An Overview on the Upper and Lower Airway Microbiome in Cystic Fibrosis Patients

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Background: In cystic fibrosis patients, the mucus is an excellent place for opportunistic bacteria and pathogens to cover. Chronic infections of upper and lower airways play a critical role in the mortality of cystic fibrosis. This study aimed to introduce the microbiota profiles in patients with cystic fibrosis.

Materials and Methods: In this study, a comprehensive literature search was done for studies on upper and lower airway microbiota in cystic fibrosis patients. International and national databases were searched for the following MeSH words: microbiota, microbiome, upper airway, lower airway, cystic fibrosis, cystic fibrosis, upper airway microbiome, lower airway microbiome, microbiome pattern in cystic fibrosis, microbiome pattern in cystic fibrosis, upper airway microbiota, lower airway microbiota, and microbiota pattern.

Results: *Streptococcus spp.* are in significantly higher relative abundance in infants and children with cystic fibrosis; however, *Pseudomonas spp.* are in higher relative abundance in adults with cystic fibrosis. Molecular diagnostic techniques can be remarkably accurate in detecting microbial strains.

Conclusion: For the detection and isolation of most bacterial species, independent-culture methods in addition to the standard culture method are recommended, and sampling should include both upper and lower airways.

Key words: Microbiome, Upper airway, Lower airway, Microbiota, Cystic Fibrosis, *Streptococcus*, *Pseudomonas*.

INTRODUCTION

More than 70,000 people worldwide are living with cystic fibrosis (CF); more than 30,000 of them are in the United States, and 1,000 new cases are diagnosed each year (1). Airways, lung tissue, and lung circulation diseases are the most critical types of lung disease (2, 3). Airway diseases, such as bronchiectasis, asthma, chronic obstructive pulmonary disease (COPD), and CF, can affect the airway tracts that carry gases (i.e. oxygen and carbon dioxide) into and out of the lungs and usually cause a narrowing or blockage of the airways. The structure of lung tissue can be affected by lung tissue diseases, such as sarcoidosis and pulmonary fibrosis, which render the lungs unable to expand fully (restrictive lung disease)

owing to scarring or inflammation of the lung tissue that affects healthy breathing. Furthermore, such type of disease makes it hard for the lungs to transmit gases (take in oxygen and release carbon dioxide), so afflicted individuals cannot breathe deeply. Lung circulation diseases affect the blood vessels in the lungs, causing clotting, inflammation, or scarring. This group of diseases also affects the ability of the lungs to take up oxygen and release carbon dioxide and may also affect heart function. Pulmonary hypertension is an example of this disease type (2, 4). CF is the most common autosomal recessive disorder and is caused by a mutation in the cystic fibrosis transmembrane regulator *(CFTR)* gene (5). With a length of 250 kb and 27 exons, this gene is located on the long arm of

chromosome No. 7 and displays high levels of polymorphism. To date, more than 1000 mutations and 200 polymorphisms have been identified in this gene, only a few of which are common (6). Mutation in the *CFTR* gene causes the CFTR protein to become dysfunctional and unable to transfer chloride to the surface of the cell. Without chloride, the cell cannot attract water to the cell surface; thus, the mucus produced in various organs becomes thick and sticky (Figure 1) (7). The incidence and symptoms of CF are different among patients. Symptoms may include thrombosis, cirrhosis, respiratory involvement, salty-tasting skin, persistent coughing, frequent lung infection, wheezing or shortness of breath, weak growth or weight gain, frequent greasy, bulky stools, male infertility, and diabetes (8-10). CF patients are generally diagnosed through newborn screening, genetic or carrier tests, signs of pulmonary obstruction, pancreatic involvement, and increased chlorine and sodium ion concentrations in sweat (sweat test)(11). A combination of therapies, such as airway clearance, inhaled medicines, pancreatic enzyme supplement, individualized fitness plan, and CFTR modulators, is generally used to manage the disease (12).

In the lungs, thick and sticky mucus clogs the airway, creating a suitable condition for microbial attachment and colonization that leads to infection and pneumonia or bronchitis, respiratory failure, inflammation, or other complications. For this reason, eliminating or reducing the conditions suitable for bacterial attachment and infections in CF patients is essential.
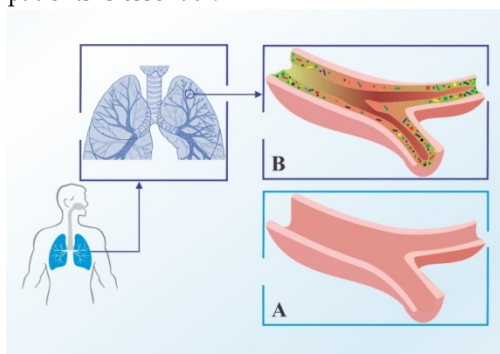

Figure 1. **A:** Airway in healthy people; **B:** Airway in CF patients. Mucoses in CF patients are sticky and can block the airway and trap various opportunistic bacteria and pathogens. In the next step, these bacteria lead to infections, one major cause of death in CF patients.

