SciMedCentral

Annals of Clinical and Medical Microbiology

Original Research

Respiratory and Gut Microbiota of Children with Cystic Fibrosis: A Pilot Study

Jannaina Ferreira de Melo Vasco*, Carlos A. Riedi[2], Camila Marconi[3], Keite S. Nogueira[4], Luiza Souza Rodrigues[5], Wellington Pine Omori[6], Rafael R. de Oliveira[6], Lilian Perreira-Ferrari[7], Nelson A. Rosário Filho[1,2]

[1]*Postgraduate Program in Child and Adolescent Health, Federal University of Paraná (UFPR), Curitiba, Brazil. Gen. Carneiro Street, 181, Curitiba-PR, Brazil, Zip code 80060-900*

[2]*Department of Pediatrics, Federal University of Paraná (UFPR), Curitiba-PR, Brazil. Padre Camargo Street, 453, Curitiba-PR, Brazil, Zip code 80030-110.*

[3]*Department of Basic Pathology, Sector of Biological Sciences, Federal University of Paraná (UFPR). Coronel Francisco H. dos Santos Avenue, 100, Curitiba-PR, Brazil, Zip code 81531-980.*

[4]*Laboratory of Bacteriology, Hospital of Clinics, Federal University of Paraná (UFPR). Padre Camargo Street, 280 Curitiba-PR, Brazil, Zip code 80060-240.*

[5]*Instituto de Pesquisa Pelé Pequeno Príncipe, Silva Jardim Avenue, 1632, Curitiba – PR, Brazil, Zip code 80250-060.*

[6]*Neoprospecta Microbiome Technologies, Sapiens Parque, Florianópolis, SC, 88056-000, Brazil, Zip code 88054-700.*

[7]*Federal University of Paraná (UFPR), Curitiba-PR, Brazil. Curitiba, Brazil. Gen. Carneiro Street, 181, Curitiba-PR, Brazil, Zip code 80060-900*

***Corresponding author**

Jannaina Ferreira de Melo Vasco, Padre Camargo Street, 453 - Alto da Glória, Curitiba-PR, Brazil, Zip code 80030-110 Tel.: +55 41 32086500; E-mail address: jannaina.vasco@gmail.com

Submitted: 23 August 2021

Accepted: 21 September 2021

Published: 23 September 2021

Keywords

- Cystic fibrosis
- Microbiota
- Respiratory microbiota
- Gut microbiota
- 16S rRNA sequencing

Abstract

Differences in the clinical presentation of cystic fibrosis (CF) may be due to microbiota components and their relationship with the host's immune system. In this pilot study, we aimed to investigate the composition of the respiratory and gut microbiota of a cohort of clinically stable children with CF, homozygous for the p.Phe508del mutation. Oropharyngeal swabs and stool samples were obtained from these children attending the CF referral clinics at the Hospital of Clinics, Federal University Paraná (CHC – UFPR). Oropharyngeal and gut microbiota were assessed by V3-V4 sequencing of the 16S ribosomal RNA, and bioinformatics analyses were performed using a proprietary pipeline. We identified a total of 456 bacterial taxa belonging to 164 genera, of which 65 (39.6 %) were common to both the respiratory and gastrointestinal tracts. Taxa from eight genera dominated more than 75 % of the microbial composition of both the niches. Among these dominant taxa, only Prevotella spp. were common to both the sites. Overall, the respiratory and gut microbiota were homogeneous among all the patients. Longitudinal studies targeting a larger cohort are important for an improved understanding of how the composition of bacterial communities is related to changes in the clinical status of CF.

ABBREVIATIONS

CF: cystic fibrosis**; CFTR:** cystic fibrosis transmembrane conductance regulator**; EL-1:** fecal elastase-1**; URT;** upper respiratory tract

INTRODUCTION

Mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene affects the respiratory and gut microbiota, even in the absence of antibiotic exposure [1–3]. Cystic fibrosis (CF) is associated with changes in the host´s microbiota, leading to lower diversity and reduced richness of bacterial communities [4–7]. Studies have highlighted that dysbiosis in individuals with CF may be related to physiological factors such as increased mucus secretion, altered dietary intake, medications such as pancreatic enzymes [4], increasing age, the use of antibiotics, decreased lung function, and disease progression [5– 7]. It has been hypothesized that differences in the clinical presentation of CF in infants and young children with the same CFTR mutation may be explained by the distinct microbiota composition and its interactions with host immunity, including the gut-lung axis [8, 9]. It is well acknowledged that the intestinal microbial colonization profile is a determinant of the microbiota of the respiratory tract. This is because both sites share several common genera, and intestinal colonization by certain species often precedes their detection in the respiratory tract [8, 10]. Longitudinal studies have identified consistent trends in the temporal dynamics of the respiratory microbiota according to the age of the host. In patients with CF aged less than two years, the respiratory tract microbiota is dominated by *Streptococcus spp., Prevotella spp., Veillonella spp., Rothia spp., and Actinomyces spp.* These species are progressively replaced by CF-associated pathogens, such

