ORIGINAL ARTICLE

Investigation of some anaerobic bacteria in the sputum of cystic fibrosis patients and healthy people in Tehran, Iran

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Abstract

Background: In Cystic Fibrosis (CF) patients, increased mucus secretion and accumulation due to genetic defect leads to decreased oxygen levels and colonization of anaerobic bacteria. *Aim and Objectives:* In addition to investigating the difference in the prevalence of anaerobic bacteria in the sputum samples of CF patients and healthy populations, this study evaluated the relationship of demographic factors with anaerobic bacteria and CF disease. *Material and Methods:* Anaerobic bacteria were investigated in sputum samples of CF patients (50 people) and healthy individuals (18 people) in Tehran, Iran by molecular method in 2021-2022. The prevalence of common anaerobic bacteria in CF patients was investigated by Polymerase Chain Reaction (PCR). *Results*: The relationship between *Bifidobacterium* and *Prevotella* in both of healthy and CF groups, as well as the disease and *Bifidobacterium* ($p = 0.0437$) and disease and *Prevotella* ($p = 0.0514$), were significant. The results of Spearman's correlation also showed an inverse relationship between age and gender (men) with disease and a direct relationship between antibiotic use and disease. *Conclusions*: The abundance of potentially beneficial species of *Bifidobacterium* and *Prevotella* was lower in CF than in healthy people. The role of anaerobic bacteria in the lungs of CF patients is different according to the bacterial genus and species.

Keywords: Anaerobic Bacteria, *Bifidobacterium*, Cystic Fibrosis, Lung infection, *Prevotella*

Introduction

Cystic Fibrosis (CF) is a progressive lung disease with a genetic cause and an autosomal recessive inheritance pattern due to a defect in the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) coding gene. This gene is located on the long arm of chromosome 7. The most common type of mutation in this disease is due to the deletion of phenylalanine at position 508 (DeltaF508), which leads to the construction of an inefficient CFTR transmembrane protein. This protein is responsible for transporting chloride anions on the surface of the epithelial cells of organs such as the lungs, intestines, kidneys, pancreas, sweat glands, and male reproductive system. The absence of efficient CFTR protein in the airway leads to disruption of water and electrolyte balance, reduction of fluid around the cilia, and increase in mucus secretion. Accumulation of mucus in the airways of CF patients causes the accumulation, growth, and colonization of anaerobic bacterial strains by providing a source of carbon and energy and creating hypoxic conditions [1].

The diversity and abundance of bacterial strains in the lungs of CF people are related to various factors such as age, genetics, Body Mass Index (BMI), disease stage, and even the diversity and abundance of other bacterial strains. Although the bacterial species *Pseudomonas aeruginosa* and *Staphylococcus aureus* are common pathogens in CF patients, *Prevotella*, *Veillonella,* and *Fusobacterium*, in the category of anaerobic bacteria, are common in the lungs of these patients.Some researchers have investigated the relationship between the presence or absence of *P. aeruginosa* and anaerobic bacteria [2].

Fusobacterium, *Prevotella*, *Veillonella*, *Bacteroides,* and *Porphyromonas* are gram-negative and obligate anaerobic bacteria that have been reported in the lungs of CF patients. Although some anaerobic genera such as *Prevotella* are usually considered as commensals, some research results have shown that some strains can be pathogenic. Anaerobic bacteria can cause purulent infections of the sinuses, central nervous system, lungs, liver, and blood vessels. Moreover, a relationship between the diversity and abundance of some specific anaerobic bacteria and the severity of lung disease in CF has been shown [3].

The difference in the diversity and abundance of bacterial strains reported in other research despite the constant genotype in patients indicates the importance of understanding and recognizing the ecological dynamics in the lungs of CF patients. Lack of adequate recognition and understanding of host-microbe and microbe-microbe communication leads to failure in the treatment of infection, progression of the disease, imposition of financial costs, and finally reduction of the patient's life span and death [2]. On the other hand, understanding the difference between potentially useful bacterial strains such as *Bifidobacterium* in the lungs of healthy and CF patients may provide a new perspective for improving the disease and quality of life of these patients [4].

While it is typically necessary to isolate the pathogenic agent by culture-based approaches to identify the bacterium for the treatment of most bacterial diseases [5], cultivating anaerobic bacteria poses a significant challenge. Furthermore, considering the function of non-cultivable anaerobic bacteria in the lung ecosystem, it appears that molecular approaches can fill the current deficiency for identifications. Therefore, the present study investigated 1) the presence or absence and the difference of anaerobic bacteria in the sputum samples of healthy and CF patients, 2) the relationship between factors such as age, and Body Mass Index (BMI) of patients with anaerobic bacteria, and 3) the importance of bacteria with probiotic role in CF disease.

Material and Methods

Subject and samples

The study was approved by the Research Ethics Committees of Alzahra University (IR.ALZAHRA.REC.1401.006). Informed consent was obtained from all participants and/or their legal guardians.

Sputum samples were collected from a group of 50 patients with CF and 18 healthy people in Tehran, Iran, between October 2021 and December 2022. The CF patients were clinically stable and referred to the Children's Medical Center in Tehran. They were diagnosed with CF through clinical and para clinical examinations. They were able to deliver sputum samples after obtaining informed consent. Socio-demographic

and clinical parameters of age, gender, BMI, suffering from other diseases and antibiotic treatments of the participants were recorded.

The mouth and throat were washed with 0.9% NaCl before excretion of sputum. For molecular analysis, the sample taken was stored in phosphate buffered saline (PBS) at -20ºC.