The microbiome is the genetic material of all the microbes, such as bacteria, fungi, protozoa, and viruses that live on and inside the human body. The composition of the microbiome is essential for immunity, nutrition, and human development (13). Protists and bacteria were first discovered by Antonie van Leeuwenhoek, who is universally acknowledged as the father of microbiology. He was the first to observe animalcules (14, 15). The diversity in microbiomes was shown by Caporaso et al. (16). The level of diversity differs in body sites, and the most diverse communities are in the gut, followed by the mouth (17).

Microbiome projects have been conducted around the world to determine the roles of microbiomes and their impact on human health (18, 19). An essential purpose of microbiome investigations is the study of microbiomes in both healthy and diseased conditions. Thus, researchers are now able to produce millions of sequences in each sample to evaluate the differences in microbial communities between environments and individuals. Aerobe and anaerobe bacteria play essential roles in the pathogenesis of CF lung disease, although it is unclear what species are responsible for protecting against pathogen virulence. Moreover, the development of the CF microbiota is induced by exogenous factors, including an introduction to antibiotics and dietary exposures such as breastfeeding (20).

The microbiota of the respiratory tract may be performances a gatekeeper role and provides resistance to colonization by respiratory pathogens. Also, the respiratory microbiota might be involved in homeostasis pathways of respiratory physiology and immunity. For millions of years, microbial communities have co-evolved with our ancestors. They inhabit all surfaces of the human body, including the respiratory tract mucosa, which contains specific bacterial communities that are thought to have a significant role in the maintenance of human health (21).

Few studies have reviewed what significant species affect CF patients, and few have considered the different aspects of airway microbiomes in CF patients. Correspondingly, the objective of this review was to report the profile of the airway microbiome in CF patients. The

best samples and methods were used to identify which dominant species form the microbiome profile in CF patients.

MATERIALS AND METHODS
Search Strategy

To identify all relevant published studies on upper and lower airway microbiomes in CF patients, the international databases of Google Scholar, PubMed, Scopus, Web of Science, Cochrane Library, Science Direct, Academic Search, MEDLINE, and Journal Storage and Iranian national scientific search engines Irandoc, Scientific Information Database (SID), Magiran, and IranMedex were searched using the keywords microbiota, microbiome, upper airway, lower airway, cystic fibrosis, CF, upper airway microbiome, lower airway microbiome, microbiome pattern in cystic fibrosis, microbiome pattern in CF, upper airway microbiota, lower airway microbiota, and microbiota pattern. Furthermore, the references cited in the identified articles were searched to find other relevant studies.

Inclusion and Exclusion Criteria

As shown in Figure 2, 109 studies relevant to the study objective and published by or before April 2019 and articles that studied upper and lower airway microbiota in CF patients were included. Studies that had irregular articles (case report, meeting report, congress report, letter to the editor, and abstract only), were published after April 2019, reported in a language other than Persian or English, or did not have an English abstract, and duplicated publications such as review reports, articles, systematic reviews, and meta-analyses were excluded.