as *Pseudomonas spp., Burkholderia spp., Stenotrophomonas spp., and Staphylococcus spp.*, which further dominate this bacterial community in patients aged 6 years and above [6,11]. Gut microbiota in patients with CF is significantly different from that in non-CF patients, with a substantial decrease in bacterial richness and diversity from infancy to late adolescence [12–14]. In these niches, a greater abundance of taxa from the Firmicutes phylum (e.g., *Staphylococcus spp., Streptococcus spp., Clostridioides difficile, and Veillonella dispar*), was observed concomitantly with decreased abundance of Bacteroidetes (e.g., Bacteroides spp.,

Bifidobacterium adolescentis, Eubacterium spp., Ruminococcus spp., and Faecalibacterium prausnitzii) [15,16]. Culture-independent studies have demonstrated that although the gut and respiratory microbiota show differences in composition, there is some concordance of the gut and respiratory microbiota in infants with CF over time [15, 17]. Thus, there is evidence of a relationship and crosstalk between the intestine and the lung (the gut-lung axis) [18, 19]. To better understand the mechanistic basis of the relationship between the host´s microbiota and the clinical presentation of CF, it is essential to characterize the microbiota of both respiratory and gastrointestinal tracts of CF. Additionally; these results could allow the identification of bacterial predictors of clinical status, including pulmonary disease in CF [20, 21]. In order to establish methods for sampling and data analysis in a larger cohort study, we conducted a pilot cross-sectional study in which we aimed to characterize the gut and respiratory microbiota of clinically stable children with CF who were homozygous for the p.Phe508del mutation.

MATERIALS AND METHODS

Patients and sampling

Ten clinically stable children with CF were enrolled for the study. Homozygosity for p.Phe508del was determined during neonatal screening and CF was confirmed by the sweat chloride test and genetic testing (**Table 1**). All participants showed pancreatic insufficiency and underwent pancreatic enzyme replacement therapy (e.g., Creon). The status of exocrine pancreatic function was defined based on steatorrhea and the levels of fecal elastase-1 (EL- 1). The values of the fecal elastase assay and enzyme linked immunosorbent assay stool test (BIOSERV Diagnostics, Rostock, Germany) for pancreatic elastase 1 were considered normal in the range of 200–500 mg/g of feces, borderline in the range of 100–200 mg/g feces, and were considered to have pancreatic insufficiency if less than 100 mg/g of feces. None of the participants had a culture positive for *Pseudomonas aeruginosa* or presented pulmonary exacerbation four weeks prior to sampling, and none of them were treated with antimicrobial drugs two weeks prior to sampling. The study protocol was approved by the Human Research Ethics Committee of Complexo Hospital de Clínicas - Federal University Paraná (CHC–UFPR), Curitiba-PR, Brazil (CEP-HC/UFPR ref no: 1.948.265). Parents of all the patients provided written informed consent for participation in the study. Stool and oropharyngeal swabs were collected from each participant using sterile flocked swabs (Copan Diagnostics, Murrieta, CA, USA) during regular scheduled visits to the CF outpatient clinic at CHC-UFPR. Each swab was placed in a separate microtube containing 1 mL of stabilization solution (NeoSampleZ, Neoprospecta, Brazil) and homogenized by microtube inversion. The samples were stored at 18–27 °C until shipment to the Neoprospecta laboratory (Florianopolis, SC, Brazil) within 30 days of sample collection. **Microbiota analysis** DNA was extracted from the preserved respiratory and fecal samples using the DNeasy PowerSoil Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. Amplification of the 16S ribosomal RNA V3/V4 region was carried out using the 341F 5′- CCTACGGGRSGCAGCAG-3′ and 806R 5′-GGACTACHVGGGTWTCTAAT-3′ primer sets, according to a previously described method [22, 23]. The libraries were prepared using a double polymerase chain reaction (PCR) protocol containing TruSeq structure adapter (Illumina, USA), allowing a second PCR with indexing sequences, as previously described [24]. The final PCR reactions were cleaned using 132 AMPureXP beads (Beckman Coulter, USA), and samples were pooled in the sequencing libraries for quantification. The library

Table 1: Overview of the clinical characteristics of the 10 study participants.

