid are both similar and different. *Ped Pulmonol*. 2018;53:475–482.
9. Zemanick ET, Wagner BD, Robertson CE, et al. Assessment of airway microbiota and inflammation in cystic fibrosis using multiple sampling methods. *Ann Am Thor Soc*. 2015;12: 221–229.
10. Boutin S, Graeber SY, Weitnauer M, et al. Comparison of microbiomes from different niches of upper and lower airways in children and adolescents with cystic fibrosis. *PLoS ONE*. 2015;10: e0116029.
11. Miller MR, Hankinson J, Brusasco V, et al. Standardisation of spirometry. *Eur Respir J*. 2005;26:319–338.
12. Pontin J, Blaylock MG, Walsh GM, Turner SW. Sputum eosinophil apoptotic rate is positively correlated to exhaled nitric oxide in children. *Pediatr Pulmonol*. 2008;43:1130–1134.
13. Twomey KB, Alston M, An S, et al. Microbiota and metabolite profiling reveal specific alterations in bacterial community structure and environment in the cystic fibrosis airway during exacerbation. *PLoS ONE*. 2013;8:e82432.
14. Moen AEF, Tannaes TM, Vatn S, Ricanek P, Vatn MH, Jahnsen J. IBD-Character Consortium. Simultaneous purification of DNA and RNA from microbiota in a single colonic mucosal biopsy. *BMC Research Notes*. 2016;9:328.
15. Kelly MS, Surette MG, Smieja M, et al. The nasopharyngeal microbiota of children with respiratory infections in Botswana. *Pediatr infect Dis J*. 2017;36:e211-–ee218.
16. Chonmaitree T, Jennings K, Golovko G, et al. Nasopharyngeal microbiota in infants and changes during viral upper respiratory tract infection and acute otitis media. *PLoS ONE*. 2017;12: e0170630.
17. Ralston SL, Lieberthal AS, Meissner HC, et al. Clinical practice guideline: the diagnosis, management, and prevention of bronchiolitis. *Pediatrics*. 2015;136:782.
18. Barbato A, Frischer T, Kuehni CE, et al. Primary ciliary dyskinesia: a consensus statement on diagnostic and treatment approaches in children. *Eur Respir J*. 2009;34:1264–1276.
19. Farrell PM, Rosenstein BJ, White TB, et al. Guidelines for diagnosis of cystic fibrosis in newborns through older adults: cystic Fibrosis Foundation consensus report. *J Pediatr*. 2008;153: S4.
20. Pringle EJ, Richardson HB, Miller D, et al. Nasal and bronchial airway epithelial cell mediator release in children. *Pediatr Pulmonol*. 2012;47:1215–1225.
21. Pitrez PMC, Brennan S, Turner S, Sly PD. Nasal wash as an alternative to bronchoalveolar lavage in detecting early pulmonary inflammation in children with cystic fibrosis. *Respirology*. 2005;10:177–182.
22. Venkatesan NN, Pine HS, Underbrink M. Laryngopharyngeal reflux disease in children. *Pediatr Clin North Am*. 2013;60: 865–878.
23. Roca-Ferrer J, Mullol J, Perez M, et al. Effects of topical glucocorticoids on in vitro lactoferrin glandular secretion: comparison between human upper and lower airways. *J Allergy Clin Immunol*. 2000;106: 1053–1062.
24. Fang FC. Antimicrobial reactive oxygen and nitrogen species: concepts and controversies. *Nature Rev Microbiol*. 2004;2:820–832.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: An S, Warris A, Turner S. Microbiome characteristics of induced sputum compared to bronchial fluid and upper airway samples. *Pediatric Pulmonology*. 2018;1–8. https://doi.org/10.1002/ppul.24037