Date Extraction

Following a planned and detailed study of the selected full-text articles, information regarding the first author, type of study, number of patients, number of samples, sample type, method, gene, and the highlights of the conclusion was extracted and then entered into Table 1.
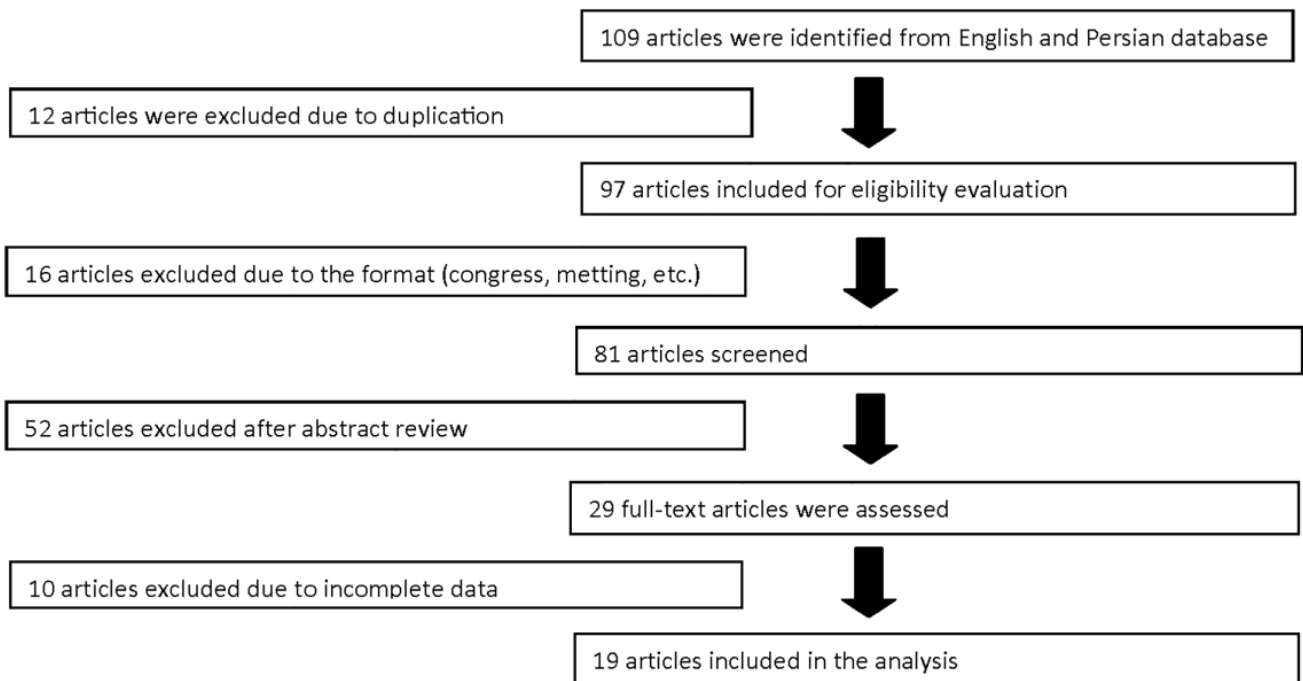

Figure 2. The flowchart for article screening.

Table 1. Studies examining the alteration of airway microbiota, use different samples and methods to identified airway microbiome profile in patients with CF

No	Author (Year of publishing)	Type of Study	No. of patients	No. of samples	Sample type	Method	Gene	Highlight	Reference
1	Zemanick (2017)	case-control	136 paediatric CF, 10 adult CF and 45 paediatric control samples	191	bronchoalveolar lavage fluid	16S rDNA sequencing	16S rDNA	The CF microbiota detected in BALF samples is differentce with age.	(25)
2	Laguna (2016)	cohort	8 infants with CF	12	BALF, nasopharyngeal (NP)/ infant pulmonary function testing	qPCR/16S rRNA sequencing	16S rRNA	Complex microbiota in asymptomatic CF infants often missed by the traditional culture of BALF.	(26)
3	Hogan (2016)	cross sectional	9 adult CF patients	-	bronchoalveolar lavage Fluid/ protected brush/ expectorated sputum	Bacterial Illumina MiSeq 16S rRNA and Fungal ITS1 Sequences	16S rRNA and Fungal ITS1	Evalute the microbiota in different regions of the mild-to-moderate CF patients.	(28)
4	Prevaes (2015)	case-control/ prospective cohort	–	324 nasopharynx samples of 20 CF infants and 45 age-matched healthy controls	nasopharynx	16S rRNA Sequences	16S rRNA	The comparision of CF infant and control group showed distinct patterns of nasopharyngeal microbiota.	(27)
5	Ahmed (2019)	cross sectional	30 infants CF patients	241	throat swab	Quantitative PCR and Illumina sequencing of the 16S rRNA	16S rRNA	Haemophilus spp. and Streptococcus spp. could play an essential role in early infant CF.	(29)
6	Coburn (2015)	cross sectional	269 CF patients	269	Expectorated sputa	16S rRNA sequencing	16S rRNA	Pseudomonas infection can correlate with age-associated trends in lung function and community diversity.	(8)
7	Prevaes (2017)	cross sectional	17 infants with CF	25	nasopharyngeal, oropharyngeal and bronchoalveolar lavage (BAL) samples	Conventional culturing and 16S rRNA sequencing.	16S rRNA	The microbiota of the upper respiratory tract and the BAL in infants has a degree of concordance within each individual	(34)