DNA extraction

At first, sputum samples were treated with enough 1N NaCl solution and vortexed. DNA extraction from the sputum sample was performed using Favorgen (FATGK001) Tissue Genomic DNA Extraction Mini Kit along with lysozyme (Bio Basic, LDB0308) according to the manufacturer's protocol.

Polymerase Chain Reaction (PCR)

PCR method and specific primers were considered for molecular identification of five bacteria *Fusobacterium*, *Bifidobacterium*, *Prevotella*, *Veillonella,* and *Bacteroides fragilis* group in sputum sample. The characteristics of the primers and PCR program are shown in Tables 1 and 2, respectively. PCR was performed in a thermal cycler (Peqlab-PEQStar) in a final volume of 25 µl, including 12.5 µl master mix 2× (Sinaclon, MM2062), 1 µl of each forward and reverse primers, 1 µl DNA and 9.5 µl distilled water.

Table 1: Primers used in this study

Bacteria	Primers	Sequence (5'→3')	PCR product length (bp)	Ref
Fusobacterium	f109V f315R	CGGGTGAGTAACGCGTAAAG GCCGTGTCTCAGTCCCCT	228	[26]
Bifidobacterium	g-Bifid-F g-Bifid-R	CTCCTGGAAACGGGTGG GGTGTTCTTCCCGATATCTACA	548 and 738	[27, 28]
Prevotella	g-Prevo-F g-Prevo-R	CACRGTAAACGATGGATGCC GGTCGGGTTGCAGACC	515	[27]
Veillonella	Veill-rpoBF Veill-rpoBR	GTAACAAAGGTGTCGTTTCTCG GCACCRTCAAATACAGGTGTAGC	700	[29]
B. fragilis group	g-Bfra-F g-Bfra-R	ATAGCCTTTCGAAAGRAAGAT CCAGTATCAACTGCAATTTTA	494-501	[27]
16S rRNA	341F 785R	CCTACGGGNGGCWGCAG GACTACHVGGGTATCTAATCC	440-500	[30]

Table 2: PCR program

	Initialization	Denaturation	Annealing	Elongation	Final Elongation
Fusobacterium	2 min-94^{0}C	30 sec-94^{0}C	30 sec-66^{0}C	1 min-68^{0}C	7 min-68^{0}C
Bifidobacterium	5 min-94^{0}C	20 sec-94^{0}C	20 sec-55^{0}C	30 sec-72^{0}C	5 min-72^{0}C
Prevotella	5 min-94^{0}C	20 sec-94^{0}C	35 sec-64^{0}C	30 sec-72^{0}C	5 min-72^{0}C
Veillonella	15 min-94^{0}C	1 min-92^{0}C	30 sec-65^{0}C	1 min-72^{0}C	5 min-72^{0}C
B. fragilis group	5 min-94^{0}C	20 sec-94^{0}C	20 sec-50^{0}C	30 sec-72^{0}C	5 min-72^{0}C
16S rRNA	5 min-95^{0}C	40 sec-95^{0}C	2 min-55^{0}C	1 min-72^{0}C	7 min-72^{0}C

The amplified PCR products were visualized by gel electrophoresis using 4 µl of PCR product and 1 µl of loading dye on 1.5% agarose gel (Sinaclon, EP5051) containing safe stain (Yektatajhiz, YT0001) in 0.5 X Tris-borate-EDTA (TBE) buffer for 5 min at 100V then switched to 80V for 40 min. The amplified DNA fragments of specific sizes were observed by Gel Documentation System. Molecular size markers (100bp, Sinaclon, SL7041) were included in each gel.

Sequencing and phylogenetic tree draw

The isolates identified by chemical method and confirmed by 16S rRNA sequencing technique were used as PCR positive control for each of the five investigated bacteria. For this purpose, DNA extraction from the identified bacterial colony was performed by chemical method, and PCR was performed with 16S rRNA primers. PCR products were sequenced by Pishgam Biotech Company in Tehran and compared with the National Center for Biotechnology Information (NCBI) database using the BLAST tool (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Multiple sequences were aligned using the MUSCLE algorithm. Phylogenetic trees were constructed by maximum composite likelihood and neighbor-joining methods in MEGA X software version 10.2.5 [6]. The stability of phylogenetic trees was assessed by a bootstrap analysis of 1000 replicates. The out group for *Fusobacterium*, *Veillonella*, *Bifidobacterium, Prevotella,* and *B. fragilis* group was considered as *Faecalibacterium prausnitzii* [7], *Dialisterpneumosintes* [8], *Gardnerella vaginalis* [9] and *Flexibacterlitoralis* [10] respectively.

Statistical analysis

Age results were reported as mean ± Standard Deviation (SD). Mann-Whitney U and Fisher's Exact tests were used. Stepwise logistic regression was used to determine risk factors for CF. For significant variables on stepwise logistic regression, we calculated Odds Ratios (OR) with 95% Confidence Intervals (95% CI). A value of p less than 0.05 was considered statistically significant. Correlation analysis between disease (CF) and other parameters (five bacteria identified by molecular method, BMI, age, gender, antibiotic treatment, and sports activity) was performed using Spearman's rank correlation, and a plot was generated. All statistical

tests were two-sided where appropriate. All analyses were performed using GraphPad Prism version 9.3 for Windows, GraphPad Software, Boston, Massachusetts USA, www.graphpad.com [11].

Results

Subjects and samples

Subject characteristics are shown in Table 3. Additionally, 12% (n=6) of CF group and 5.56% (n=1) of the healthy group were diabetic.