Participant ID	Sex	Age (years)	Pancreatic insufficiency, treatment given	Levels of EL-1 (µ/g)	PA	DNase	Vitamin supplement (ADEK)	GI findings
					Culture[a]			Steatorrhea
CF 1	Female	6.4	Yes, Creon	< 5,50	Negative	Yes	Yes	Yes
CF 2	Male	2	Yes, Creon	< 5,50	Negative	No	Yes	Yes
CF 3	Male	6.3	Yes, Creon	< 5,50	Negative	No	Yes	Yes
CF 4	Female	6.2	Yes, Creon	< 5,50	Negative	Yes	Yes	Yes
CF 5	Male	4	Yes, Creon	< 5,50	Negative	Yes	Yes	Yes
CF 6	Female	3	Yes, Creon	< 5,50	Negative	Yes	Yes	Yes
CF 7	Female	4.8	Yes, Creon	< 5,50	Negative	Yes	Yes	Yes
CF 8	Female	2	Yes, Creon	< 5,50	Negative	Yes	Yes	Yes
CF 9	Male	5.9	Yes, Creon	< 5,50	Negative	Yes	Yes	Yes
CF 10	Female	12.6	Yes, Creon	< 5,50	Negative	Yes	Yes	Yes

Abbreviations: CF; cystic fibrosis**; EL-1**; fecal elastase-1; **PA**; *Pseudomonas aeruginosa*; **GI;** Gastrointestinal a within the last month.

pools were adjusted to a final concentration of 11.5 pM and sequenced using a MiSeq system (Illumina Inc., San Diego, CA), using the V2 kit (2 × 250 cycles) [25]. Raw read sequences were converted into FASTQ files, quality-filtered (Phred quality score < 20), and trimmed to remove primers. A final 283-bp read was thus obtained using the Sentinel pipeline (Neoprospecta Microbiome, Florianopolis, SC, Brazil). Identical reads (99 % similarity) were grouped into clusters. Clusters present in less than five samples were removed, since such structures are often related to chimeric sequences [26]. Operational taxonomic units (OTUs) were selected using the BLASTN v.2.6.0+ tool [27] with the 16S ribosomal gene database created by Neoprospecta Microbiome. For taxonomic assignments, only sequences with one of the following conditions were used in subsequent analyses: high bit score, low e-value, or taxonomies with greater representation. A total of 1,056,505 reads (average of 42,010 reads per sample) were retrieved by sequencing analyses. Finally, a bacterial profile for each sample was created based on the relative abundance of the bacterial genera. Relative abundance was calculated after normalization using the median values of all the reads retrieved from each sample. Graphs of the proportion of taxa in each patient and all the samples; Venn diagrams were constructed using the OTU table normalized by the median and Venn Diagram packages in R [28,29]. To determine the relative abundance of bacterial taxa at each site, the numbers of reads of an individual taxon were divided by the sum of all taxa reads from the sample in each participant. reads (average of 42,010 reads per sample)

RESULTS AND DISCUSSION

We identified 456 bacterial taxa representing 164 genera, of which 65 (39.6 %) were shared between the gastrointestinal and respiratory tracts Venn diagram (Figure 1). Gut microbiota showed greater richness (130 genera) than respiratory microbiota (99 genera). The gut microbial community comprised of nine genera: *Bacteroides*, *Blautia*, *Anaerostipes*, *Lachnoclostridium*, *Tyzzerella*, *Veillonella*, *Streptococcus*, *Clostridium*, and *Bifidobacterium.* As shown in (Figure 2), eight genera represented more than 75 % of the *taxa* found in either the gut or the respiratory niches. Among the most abundant genera, only *Prevotella* spp. was common to both the sites. Gut microbiota analysis revealed that the genus *Bacteroides* from the phylum Bacteroidetes showed the highest relative abundance in all the patients, except patient 10, who showed *Prevotella* spp. as the most abundant genus (Figure 2). The initial years of life are critical for the development of the immune system, and bacterial species play a critical role in this process [30]. Progressively, the relatively simple neonatal intestinal microbiota matures and increases the complexity of the microbiota, with enrichment in Firmicutes and Bacteroidetes. However, at the genus level, intestinal microbiota are significantly different in children and adults with CF [31]. External factors, such as antibiotics, seem to alter the bacterial composition in children below two years of age, and are associated with a decrease in the relative abundance of *Bacteroides* spp. [14]. Age is certainly considered as the main predictor of fecal bacterial composition and is positively associated with an increase in the relative abundance of the genus *Blautia* (phylum Firmicutes) [10, 32]. All the fecal samples showed a high abundance of this genus. The respiratory "core" microbial community was composed of five genera: *Fusobacterium*, *Rothia*, *Streptococcus, Veillonella*, and

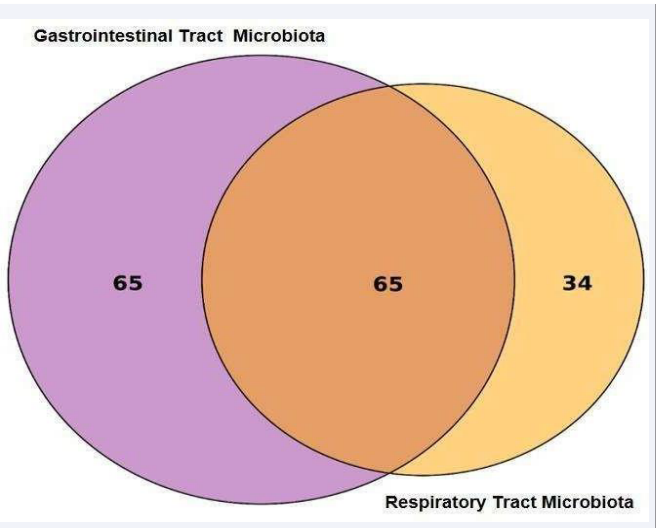

Figure 1 Distribution of the genera identified in the sequencing analyses, based on the assessment of the two body sites (gastrointestinal and respiratory tracts).