No	Author (Year of publishing)	Type of Study	No. of patients	No. of samples	Sample type	Method	Gene	Highlight	Reference
8	Zemanick (2016)	cross sectional	16 children with CF_	61	Oropharyngeal (OP) swabs/ induced sputum (IS)/ expectorated sputum (ES)/ saliva	pyrosequencing / quantitative PCR	-	Microbiota composition in IS be similar to Es samples, although, OP samples coldn't represent microbiota composition with airway inflammation	(35)
9	Whelan (2017)	cross sectional	6 knowledgeable and compliant CF	510	sputum	illumina sequencing	16S rRNA	The universal indicator within the lung microbiome of exacerbation doesn't found.	(36)
10	Pittman (2017)	the prospective, observational,multicenter study	32 infants with CF	32	BALF/ OP secretions	16S rRNA Sequences-16S rRNA q_PCR	16S rRNA	There was an association between the prophylactic antibiotics and reduction of bacterial diversity in the upper and lower airways in infant CF	(37)
11	Boutin (2017)	case-control	healthy children = 62 asthma children = 27 CF children =57	healthy children = 62 asthma children = 27 CF children =57	Throat swab	16S rRNA amplicon sequencing	16S rRNA	Exist of the same core microbiome in the respiratory tract of asthma, CF, and healthy children showed host regulation growth of commensals.	(30)
12	Hahn (2016)	cross-sectional	150 CF Patients	12 samples	extracted bacterial DNA	equenced on both MiSeq and PacBio NGS platforms	V4 region of the 16S rRNA	Comparing the results of microbiome diversity in CF patients with MiSeq, 16S sequences, and PacBio NGS platforms showed different results in microbial composition and structure	(38)
13	Botterel (2018)	cross-sectional	4 CF patients	4	Sputum	ribosomal gene (rDNA) fragments and cloning plus sequencing of part of fungal rRNA genes	16S rDNA	Use of USD remains difficult because of the cost of this method and lack of standardization	(39)
14	de Dios Caballero (2017)	cross-sectional	15 CF patients	15	Sputum	cultivation and NGS techniques		The new computational model can help us to hypothesize that the inoculation of predators into the lung microbiome may play a role in chronic colonization by CF pathogens during the early colonization stages.	(40)

No	Author (Year of publishing)	Type of Study	No. of patients	No. of samples	Sample type	Method	Gene	Highlight	Reference
15	Keravec (2015)	cross-sectional	5 CF patients	20	Sputum	pyrosequencing and cloning-sequencing	16S rRNA	Some bacterial genera could have a potential as a biomarker of pulmonary infection state.	(41)
16	Twomey (2013)	cross-sectional	75 CF patients and 5 non-CF patients	110 lower airway expectorated	Sputum	16S rRNA PCR/ Roche 454 sequencing	16S rRNA	New organisms were identified in this study, which wasn't reported hithero and also, has introduced a potential metabolic biomarkers to exacerbation.	(42)
17	Hauser (2014)	cross sectional	two cystic fibrosis patients	–	sputa	whole genome sequencing (WGS) using the Illumina high-throughput technology/ PCR/ Culture	16S rRNA	In the future, characterization of microbiota by WGS method was common if the increase of reads' sizes and decreases in cost occurred.	(43)
18	Rudkjøbing (2011)	cross sectional	five Danish end-stage CF patients	34 lung tissue	pus/sputum	standard culturing and 16S rRNA gene analysis	16S rRNA	Commen cultivation method could be reliable for the identification of the composition of the microbiota	(44)
19	Mounier (2014)	cross sectional	4 CF patients	4 CF patients	sputum	denaturing high-performance liquid chromatography/ cultural methods/ cloning-sequencing	16S rRNA	DHPLC could be considered as a complementary method inside the culture-dependent analyses to identified the composition of the microbiota	(45)

ASSOCIATIONS OF AIRWAY MICROBIOTA AND CF

Impact of Age on Airway Microbiota Profile

The profiles of respiratory tract microbiota may change with age and/or antibiotic use and evolve in young children (22-24). Moreover, the pattern of bronchoalveolar lavage fluid (BALF) microbiota in CF patients varies among individuals of different ages. Zemanick et al. (25) showed that in BALF samples in CF patients aged<2 years, *Streptococcus, Veillonella*, and *Prevotella* constituted ~50% of the pulmonary microbiota that was inversely associated with airway inflammation. In CF patients aged ⩾six years, *Pseudomonas, Stenotrophomonas*, and S*taphylococcus* were predominant. Also, *Streptococcus* or *Prevotella* were detected by sequencing in 20% of CF BALF.