Table 3: Demographic and clinical data

Data		Healthy Controls N (%)	Patients (CF) N (%)
Gender	Female	3 (16.66 %)	29 (58 %)
	Male	15 (83.33 %)	21 (42 %)
	Overall	18	50
Age	Mean ± SD	31.5 ± 7.350	16.76 ± 9.169
	Median (range)	33 (22)	15 (53)
Age group (years)	3 - < 7	0 (0%)	1 (2 %)
	7 - < 13	0 (0%)	16 (32%)
	13 - < 19	0 (0%)	20 (40%)
	19 - < 25	6 (33.33%)	7 (14%)
	25 - < 31	1 (5.55%)	2 (4%)
	⩾ 31	11 (61.11%)	4 (8%)
Body-mass index (kg/m^2)[a]	Underweight	0 (0%)	35 (70%)
	Normal weight	11 (61.11%)	12 (24%)
	Overweight	6 (33.33%)	2 (4%)
	Obese	1 (5.55%)	1 (2%)
Antibiotic therapy	Azithromycin	0 (0%)	21 (42%)
	Tobramycin	0 (0%)	11 (22%)
	Other	1 (5.55%)	12 (24%)
	No consumption	17 (94.44%)	19 (38%)
Sports activity (hr/wk)	Mean ± SD	3.278 ± 2.803	2.560 ± 3.764

SD: Standard deviation, Underweight: BMI < 18.5; Normal weight: BMI = 18.5-24.9; Overweight: BMI ⩾ 25; Obese: BMI ⩾ 30.

Molecular identification

All 68 samples (50 CF samples and 18 healthy samples) were analyzed using five specific primers. Figure 1 shows the results of molecular identification of five anaerobic bacterial strains using specific primers based on healthy and CF groups, and age groups in CF. According to the results shown in this figure, the prevalence of all studied anaerobic bacteria except *B. fragilis* group was higher in healthy people than in CF patients. The phylogeny tree of clinical strains drawn through 16S rRNA sequencing that was used as a positive control in PCR is shown in Figure 2. In addition, Figure 3 shows the PCR product of five specific primers and 16S rRNA primer on 1.5% agarose gel, and five clinical isolates used as positive control in PCR were sequenced, and the phylogeny tree of these strains were represented.

Statistical analysis

The results of statistical analysis of logistic regression (Table 4) revealed that two parameters of BMI and detection by molecular method of *Bifidobacterium* have a significant relationship with CF disease. The *Bifidobacterium* parameter has an inverse relationship with the disease with an estimated value of -1.973. Its OR value shows that non-identification of *Bifidobacterium* in the molecular method increases the chance of CF disease by 0.1390 times. Additionally, BMI with an estimated value of -0.4035 shows the inverse relationship of this parameter with CF disease. Its OR value shows that a high BMI reduces the chance of disease by 0.6680 times. The result of correlation analyses using Spearman's non-parametric correlation coefficient (Figure 4) showed that CF disease had a significant relationship with the presence of *Bifidobacterium* ($p < 0.0001$, r = -0.4739), *Prevotella* ($p = 0.0187$, r = -0.2844), BMI ($p < 0.0001$, r = -0.6471), age ($p < 0.0001$, r = -0.6426), male gender ($p = 0.0022$, r = -0.3653) and antibiotic treatment ($p < 0.0001$, r = 0.4989). However, the relationships of CF disease with the identification of *B. fragilis* group, *Fusobacterium*, *Veillonella,* and the amount of sports activity (hr/wk) were not significant.