Haemophilus. The anaerobic *Prevotella* genera were found in the respiratory tracts of nine children and were the predominant taxon in three of them. The abundance of *Prevotella* spp. in the airways of patients with CF is unclear, and in this study, all patients were stable. Further characterization of the isolates will aid in determining the contribution of *Prevotella* spp. to airway disease associated with CF. Considering the respiratory "core" genera, *Streptococcus* spp. was the most abundant genus in the three samples. The composition of a healthy lung microbiota is determined by the balance of microbial immigration from the upper respiratory tract (URT) and its elimination capacity. In healthy children, although the lung microbiota is distinct from the URT microbiota, it is dominated by species that are also present in the nasopharynx and oropharynx, including *Moraxella* spp., *Haemophilus* spp., *Staphylococcus* spp., and *Streptococcus* spp. [33]. The adult lung microbiota seems to be dominated by the phyla Firmicutes and Bacteroidetes, including the genera *Streptococcus* spp., *Veillonella* spp., and *Prevotella* spp. [34]. *Veillonella* spp. have been previously associated with positive interactions with *Streptococcus* spp., *Moraxella catarrhalis,* and *Haemophilus influenzae* [35, 36] Except *Moraxella* spp., all other genera were detected in the respiratory samples. Although none of the study participants presented symptoms of respiratory exacerbation or a positive culture for *P. aeruginosa*, four of them (40 %) showed the presence of *Pseudomonas* spp. in the oropharyngeal microbiota. It is known that the genus *Pseudomonas* is enriched in the airways of clinically stable children with CF when compared to non-CF children [37, 38, and 39]. A previous study showed that decrease in the relative abundance of *Parabacteroides* spp. in the gut microbiota precedes chronic colonization by *Pseudomonas aeruginosa* [14,40]. Patient 01 showed less diversity in the respiratory microbiota compared to the other patients, with *Staphylococcus aureus* being the most abundant species. Although lower microbial diversity is associated with lower quality of respiratory microbiota, and *S. aureus* is an opportunistic pathogen in CF, interactions between species and with the host interfere in these dynamics [33]. Available data support the hypothesis that