Laguna et al.   (26) reported that *Streptococcus, Burkholderia, Gemella, Neisseria, Prevotella, Haemophilus, Porphyromonas, Methylobacterium*, and *Veillonella* had the highest levels of abundance, and *Stenotrophomonas, Pseudomonas*, and *Staphylococcus* had lower prevalence rates in the BALF of asymptomatic CF infants. It was further shown that these bacterial communities could be dynamic and could change.

The results of a prospective cohort that considered 324 nasopharynx samples from 20 CF infants indicated that the airway microbiota in these infants consisted of *Staphylococcus aureus, Corynebacterium spp.*, and *Moraxella spp*. Subsequently, after three months of age, the microbiota changed to *Streptococcus mitis*. In the control group, it was altered to *Moraxella spp* during the early six months (27). In general, *S. mitis, S. aureus, bacilli*, and *Corynebacterium* were more dominant in CF infants than in control infants, in whom *Haemophilus influenza, Corynebacterium pseudodiphtericum*, and *Corynebacterium propinquity* were more dominant. An antibiotic regimen causes a reduction in colonization and in the presence of commensal bacteria. Moreover, it is independently related to the attachment and colonization of some gram-negative bacteria, such as *Burkholderia* and *Enterobacteriaceae spp*. Hogan et al. (28) investigated nine clinical individuals who had not experienced a severe period of disease within four weeks of the study to determine the microbiome profile in the lungs of adult CF patients. The results of BALF and protected brush (PB) samples found *Pseudomonas aeruginosa*, methicillin-sensitive *S. aureus, Mycobacterium avium* complex, *Achromobacter, Stenotrophomonas*, and *Candida lusitaniae* to be the most abundant microbiomes. Damage in the upper, middle, and lower lobes of both lungs was not correlated with any particular microbial genera, level of diversity in the community, or amount of bacterial genome.

Ahmed et al. (29) confirmed that bacterial load was increased from 12 up to 21 months, and *Streptococcus spp*. (55.0%) and *Haemophilus spp*. (12.5%) were the most common genera which presented in the first two years of life in CF infants. They also showed that oropharyngeal microbiota in CF infants became less homogenous with age.

Some studies have indicated that no significant association exists between age and the airway microbiota of CF patients. Coburn et al. (8) considered 76 pediatric (<18 years old) and 193 adult CF patients. After different analyses, 87 genera were ascertained across all samples; core genera in the adult group were *Prevotella, Streptococcus, Veillonella, Rothia*, and *Actinomyces, and in the pediatric group were Haemophilus, Neisseria*, and *Gemella*. Furthermore, *Pseudomonas* was the unique core genera in the adult group. Correspondingly, diversity and lung function was lower in the adult group than in the pediatric group, which is in concordance with a higher proportion of *Pseudomonas* or *Burkholderia* and lower proportion of *Streptococcus*.

The results achieved by Boutin et al. (30) showed high levels of similarities in the core microbiota of CF patients

and healthy individuals, especially in *Streptococcus, Haemophilus, Veillonella, Prevotella*, and *Neisseria*. Even if the CF airway microbiota profile compared with the healthy group as a control showed an increasing trend in *Veillonella, Prevotella, Streptococcus*, and *Neisseria*, likewise, the analysis showed that *Pseudomonas* and *Phyllobacterium* genus were present, and *Moraxella* was absent in CF patients.

Chronic airway infection and inflammation are some of the most important causes of morbidity and mortality in CF patients (31). *S. aureus* and *P. aeruginosa*, along with other gram-negative bacteria (*H. influenza, Achromobacter spp., B. cepacia* complex, and *Stenotrophomonas maltophilia*) are the predominant bacteria isolated from CF airway samples, and they are associated with pulmonary decline and chronic inflammation, causing progressive lung damage (31-33). Other details of these studies, including the methods, samples, sample numbers, etc. are reported in Table 1. The prevalence percentages of microbiota in infant and pediatric CF and adult CF are shown in Figures 3 and 4. Each of the 20 studies considered reported specific bacterium or bacteria as the most common in infant and pediatric CF and adult CF. Subsequently, the results were reported as the prevalence percentage.
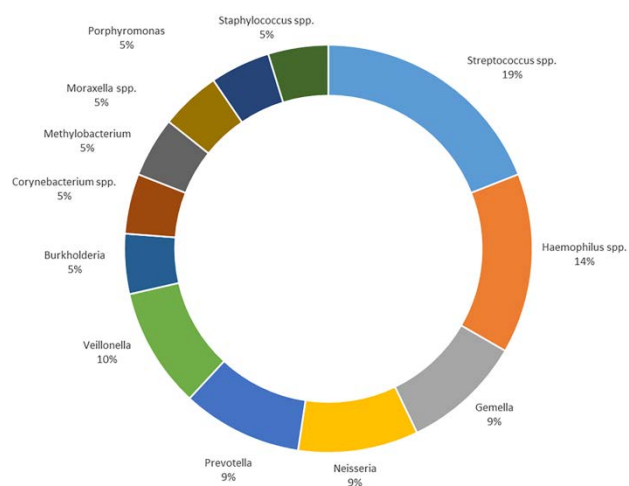