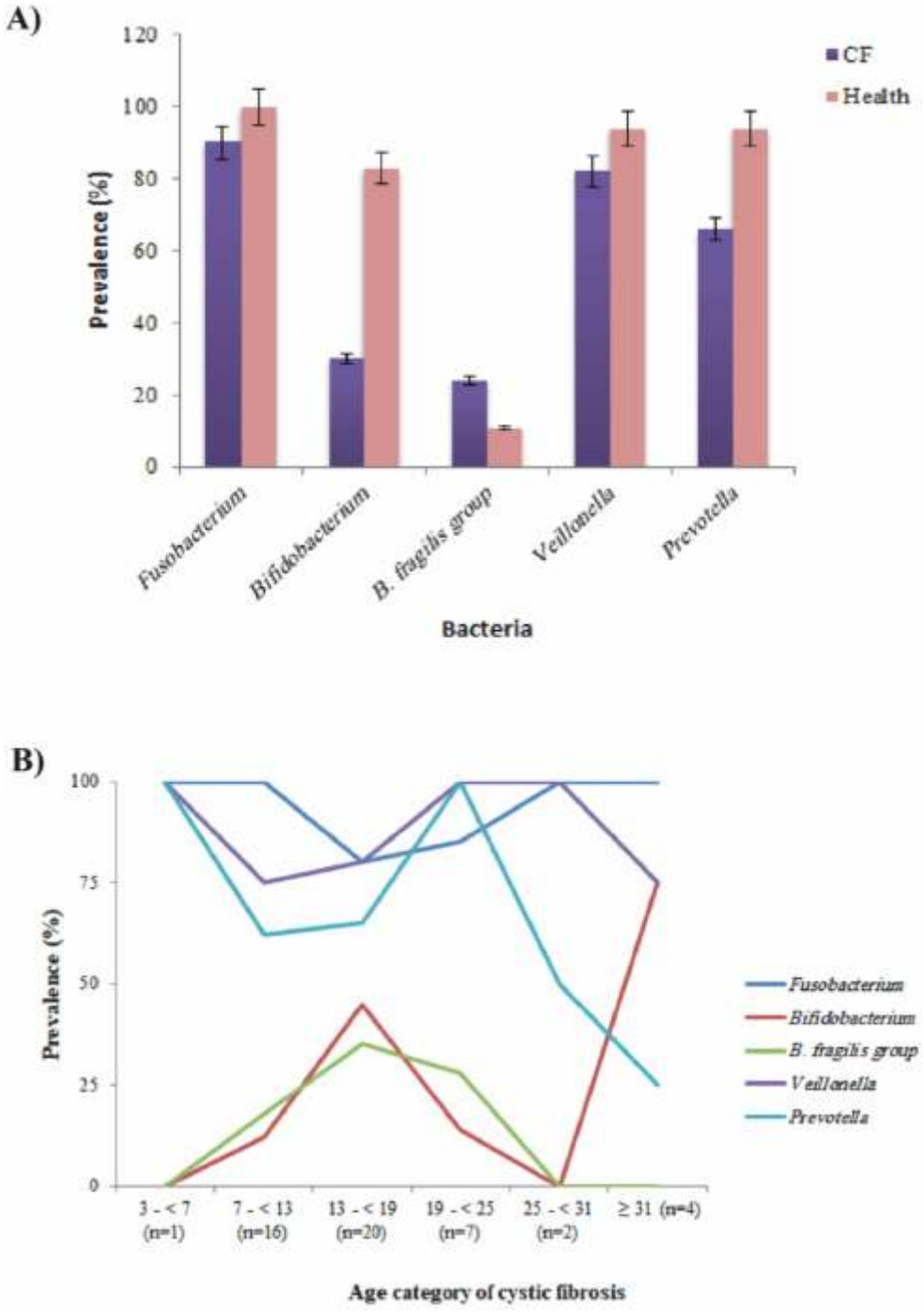

Figure 1: Molecular identification results. A) The description of the identification of anaerobic bacteria investigated by PCR method in two healthy (pink) and CF (purple) groups is shown in prevalence. B) Changes in bacterial communities and prevalence with subject age in CF. Prevalence for bacteria are shown by age group. The number of samples for each age group is given in parentheses

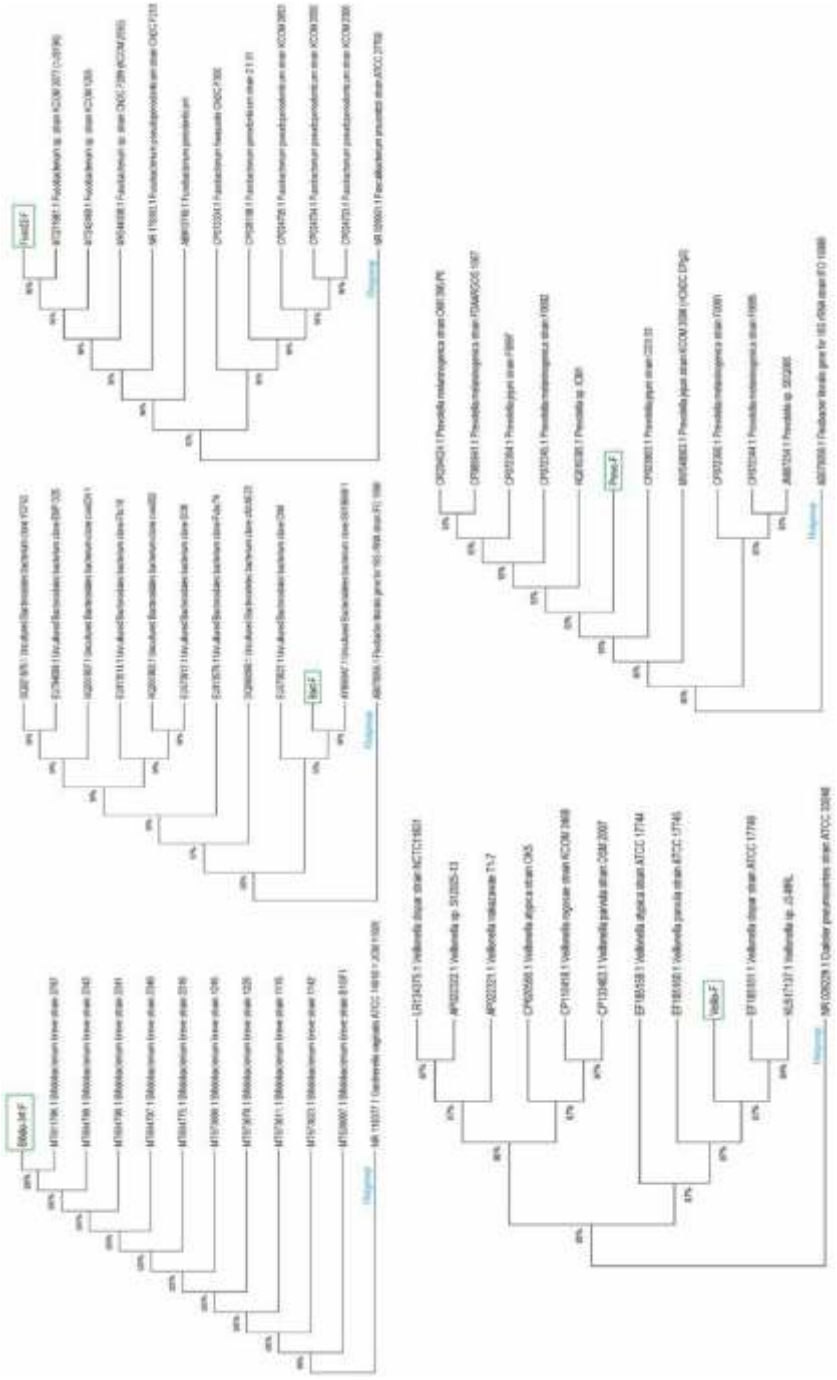

Figure 2: Phylogeny tree topology of five anaerobic clinical isolates of *Fusobacterium*, *Prevotella*, *Veillonella*, *Bacteroides* and *Bifidobacterium* used as positive control in molecular identification by PCR method. The studied isolates are indicated by green boxes in each tree. The accession number of each strain in NCBI is mentioned before its name. The analysis was run in bootstrap 1000