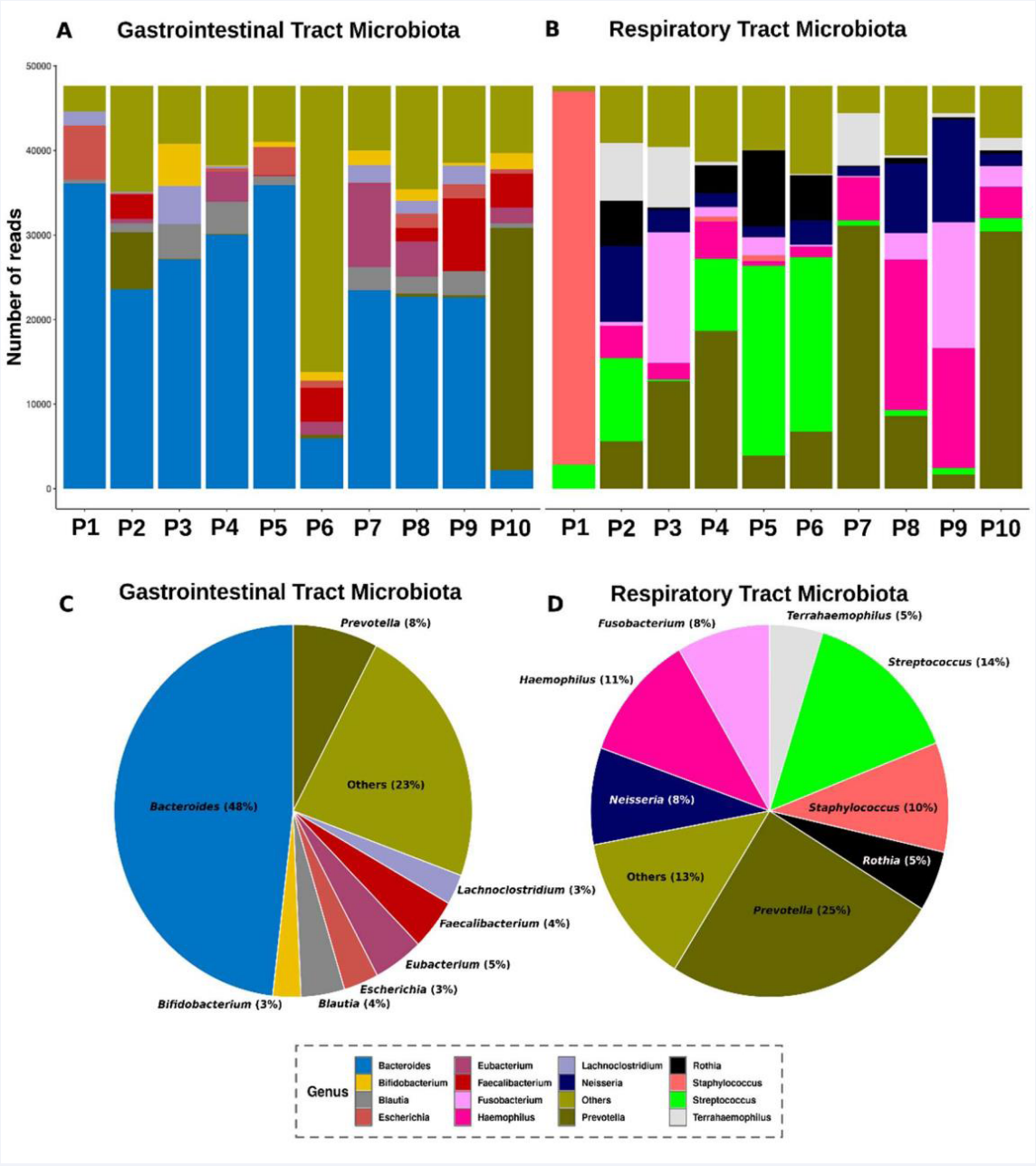

Figure 2 Distribution of the most abundant bacterial taxa, at the genus level, of the microbiomes in the intestinal and respiratory tracts. (A) and (B) – Stacked bar plots displaying the relative abundance (y-axis) of the eight most abundant taxa observed in the
(A) Intestinal tracts and (B) respiratory tracts of the study population; the x-axis in each
Graph corresponds to the 10 study participants. (C) And (D) – Pie chart of the eight most abundant genera across all the (C) intestinal tract samples and (D) respiratory tract samples

S. aureus alters its virulence to a commensal state when exposed to *Corynebacterium* species [41, 42, 43], genera of which were also detected in the respiratory sample of patient 01. Diversity and richness measurements are important for understanding the bacterial community structure and dynamics. Here, were identified 64 important genera in differential abundance between the gut and respiratory microbiota: *Rothia, Bifidobacterium, Actinomyces, Atopobium, Collinsella, Slackia, Fusobacterium, Leptotrichia, Parasutterella, Sutterella, Lautropia, Achromobacter, Neisseria, Kingella, Eikenella, Escherichia, Enterobacter, Salmonella, Haemophilus, Terrahaemophilus, Aggregatibacter, Peptostreptococcus, Intestinibacter, Terrisporobacter, Peptoclostridium, Clostridium, Eubacterium, Mogibacterium, Peptoniphilus, Parvimonas, Mycoplasma, Granulicatella, Abiotrophia, Enterococcus, Staphylococcus, Streptococcus, Erysipelatoclostridium, Coprobacillus, Solobacterium, Veillonella, Dialister, Megasphaera, Tyzzerella, Roseburia, Coprococcus, Blautia, Lachnoclostridium, Catonella, Stomatobaculum,*