Figure 3. The prevalence percentage microbiota in the upper and lower airway in CF infant and pediatric. The highest prevalence percentage belongs to *Streptococcus spp.* Follow by *Haemophilus spp.* and the lowest prevalence percentage is related to *Burkholderia, Corynebacterium spp., Methylobacterium, Moraxella spp., Porphyromonas,* and *Staphylococcus spp.*
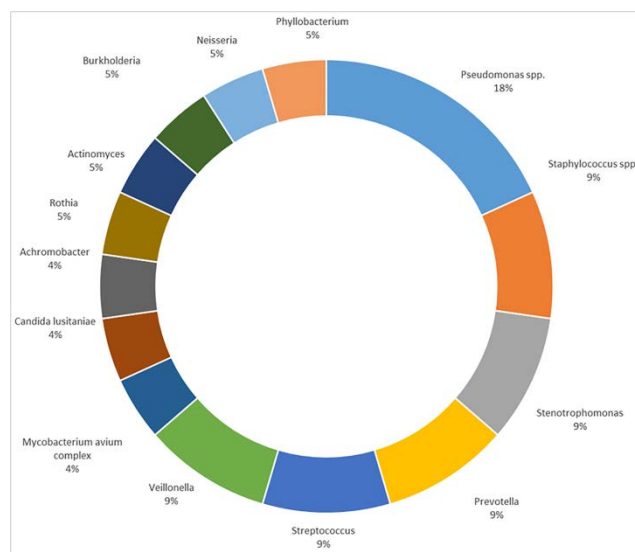

Figure 4. The prevalence percentage microbiota in the upper and lower airway in CF adults. The highest prevalence percentage belongs to *Pseudomonas spp.* Follow by *Staphylococcus spp.* and the lowest prevalence percentage is related to Mycoba*terium avium complex, Candida lusitaniae,* and *Achromobacter.*

Microbiota Composition of Different Sample

Bacterial communities may differ based on the type of samples. Prevaes et al. (34) compared the concordance between oropharyngeal (OP), nasopharyngeal (NP), and BAL microbiota using the Bray–Curtis similarity measures. They also compared the concordance between upper and lower respiratory microbiota in CF infants. Clustering analyses revealed that BAL microbiota profiles were illustrated by a mixture of nasopharyngeal and oral bacteria. Commensals like *Veillonella, Neisseria, Streptococcus*, and *Rothia spp.* Furthermore, pathogens like *H. influenzae, Moraxella spp.,* and *S. aureus* were observed. OP microbiota profiles were similar to BAL profiles with the addition of *Gemella, Veillonella,* and *Prevotella spp.* In NP samples, *Corynebacterium* and *Dolosigranulum spp.* were predominant in the microbiota profiles. *Pseudomonas ssp.* abundance was low for all samples.

Upper airway samples (oropharyngeal swabs and saliva) could reflect different microbiota than those in lower airway samples (induced sputum [IS] and expectorated sputum [ES]). Zemanick et al. (35) collected 61 airway samples from 16 children with CF: 16 OP swabs,

15 ES, 16 IS, and 14 saliva samples. Their results showed that bacterial communities detected from IS by the molecular technique are closer to ES than OP swabs and saliva samples. Although upper and lower microbiota communities have high similarity among some patients, in other samples, different communities were detected. *Pseudomonas, Staphylococcus*, and *Enterobacteriaceae* had a higher prevalence in lower airways than upper airways. Also, *Veillonella, Streptococcus, Prevotella*, and *Rothia* were higher in upper airway samples (OP and saliva) compared with lower airway samples (sputum). Furthermore, *Streptococcus, Rothia, Veillonella*, and *Prevotella* had negative associations with inflammation.

Whelan et al. (36) examined 3 sample groups as follows: intermediate, treatment, and stable period. They found that sample type had a significant effect on microbial composition. All of the participants were colonized with *P. aeruginosa*, and 66% of them were colonized with *Streptococcus agalactiae, Mycobacterium abscessus, S. aureus, Cupriavidus sp., Streptococcus anginosus*, or *S. Milleri*, which were targeted with antibiotic therapy.