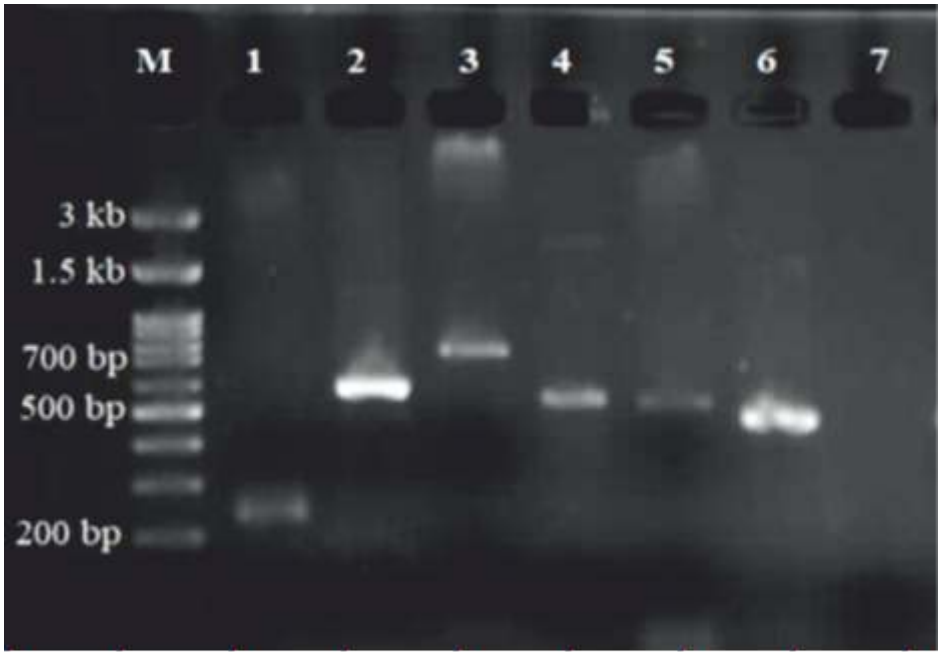

Figure 3: PCR products obtained for five species with specific primers and 16S rRNA primers. M: DNA size marker 100 bp, Lan 1: Fusobacterium (228 bp), Lan 2: Bifidobacterium (548 bp), Lan 3: Veillonella (700 bp), Lan 4: Prevotella (515 bp), Lan 5: B. fragilis group (500 bp), Lan 6:16S rRNA (500 bp), Lan 7: negative control (distilled water)

Table 4: Multivariate analysis with logistic regression modeling

Parameters	OR (95% confidence interval)	*p*-value
B. fragilis group	2.547 (0.3232 - 27.95)	0.3960
Veillonella	3.869 (0.09927 - 108.2)	0.4134
Prevotella	0.03679 (0.0007012 - 0.7197)	0.0514
Bifidobacterium	0.1390 (0.01590 - 0.8548)	0.0437
BMI	0.6680 (0.4923 - 0.8266)	0.0018