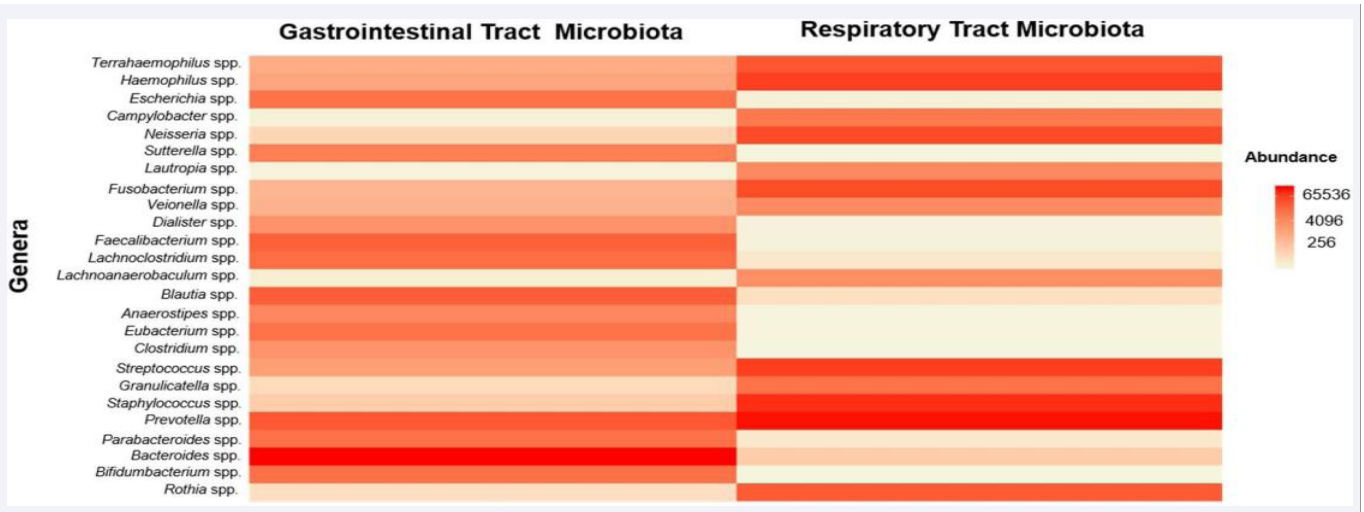

Figure 3 Top 25 genera identified with differential abundance in the groups.

Lachnoanaerobaculum, Fusicatenibacter, Oribacterium, and *Anaerostipes.* These genera have increased abundance in the respiratory tract and reduced or null abundance in the intestinal tract, or vice versa, as exemplified in Figure 3. Although clinical evidence of the impact of gut microbiota on lung infections and disease is not fully understood, studies have shown that lung diseases are associated with disorders of the gut microbiome [44, 45]. Studies indicate that alterations in gut microbial diversity and abundance have been linked to immune response, inflammation, and lung disease development. Reduced diversity of intestinal bacteria was previously associated with CF [37, 46], and gut colonization patterns were considered as determinants in the development of the respiratory tract microbiota in children, related to airway exacerbations [10]. A significant over-representation of *Streptococcus* spp. in the intestinal microbiota was also noted in the current study. Children with intestinal inflammation seem to be prone to CF and provide a basis for the important role of the gut-lung axis in the evolution of this systemic disease [38]. Schippa et al. [47] found that homozygosity of p.Phe508del is more closely associated with a distinct profile of the gut microbiota than other *CFTR* allelic variants, with an increased abundance of potentially pathogenic species such as *Escherichia coli* and *Eubacterium biform*. The bacterial genus *Escherichia* was detected in all the fecal samples and *Eubacterium* was detected in 70 % of the top 10 genera of the gut microbiota (Figure 2).

CONCLUSION

Despite the limited sample size of this pilot study, we successfully analyzed the microbial community of children with highly homogeneous CF without pulmonary exacerbation. The microbiota composition of the study population was fairly homogeneous among the patients, and the identified taxa corresponded to trends consistent with studies for the same age group in the CF patient homozygote DelF508. Current data provide information regarding the colonization of well-acknowledged respiratory and intestinal pathogens in this population, warranting further longitudinal studies based on larger cohorts. These future studies will allow a better understanding of how mutations in CF may affect the airway and intestinal microenvironment, which could lead to non-optimal microbial colonization. These studies will provide important information for the potential use of microbiota components as prognostic tools for determining the course of CF, response to treatment, and/or prevention of lung disease.

ACKNOWLEDGEMENTS

We gratefully acknowledge the participation of the patients and their families in this study. We would like to thank Wellington Pine Omori, at Neoprospecta® Microbiome Technologies, for aiding with the bioinformatics analyses and Elessandra S. Bitencourt, a nurse at the Federal University of Paraná, for aiding with sample collection. We would like to thank Editage (www.editage.com) for English language editing.

DECLARATIONS OF INTEREST FUNDING

This work was supported by Vertex® Pharmaceuticals Inc (Tracking Number: IIS-2017- 106050) for conducting the research and collecting and analyzing of data.

REFERENCES

1. Klepac-Ceraj V, Lemon KP, Martin TR, Allgaier M, Kembel SW, Knapp AA, et al. Relationship between cystic fibrosis respiratory tract bacterial communities and age, genotype, antibiotics and Pseudomonas aeruginosa. Environ Microbiol. 2010; 12: 1293– 1303.
2. Miragoli F, Federici S, Ferrari S, Minuti A, Rebecchi A, Bruzzese E, et al. Impact of cystic fibrosis disease on archaea and bacteria composition of gut microbiota. FEMS Microbiol Ecol. 2017; 93: 230.
3. Cox MJ, Allgaier M, Taylor B, Baek MS, Huang YJ, Daly RA, et al. Airway microbiota and pathogen abundance in age-stratified cystic fibrosis patients. PLoS One. 2010; 5: 11044.
4. Zhao J, Schloss PD, Kalikin LM, Carmody LA, Foster BK, Petrosino JF, et al. Decade-long bacterial community dynamics in cystic fibrosis airways. Proc Natl Acad Sci U S A. 2012; 109: 5809–5814.
5. Muhlebach MS, Hatch JE, Einarsson GG, Mcgrath SJ, Gilipin DF, Mirkovic B, et al. Anaerobic bacteria cultured from cystic fibrosis airways correlate to milder disease:a multisite study. 2018; 52: 1800242.