In a study by Pittman et al. (37), *Streptococcus spp.* was the dominant taxa found in BAL and OP samples. Microbial cultures showed the growth of normal flora and additional bacterial, such as methicillin-sensitive and methicillin-resistant *S. aureus, P. aeruginosa, S. maltophilia, H. influenzae, Moraxella catarrhalis, Escherichia coli, Klebsiella pneumonia,* and *Morganella morganii*. The researchers' results further revealed an association between bacterial diversity in respiratory tract microbiota and antibiotic exposure in young CF infants.

Different Method of Microbiota Detection

The use of different analysis methods, like 16S sequences, PacBio, and MiSeq NGS platforms, leads to different results in identifying microbial communities and alpha-diversity in CF patients. The MiSeq sequence results on 16s rRNA V4 regions presented higher Chao1 and Shannon indices than the PacBio RSII and many more operational taxonomic units (OTUs) because of the depth of coverage (38). In a study by Hahn et al. (38), 16s rRNA

sequencing was done using the PacBio and MiSeq NGS platforms. *Escherichia,* an unusual pathogen in the airway community, was detected, and *Burkholderia* was identified as an essential CF pathogen only by the PacBio RSII NGS platform. Results of CF samples that were sequenced via PacBio were the following genera, ranked from most to least prevalent, respectively: *Escherichia* or *Shigella, Pseudomonas, Streptococcus, Burkholderia, Haemophilus, Prevotella, Staphylococcus*, and *Gemella*. Similarly, the results of CF samples that were sequenced via MiSeq were of the following genera, ranked from most to least prevalent, respectively: *Enterobacteriaceae* (unclassified genus), *Pseudomonas, Prevotella, Streptococcus, Haemophilus, Staphylococcus, Bacteroides*, and *Gemella*.

Botterel et al. (39) collected sputum samples from 4 CF patients, in which a total of 18 fungal and 27 bacterial genera were detected. Different analysis methods (culture, first-generation sequencing [FGS], and ultra-deep-sequencing [UDS]) produced different results in bacterial and fungi detection. The culture method detected five bacterial genera (18%) and 3 (16%) fungal genera; FGS methods detected 9 bacterial genera (33%) and 3 (16%) fungal genera. UDS distinguished 26 bacterial genera (96%) and 18 fungal genera (100%), which were the highest numbers detected per patient.

de Dios Caballero et al. (40) considered 15 CF patients clustered in mild, moderate, and severe groups. Their microbiological cultures detected *P. aeruginosa, S. aureus, Burkholderia*, and *Pandoraea* species. In the eight patients who had the most inferior lung function, *P. aeruginosa* and *S. aureus* were observed. For the first time, using NGS analysis, *Bdellovibrio, Vampirovibrio,* and bacterial parasites of the phylum *Parcubacteria* were detected in CF lung microbiota.

Conversely, Keravec et al. (41) showed no remarkable differences between cloning-sequencing and pyrosequencing in relative abundance at the phylum or genus level in CF patients. Both methods detected five phyla, including *Proteobacteria, Firmicutes, Fusobacteria,*

Actinobacteria, and *Bacteroidetes,* and 13 predominant genera.

In another study, the ten most prevalent bacteria detected by DNA-based analyses, ranked sequentially, were *Pseudomonadales, Burkholderiales, Chrysiogenales, Chlamydiales, Flavobacteriales Clostridiales, Bacillales, Bacteroidales, Xanthomonadales*, and *Methanosarcinales*. The predominant bacteria detected by culture were *Pseudomonadales* and *S. aureus, Burkholderiales*, and *Haemophilus* genera. Furthermore, *P. aeruginosa* was the principal and dominant bacteria in all patients. *Streptococcus* and *Mycobacterium spp.* were less detected in CF patients. The RNA-based and culture-based analyses of sputum samples demonstrated that both methods could detect dominant pathogens in CF patients. Still, the RNA-based analysis afforded a broader definition of the CF airway microbiome (42).

Hauser et al. (43) considered the microbiota profile by whole genome sequencing (WGS) using three different analysis methods, cultivation, and PCR for two CF patients. WGS provided the same bacteria (e.g., *Achromobacter spp.*, *Gemella spp.*, *Staphylococcus spp.*, *Streptococcus spp.*, *Neisseria spp.*, *Haemophilus spp.*, *Veillonella, Prevotella, Fusobacterium,* and *Granulicatella*). And could also detect some genera, such as *Haemophilus spp., Gemella spp.*, and *Neisseria spp*, which were not detected by culture or PCR. Thus, WGS provided a better qualitative and quantitative estimation of the microbiota profile than PCR and culture.