OR: Odds ratio; BMI: Body-mass index

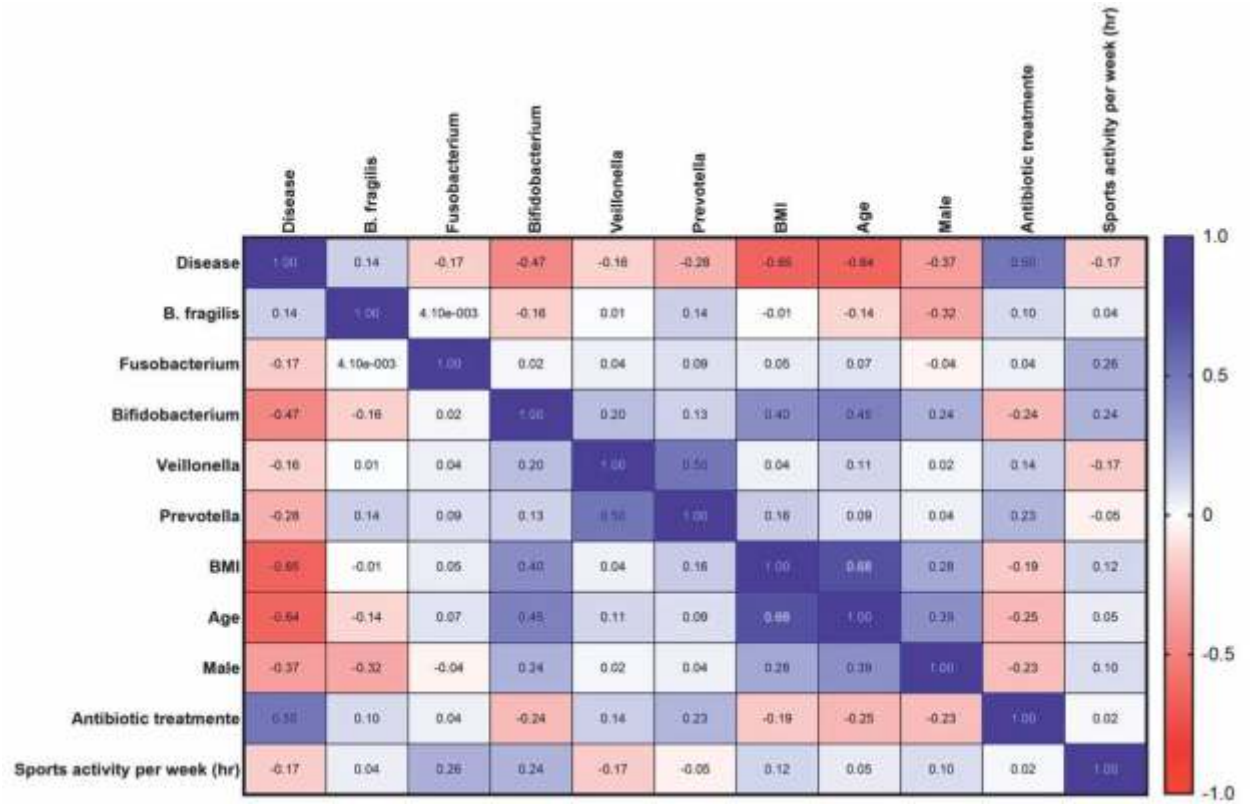

Figure 4: Spearman's correlation test heatmap. The correlation coefficient r of both parameters is displayed. Blue: direct correlation, red: reverse correlation, and white: lack of correlation

Discussion

In the past, the human lungs were assumed to be sterile. However, as we know today, there are colonies of microorganisms in both the respiratory and digestive systems. Microbiome characteristics change at different ages according to genetic and environmental factors. Presumably, the presence and colonization of anaerobic bacteria are possible in the lungs of CF patients due to the nature of the disease, which is the accumulation of mucus and the creation of hypoxic conditions [12-13].

The present study is among the first research studies conducted on anaerobic bacteria in CF patients in Iran. This research has compared the anaerobic bacteria of sputum in healthy and CF groups. The patients referred to the CF center were between 4 and 57 years old with an average age of 16 years old indicating an increase in life expectancy in these patients. However, most patients were in the age range of 7 to 18 years old. Sampling from healthy population was considered without the use of special tools and this group of people did not often produce sputum. Since it was easier for men to expel sputum, most of the control samples were from men and were aged over 31 years old (Table 3).

Previously, Moogahi *et al*. [14] had investigated anaerobic bacteria in bronchoalveolar lavage and sputum samples of CF patients in Ahvaz, Iran, but they did not compare their results with healthy controls. In addition, the relationship of a specific

genus of anaerobic bacteria with other non-bacterial factors in CF patients, like in this study, had not been investigated by them.

Like the results obtained in the present study, some studies have also reported anaerobic bacteria in the lungs of healthy people [15]. In both healthy and CF groups, the largest value for the anaerobic bacteria in the present study was related to *Fusobacterium*. *Fusobacterium*, *Prevotella* and *Veillonella* species in the lungs of both the healthy and CF groups are known as anaerobic bacterial species. The role of *Prevotella* and *Veillonella* as key species during CF disease progression has been demonstrated previously [16]. Different reports have been published on the relationship between the prevalence of specific bacterial strains and disease progression. Many of these associations are defined by the presence or absence of *P. aeruginosa*. As shown in the report of Li *et al*., *Fusobacterium nucleatum* can encourage the proliferation of *P. aeruginosa* [17]. In this regard, the results of Tunney *et al*. study showed that anaerobic bacteria are present in higher or equal amounts than *P. aeruginosa* bacteria [15]. However, in another study, it was found that the microbial community in CF people may be divided into three groups: gram-positive anaerobes, *P. aeruginosa* and *S. aureus*. *P. aeruginosa* and *S. aureus* have an antagonistic relationship with the anaerobes group [18]. Also, the results of Bertelsen *et al*. study showed that *Prevotella spp*. can reduce the inflammation caused by *P. aeruginosa* in CF bronchial epithelial cells [19]. Therefore, the interaction of anaerobic bacteria with other aerobic bacteria seems to be important in the pathogenesis and progression of lung disease.

In this study, there was no significant relationship between CF and healthy groups considering the detected *Fusobacterium*, *Veillonella,* and *B. fragilis* group. The relationship between *Bifidobacterium* and *Prevotella* in both healthy and CF groups, as well as the disease and *Bifidobacterium* (p = 0.0437), and disease and *Prevotella* (p = 0.0514) were significant, in such a way that the absence of *Prevotella* increased the disease by 0.03679 times and the presence of *Bifidobacterium* decreased the disease by 0.1390 times (Table 4 and Figure 4). According to the obtained results, it seemed that the presence of *Bifidobacterium* and the absence of *Prevotella* were beneficial to health.

Bacterial strains of *Lactobacillus* and *Bifidobacterium* are among the group of probiotic bacteria that have potential impact on human health. The results of Ray *et al*. indicated that the enrichment of intestinal bifidobacteria following oral *Lactobacillus* supplementation was associated with clinical improvement in children with cystic fibrosis [4]. Although the results of this research determined the significant relationship of *Bifidobacterium*, however, the issue of lung probiotics is relatively new, and whether oral probiotics can reduce the respiratory symptoms of CF patients and improve the quality of life of these people needs more research [20].