6. Coburn B, Wang PW, Caballero JD, Clark ST, Brahma V, Donaldson S, et al. Lung microbiota across age and disease stage in cystic fibrosis. Sci Rep. 2015; 5: 1–12.

7. Carmody LA, Caverly LJ, Foster BK, Rogers MA, Kalikin LM, Simon RH, et al. Fluctuations in airway bacterial communities associated with clinical states and disease stages in cystic fibrosis. PLoS one. 2018; 13: 0194060.

8. Madan JC. Neonatal gastrointestinal and respiratory microbiome in cystic fibrosis: potential interactions and implications for systemic health. Clinical Therapeutics. 2016; 38: 740–746.

9. Rogers GB, Carroll MP, Hoffman LR, Walker AW, Fine DA, Bruce KD. Comparing the microbiota of the cystic fibrosis lung and human gut. Gut Microbes. 2010; 1: 85–93.

10. Madan JC, Koestle DC, Stanton BA, Davidson L, Moulton LA, Housman ML, et al. Serial analysis of the gut and respiratory microbiome in cystic fibrosis in infancy: interaction between intestinal and respiratory tracts and impact of nutritional exposures. MBio. 2012; 3: 00251-12.

11. Zemanick ET, Wagner BD, Robertson CE, Ahrens RC, Chmiel JF, Clancy JP, et al. Airway microbiota across age and disease spectrum in cystic fibrosis. Eur respir j. 2017; 50: 1700832.

12. Fouhy F, Ronan NJ, Sullivan OO, Mccarthy Y, Walsh AM, Murphy DM. A pilot study demonstrating the altered gut microbiota functionality in stable adults with Cystic Fibrosis. Sci Rep. 2017; 7: 1–12.

13. Nielsen S, Needham B, Leach ST, Day AS, Jaffe A, Thomas T, et al. Disrupted progression of the intestinal microbiota with age in children with cystic fibrosis. Sci Rep. 2016; 6: 24857.

14. Kristensen M, Prevaes SMPJ, Kalkman G, Tramper-Stranders GA, Hasrat R, de Winter- de Groot KM, et al. Development of the gut microbiota in early life: The impact of cystic fibrosis and antibiotic treatment. J Cyst Fibros. 2020; 19: 553–561.

15. Enaud R, Hooks KB, Barre A, Barnetche T, Hubert C, Massot M, et al. Intestinal Inflammation in Children with cystic fibrosis is associated with Crohn's-Like microbiota disturbances. J Clin Med. 2019; 8: 645.

16. Vernocchi P, Del Chierico F, Russo A, Majo F, Rossitto M, Valerio M, et al. Gut microbiota signatures in cystic fibrosis: Loss of host CFTR function drives the microbiota enterophenotype. PLoS one. 2018; 13: 0208171.

17. Li L, Somerset S. The clinical significance of the gut microbiota in cystic fibrosis and the potential for dietary therapies. Clin Nutr. 2014; 33: 571-580.

18. Frati F, Salvatori C, Incorvaia C, Bellucci A, Di Cara G, Marcucci F, et al. The role of the microbiome in asthma: The gut–lung axis. Int J Mol Sci. 2019; 20: 123.

19. Chunxi L, Haiyue L, Yanxia L, Jianbing P, Jin S. The gut microbiota and respiratory diseases: New Evidence. J Immunol Res. 2020; 2020. doi: 10.1155/2020/2340670.

20. Ahmed B, Cox MJ, Cuthbertson L, James P, Cookson WOC, Davies JC, et al. Longitudinal development of the airway microbiota in infants with cystic fibrosis. Sci Rep. 2019; 9: 1-10.

21. Cuthbertson L, Walker AW, Oliver AE, Rogers GB, Rivett DW, Hampton TH, et al. Lung function and microbiota diversity in cystic fibrosis. Micro biome. 2020; 8: 45.

22. Wang Y, Qian P-Y. Conservative fragments in bacterial 16S rRNA genes and primer 548 design for 16S ribosomal DNA amplicons in metagenomic studies. PLoS ONE. 2009; 4: 7401.

23. Caporaso JG, Lauber CL, Walters WA, Berg-Lyons D, Huntley J, Fierer N, et al. Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. The ISME Journal. 2012; 6: 1621–1624.

24. Caporaso JG, Lauber CL, Walters WA, Berg-Lyons D, Lozupone CA, Turnbaugh PJ, et al. Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. PNAS. 2011; 108: 4516–4522.

25. Christoff AP, Flores Cruz GN, Sereia AFR, Yamanaka LE, Silveira PP, de Oliveira LFV. End-to-end assessment of fecal bacteriome analysis: from sample processing to DNA sequencing and bioinformatics results. BioRxiv. 2019; 1–22.

26. Smyth RP, Schlub TE, Grimm A, Venturi V, Chopra A, Mallal S, et al. Reducing chimera formation during PCR amplification to ensure accurate genotyping. Gene. 2010; 469: 45–51.

27. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic local alignment search tool. J Mol Biol. 1990; 215: 403–410.

28. Chen H, Boutros PC. VennDiagram: A package for the generation of highly- customizable Venn and Euler diagrams in R. BMC Bioinformatics. 2011; 12: 1–7.

29. McMurdie PJ, Holmes S. phyloseq: an R Package for reproducible interactive analysis and graphics of microbiome census data. PLoS one. 2013; 8: e61217.

30. Gaufin T, Tobin NH, Aldrovandi GM. The importance of the microbiome in pediatrics and pediatric infectious diseases. Curr Opin Pediatr. 2018; 30: 117–124.

31. Ihekweazu FD, Versalovic J. Development of the pediatric gut microbiome: impact on health and disease. Am J Med Sci. 2018; 356: 413–423.

32. Loman BR, Shrestha CL, Thompson R, Groner JA, Mejias A, Ruoff KL, et al. Age and environmental exposures influence the fecal bacteriome of young children with cystic fibrosis. Pediatr Pulmonol. 2020; 55: 1661–1670.

33. Man WH, De Steenhuijsen Piters WA, Bogaert D. The microbiota of the respiratory tract: gatekeeper to respiratory health. Nat Rev Microbiol. 2017; 15: 259–270.

34. Voronina OL, Ryzhova NN, Kunda MS, Loseva EV, Aksenova EI, Amelina EL, et al. Characteristics of the airway microbiome of cystic fibrosis patients. Biochem. 2020; 85: 1–10.

35. Mashima I, Nakazawa F. The influence of oral Veillonella species on biofilms formed by Streptococcus species. Anaerobe. 2014; 28: 54-61.

36. Caverly LJ, Huang YJ, Sze MA. Past, Present, and future research on the lung microbiome in inflammatory airway disease. Chest. 2019; 156: 376–382.

37. de Almeida OGG, da Costa Capizzani CP, Tonani L, Grizante Barião PH, da Cunha AF, De Martinis ECP, et al. The lung microbiome of three young brazilian patients with cystic fibrosis colonized by fungi. Front Cell Infect Microbiol. 2020; 10. doi: 10.3389/fcimb.2020.598938.

38. Rossi GA, Morelli P, Galietta LJ, Colin AA. Airway microenvironment alterations and pathogen growth in cystic fibrosis. Pediatr Pulmonol. 2019; 54: 497–506.

39. Vermeulen F, Proesmans M, Vermaelen M, Boon M, De Boeck K. Isolation of Enterobacteriaceae in airway samples is associated with worse outcome in preschool children with cystic fibrosis. J Cyst Fibros. 2020; 19: 365–369.

40. Hoen AG, Li J, Moulton LA, O'Toole GA, Housman ML, Koestler DC, et al. Associations between gut microbial colonization in early life and respiratory outcomes in cystic fibrosis. J Pediatr. 2015; 167: 138–147.

41. Ramsey MM, Freire MO, Gabrilska RA, Rumbaugh KP, Lemon KP. Staphylococcus aureus shifts toward commensalism in response to Corynebacterium species. Front Microbiol. 2016; 7: 1230.

42. Pallett R, Leslie LJ, Lambert PA, Milic I, Devitt A, Marshall LJ. Anaerobiosis influences virulence properties of Pseudomonas aeruginosa cystic fibrosis isolates and theinteraction with Staphylococcus aureus. Sci Rep. 2019; 9: 1–18.

43. Garcia-Nuñez M, Garcia-Gonzalez M, Pomares X, Montón C, Millares L, Quero S, et al. The respiratory microbiome in cystic fibrosis: compartment patterns and clinical relationships in early stage disease. Front Microbiol. 2020; 11:1463.

44. Burke DG, Fouhy F, Harrison MJ, Rea MC, Cotter PD, O'Sullivan O, et al. The altered gut microbiota in adults with cystic fibrosis. BMC Microbiol. 2017; 17: 58.

45. Ghorban Movahed M, Abdi Ali A. A Review of the Role of The Microbiome on Immune Responses and Its Association With Cystic Fibrosis. Immunoregulation. 2020; 3: 75–88.

46. Zhang D, Li S, Wang N, Tan HY, Zhang Z, Feng Y. The Cross-Talk Between Gut Microbiota and Lungs in Common Lung Diseases. Front Microbiol. 2020; 25; 11: 301.

47. Schippa S, Iebba V, Santangelo F, Gagliardi A, De RV, Stamato A, et al. Cystic fibrosis transmembrane conductance regulator (CFTR) allelic variants relate to shifts in faecal microbiota of cystic fibrosis patients. PLoS one. 2013; 8: 61176.

Cite this article

Vasco JFM, Riedi CA, Marconi C, Nogueira KS, Rodrigues LS, et al. (2021) Respiratory and Gut Microbiota of Children with Cystic Fibrosis: A Pilot Study. Ann Clin Med Microbiol 5(2): 1028.