Rudkjøbing et al. (44) considered sputum samples by standard culturing and 16s rRNA gene. *Achromobacter xylosoxidans*, *P. aeruginosa*, and *S. maltophilia* were detected by culture. No growth of anaerobic bacteria was reported. *A. xylosoxidans*, *Burkholderia fungorum, Bacillus cereus, P. aeruginosa, Lactobacillus mucosae, S. maltophilia, Polaromonas spp.*, uncultured *Saprospiraceae* bacterium, uncultured *Flavobacterium*, uncultured *betaproteobacterium*, and uncultured *Bacteroidetes* bacterium were detected by blasting 16s rRNA gene.

Mounier et al. (45) compared two methods: denaturing high-performance liquid chromatography (DHPLC) and standard culture. The DHPLC method could detect, overall, 15 genera from the *Proteobacteria (Pseudomonas, Haemophilus, Escherichia,* and *Neisseria)*, *Firmicutes* (*Staphylococcus, Streptococcus, Gemella, Granulicatella, Veillonella,* and *Parvimonas*), and *Actinobacteria (Rothia, Granulicatella, Micrococcus, Actinomyces,* and *Scardovia*) phyla. Correspondingly, *S. aureus, P. aeruginosa, E. coli, M. abscessus*, and *S. pneumonia* were detected using the standard cultivation method. Standard microbiological cultures are used in standard clinical practice to detect cultivable pathogens in CF patients. However, culture-based approaches can identify a small species of pathogens within airway samples, and this approach is limited due to the misidentification of organisms. Recently, culture-independent techniques, 16s rRNA, WGS, NGS, etc., have classified many more diverse microbial communities within CF.

CONCLUSION

The two last decades have brought significant advancements in our knowledge about the role of the human microbiome in health and disease. Recent longitudinal studies have supported new evidence for altered respiratory microbiota in CF patients compared with healthy individuals. The airways of an individual with CF are infected with a variety of bacterial species. The most prevalent bacteria detected in infants and small children with CF is *Streptococcus spp.*, followed by *Haemophilus spp.*, *Gemella, Neisseria, Prevotella, Veillonella, Burkholderia, Corynebacterium spp.*, *Methylobacterium, Moraxella spp.*, *Porphyromonas*, and *Staphylococcus spp.* Likewise, the most prevalent bacteria in adults with CF is *Pseudomonas spp.*, followed by *Staphylococcus spp.*, *Stenotrophomonas, Prevotella, Streptococcus, Veillonella, Mycobacterium avium* complex, *C. lusitaniae, Achromobacter, Rothia, Actinomyces, Burkholderia, Neisseria*, and *Phyllobacterium*. Therefore, it is vital to understand the

initial steps of *Streptococcus spp.* and *Pseudomonas spp.* infections.

In infants, children, and adults with CF, airway infection and inflammation are associated with adverse respiratory outcomes, which could lead to decreased lung function. It is noteworthy that microbiota profiles could differ based on the sample type. Not only can the bacterial composition of OP, NP, BAL, IS, ES, upper and lower airway, saliva, oropharyngeal, and sputum samples have different species, but they can also have overlapping species. According to various articles, samples taken from the upper airways (such as nasopharyngeal and oropharyngeal samples) are more likely to be contaminated than samples taken from the lower airways (such as BALF). However, BALF is an aggressive method of sampling. One should bear in mind that samples from the upper airways can only identify the microbial community of the upper airways, and the same is true for lower airway samples. As a result, the type of samples should be determined based on the purpose of the study.

In addition to developing methods for isolating and diagnosing bacterial species, scientists are looking for ways to isolate and categorize all bacterial species in different samples. Among them, cultivation, 16S rRNA sequences, PacBio, MiSeq, WGS, FGS, UDS, NGS, DHPLC, etc. are methods used to isolate and detect airway microbiome profiles. Given that some bacteria are non-cultivable or require specific conditions for growth, based on the culture of the bacteria, only some species and not all species can be isolated and detected. With molecular methods such as DNA-based ones, isolated bacteria may be that which can be killed by antibiotic treatment. However, their genomes remain and can only be detected by molecular methods. Therefore, it is suggested that culture and molecular methods be used simultaneously to study microbial profiles. Research into how airway microbiomes and their metabolites influence human health and disease is ongoing.

Conflict of interest

The authors have no conflict of interest.

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