Since the sampling in this research was done during the coronavirus pandemic, a large percentage of the participants were not present in society like in non-pandemic conditions. Franciosi *et al*. [21] had investigated the effect of the coronavirus pandemic on CF patients from a microbiological perspective, and found difference between the detection of *Burkholderia Cepacia* complex, Methicillin-resistant *S. aureus*, *Stenotrophomonas maltophilia* and *P. aeruginosa* bacteria before the pandemic, and the detection of only *P. aeruginosa* during the pandemic. Nath *et al*. [22] reported a gradual

decrease in the frequency of some anaerobic bacteria from upper respiratory tract samples in COVID-19 patients compared to healthy people. Some published results indicate a possible inverse relationship between the coronavirus (or the coronavirus pandemic) and anaerobic bacteria in respiratory samples. Since this sampling in the current study was also done during the coronavirus pandemic, the difference in the results obtained with other published studies is probably due to this reason.

In the study by Tunney *et al*., anaerobic bacteria were found in 50% of the sputum samples of healthy people while the total amount of anaerobic bacteria [Colony-Forming Unit (CFU)] in the sputum samples of CF was found to be higher than that of healthy people [15]. Determining the CFU of anaerobic bacteria was not the aim of this study, and the difference in prevalence of common anaerobic bacteria in CF patients and healthy people was investigated. The results of the present study showed a higher prevalence of anaerobic bacteria in healthy people than in the CF group (Figure 1). It seems that the abundance and CFU of anaerobic bacteria are different in CF and healthy groups. Therefore, in addition to the bacterial species and its presence, CFU values are also important in determining the role of anaerobic bacterial species.

The results of the statistical analysis of the present study showed an inverse relationship between BMI and CF disease. It is obvious that due to deficiency in CFTR, most patients have digestive problems, especially in the pancreas, and they are often underweight according to their BMI, despite the use of drugs containing pancreatic enzymes. A significant difference in BMI ($p < 0.0001$) was also found between CF and healthy groups. The results of Spearman's correlation also showed an inverse relationship between age and gender (men) with disease and a direct relationship between antibiotic use and disease (Figure 4). Most of the participants in the CF group were between 13 to < 19 years old (40%) (Table 3). Probably due to the young age of the people being referred to the medical center in Tehran, the inverse relationship between age and disease was noted in the statistical analysis.

The limitations of this research included not stopping the use of antibiotics when collecting samples from the patient group and the single center for collecting samples.

Anaerobic bacteria in the lungs of CF patients can be different in terms of diversity, abundance, and number of bacterial colonies based on the clinical condition of the patient and age. In general, the diversity of anaerobic bacteria is lower in healthy people than CF, while in the CF group, the diversity decreases with age. In this way, the diversity of anaerobic bacteria decreases from CF patients with younger age to CF patients with older age, and healthy people, respectively [23-24]. The present study compared the abundance of anaerobic bacteria in CF patients and healthy people. Some species of anaerobic bacteria such as *Prevotella* can be pathogenic by producing protease [12] while some other bacterial anaerobes improve lung function by reducing inflammation [25].

According to the results of the present study and other similar studies, it seems that the role of anaerobic bacteria in CF depends on various factors in addition to the bacterial species. For example, in the case of infection with *Fusobacterium* and *Prevotella*, infection with *P. aeruginosa* should also be considered. It is also possible that the administration of probiotic microorganisms can be useful in preventing infection or helping to treat the infection.

Conclusion

This study showed the presence of anaerobic bacteria in the lungs of healthy people and CF patients. However, although a significant association between *Bifidobacterium*, *Prevotella* and CF disease was shown, the role of anaerobic bacteria in the progression of the disease and the relationship with other lung microorganisms need further investigation. Based on the current findings, it appears that these roles vary among different genera and species of bacteria. Therefore, generalizing a specific feature to all members of the anaerobic bacteria group may not be appropriate.

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References

1. Lambiase A, Catania MR, Rossano F. Anaerobic bacteria infection in cystic fibrosis airway disease. *New Microbiol* 2010;33(3):185-194.
2. Thornton CS, Acosta N, Surette MG, Parkins MD. Exploring the cystic fibrosis lung microbiome: Making the most of a sticky situation. *J Pediatric Infect Dis Soc* 2022;11(Supplement_2):S13-S22.
3. Thornton CS, Caverly LJ, LiPuma JJ. Coming up for air: the role of anaerobes in cystic fibrosis. *Ann Am Thorac Soc* 2022;19(5):713-716.
4. Ray KJ, Santee C, McCauley K, Panzer AR, Lynch SV. Gut Bifidobacteria enrichment following oral Lactobacillus-supplementation is associated with clinical improvements in children with cystic fibrosis. *BMC Pulm Med* 2022;22(1): 287.
5. Shah SJ, Mehta N, Shah S. Study of sputum bacteriology of patients with acute exacerbation of chronic obstructive pulmonary disease. *J Krishna Inst Med Sci Univ* 2021;10(2): 130-138.
6. Kumar S, Stecher G, Li M, Knyaz C, Tamura K. MEGA X: molecular evolutionary genetics analysis across computing platforms. *Mol Biol Evol* 2018;35(6):1547.
7. Conrads G, Claros MC, Citron DM, Tyrrell KL, Merriam V, Goldstein EJ. 16S-23S rDNA internal transcribed spacer sequences for analysis of the phylogenetic relationships among species of the genus Fusobacterium. *Int J Syst Evol Microbiol* 2002;52(Pt 2): 493-499.
8. Arif N, Do T, Byun R, Sheehy E, Clark D, Gilbert SC, *et al*. Veillonella rogosae sp. nov., an anaerobic, Gram-negative coccus isolated from dental plaque. *Int J Syst Evol Microbiol* 2008;58(Pt 3):581-584.
9. Leblond-Bourget N, Philippe H, Mangin I, Decaris B. 16S rRNA and 16S to 23S internal transcribed spacer sequence analyses reveal inter- and intraspecific Bifidobacterium phylogeny. *Int J Syst Bacteriol* 1996; 46(1):102-111.
10. Kobayashi A, Sano D, Hatori J, Ishii S, Okabe S. Chicken-and duck-associated Bacteroides–Prevotella genetic markers for detecting fecal contamination in environmental water. *Appl Microbiol Biotechnol* 2013; 97(16):7427-7437.
11. Swift ML. GraphPad prism, data analysis, and scientific graphing. *J Chem Inf Comput Sci* 1997;37(2): 411-412.
12. Thornton CS, Surette MG. Potential contributions of anaerobes in cystic fibrosis airways. *J Clin Microbiol* 2021;59(3):10.1128/jcm. 01813-19.
13. Utpat K, Nanaware S, Desai U, Joshi JM. Clinical profile and aetiology of bronchiectasis. *J Krishna Inst Med Sci Univ* 2017;6(4):28-37.
14. Moogahi S, Beni FT, Hashemzadeh M, Dezfuli AAZ. Molecular identification and biofilm formation of aerobic and anaerobic coinfection bacterial isolated from cystic fibrosis patients in southwest Iran from 2014 to 2022. *Mol Biol Rep* 2023;50(10):8225-8235.

15. Tunney MM, Field TR, Moriarty TF, Patrick S, Doering G, Muhlebach MS, *et al*. Detection of anaerobic bacteria in high numbers in sputum from patients with cystic fibrosis. *Am J Respir Crit Care Med* 2008;177(9):995-1001.
16. Silveira CB, Cobián-Güemes AG, Uranga C, Baker JL, Edlund A, Rohwer F, *et al*. Multi-omics study of keystone species in a cystic fibrosis microbiome. *Int J Mol Sci* 2021;22(21):12050.
17. Li Q, Tan L, Wang H, Kou Y, Shi X, Zhang S, *et al.* Fusobacterium nucleatum interaction with Pseudomonas aeruginosa induces biofilm-associated antibiotic tolerance via Fusobacterium adhesin A. *ACS Infect Dis* 2020;6(7):1686-1696.
18. Quinn RA, Whiteson K, Lim YW, Zhao J, Conrad D, LiPuma JJ, *et al*. Ecological networking of cystic fibrosis lung infections. *Nat Biofilm Microbio* 2016; 2(1):1-11.
19. Bertelsen A, Elborn JS, Schock BC. Microbial interaction: Prevotella spp. reduce P. aeruginosa induced inflammation in cystic fibrosis bronchial epithelial cells. *J Cyst Fibros* 2021;20(4):682-91.
20. Françoise A, Héry-Arnaud G. The microbiome in cystic fibrosis pulmonary disease. *Genes* 2020;11(5): 536.
21. Franciosi AN, Wilcox PG, Quon BS. Cystic fibrosis respiratory microbiology monitoring during a global pandemic: lessons learned from a shift to telehealth. *Ann Am Thor Soc* 2022;19(3):498-500.
22. Nath S, Sarkar M, Maddheshiya A, De D, Paul S, Dey S, *et al*. Upper respiratory tract microbiome profiles in SARS-CoV-2 Delta and Omicron infected patients exhibit variant specific patterns and robust prediction of disease groups. *Microbiol Spectr* 2023:e02368.
23. Linnane B, Walsh AM, Walsh CJ, Crispie F, O'Sullivan O, Cotter PD, *et al*. The lung microbiome in young children with cystic fibrosis: A prospective cohort study. *Microorganisms* 2021;9(3):492.
24. Lamoureux C, Guilloux C-A, Beauruelle C, Jolivet-Gougeon A, Héry-Arnaud G. Anaerobes in cystic fibrosis patients' airways. *Critical Rev Microbiol* 2019; 45(1):103-117.
25. Zemanick ET, Harris JK, Wagner BD, Robertson CE, Sagel SD, Stevens MJ, *et al*. Inflammation and airway microbiota during cystic fibrosis pulmonary exacerbations. *PLoS One* 2013;8(4):e62917.
26. Walter J, Margosch D, Hammes WP, Hertel C. Detection of Fusobacterium species in human feces using genus-specific PCR primers and denaturing gradient gel electrophoresis. *Microb Ecol Health Dis* 2002;14(3):129-132.
27. Matsuki T, Watanabe K, Fujimoto J, Miyamoto Y, Takada T, Matsumoto K, *et al*. Development of 16S rRNA-gene-targeted group-specific primers for the detection and identification of predominant bacteria in human feces. *Appl Environ Microbiol* 2002;68(11): 5445-5451.
28. Matsuki T, Watanabe K, Tanaka R. Genus-and species-specific PCR primers for the detection and identification of bifidobacteria. *Curr Issues Intest Microbiol* 2003; 4(2):61-69.
29. Mashima I, Kamaguchi A, Nakazawa F. The distribution and frequency of oral Veillonella spp. in the tongue biofilm of healthy young adults. *Curr Microbiol* 2011; 63(5):403-407.
30. Abellan-Schneyder I, Matchado MS, Reitmeier S, Sommer A, Sewald Z, Baumbach J, *et al*. Primer, pipelines, parameters: issues in 16S rRNA gene sequencing. *Msphere* 2021;6(1):e01202-20.

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