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Original Research

The first study characterizing the respiratory microbiome in cystic fibrosis patients in Jordan

Ahmad R. Alsayed, Anas Abed, Malek Zihlif, Mahmoud S Abu-Samak, Rana Ahmad Almuhur, Mohammad Alkhatib

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Abstract

Cystic fibrosis (CF) is most commonly seen in Caucasians and is uncommon in the Middle East. This study, based in Jordan, aimed to describe the association between lung exacerbation in CF patients and the respiratory microbiome composition. Using the 16S rRNA marker-gene sequencing, we investigated the microbiota in sputa during exacerbation (E1) and 14 days after the exacerbation (E2) of two CF patients admitted to the hospital. Detected genera with high abundance in the E1-related sputa of the first patient included Achromobacter and Streptococcus. At E2, Achromobacter and Staphylococcus were the highest abundant genera. Regarding the second patient, Veillonella and Streptococcus, were the highest abundant genera at E1. Whereas, Streptococcus and Veillonella were the highest abundant genera. This is the first study, based in Jordan, to report and describe the respiratory microbiome during and after the exacerbation of CF patients. This study suggests that pulmonary exacerbation in CF patients can result in alterations in their respiratory microbiome. A better knowledge of this link could allow more focused use of antibiotics, especially during exacerbations, improving clinical efficacy and patient outcomes.

Keywords: cystic fibrosis; respiratory; microbiome; Jordan; epidemiology

INTRODUCTION

Cystic fibrosis (CF) is an autosomal recessive disease caused by loss of CFTR (CF transmembrane conductance regulator) function due to a mutation in its encoding gene. CF is characterized by profound dysbiosis, particularly in respiratory and gut microbiota, resulting in lower airway infection, respiratory failure, and death.[1,2] CF is most commonly seen in Caucasians' live birth.[1] Multiple organs are affected by CF, and among them are the upper and lower respiratory tracts. CF's lethality results from end-stage lung disease. Changes in the airway surface liquid, caused by truant or non-functional CFTR, make patients vulnerable to chronic airway infections and airway destruction.[3,4] CF patients have frequent acute or subacute respiratory exacerbations. This deterioration of the respiratory manifestations is critical to the extent that it justifies intravenous or oral antibiotics. Such patients tend to encounter fast deterioration of their lung function hence increasing their mortality and morbidity.[5]

Chronic endobronchial infection is the defining characteristic of CF lung disease.[1] Conventional culture-based microbiological methods have evolved to identify "typical CF pathogens" with the highest therapeutic importance. According to this method, staphylococcus aureus and Haemophilus influenzae are the major pathogens in children with CF. With increasing age, Pseudomonas aeruginosa infection becomes the most frequent pathogen in adults with CF. Other opportunistic bacteria, mycobacteria, and fungi may also be detected in the respiratory tracts of CF patients.[3] However, culture-independent examination of CF respiratory samples has revealed that the microbial populations within the CF lung are substantially more complicated than suggested by classical culture.[2]

In CF, it has been shown that the administration of antibiotics and age influence microbial diversity.[6-9] Further study, especially with longitudinal sampling, promises to identify other diversity drivers and unfold their clinical significance.

CF is uncommon in the middle east.[10,11] This is the first study, based in Jordan, describes the association between the lung exacerbation in CF patients and the respiratory microbiome composition. A better knowledge of this association could allow more focused use of antibiotics, especially during pulmonary exacerbations, which could improve clinical efficacy and patient outcomes.

Ahmad R. ALSAYED*. Ph.D., MSc, PharmD, Associate Professor in Clinical Pharmacy, Ph.D. in Therapeutics and Precision Medicine, Department of Clinical Pharmacy and Therapeutics, Faculty of Pharmacy, Applied Science Private University, Jordan; a_alsayed@asu.edu.jo
Anas ABED. Pharmacological and Diagnostic Research Centre, Faculty of Pharmacy, Al-Ahliyya Amman University, Amman 1193-166, Jordan. a.abed@ammanu.edu.jo
Malek ZIHLIF. Department of Pharmacology, School of Medicine, The University of Jordan, Amman, Jordan. m.zihlif@ju.edu.jo
Mahmoud S ABU-SAMAK. Department of Clinical Pharmacy and Therapeutics, Faculty of Pharmacy, Applied Science Private University, Amman 11931-166, Jordan. m_abusamak@asu.edu.jo
Rana Ahmad ALMUHUR. Department of Biological Science, Al al-Bayt University, Mafraq, Jordan. raa076@aabu.edu.jo
Mohammad ALKHATIB. Department of Experimental Medicine, University of Rome "Tor Vergata", Roma 00133. mohammad--alkhatib@hotmail.com

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MATERIALS AND METHODS

Patients and sputum samples

Two of the 165 patients admitted to the hospital due to lower respiratory tract infection during 2021 presented with CF diagnosis. The ethical approval is obtained from the Islamic hospital in Amman, Jordan (IRB: 101/2021/1053). Informed consent was obtained from the parents of these two patients.

The diagnosis of CF was primarily based on clinical evidence, with supporting evidence provided through sweat chloride measurements, genetic analysis, nasal/rectal potential difference investigations, or testing for neonatal screening for trypsinogen.[12-14] In addition to productive cough, other common symptoms included shortness of breath, hemoptysis, and pleuritic chest pain.

Malaise, increased sputum production, anorexia, weight loss, and a decline in lung function are typical symptoms of a pulmonary exacerbation in CF. However, no universally acknowledged description of what constitutes an exacerbation exists.[15]

Expectorated sputum samples from patients with CF were acquired during their pulmonary exacerbation. The expectorated sputum samples were taken within 24 hours from the onset of the exacerbation (E1), and 14 days after the exacerbation episode and taking the appropriate treatment (E2). After collecting and processing the samples, they were kept in a freezer at -80 degrees Celsius for later use in a retrospective analysis after the study was finished. On dry ice, samples that had been frozen were transferred.

Microbiome analysis

As detailed in our previous studies,[16-18] we extracted nucleic acid from 200µL sputum samples. A volume of 1.8ml of sterile Phosphate Buffered Saline (PBS) was added to the corresponding sample tube of pre-aliquoted Sputolysin stock to obtain a 10% Sputolysin solution. After extraction, the eluted nucleic acid samples (100µL) were frozen at -80°C until ready for library preparation. DNA extracts of the sputum samples were measured for DNA concentration.[16]

Variable V4 primer pair sequences create a 300bp amplicon. Adapters are appended to primer pair sequences for compatibility with Illumina MiSeq index and sequencing adapters. As discussed in our previous studies,[19,20] the sample preparation protocol for sequencing the 16S rRNA gene V4 region consisted of four main PCR steps. MiSeq v3 reagents and paired 300-bp reads were used to create full-length, high-quality V4 reads in a single 65-hour run.

Paired-end Illumina MiSeq sequences were processed using QIIME version 1.9.1).[21] UCLUST grouped sequences into OTUs at 97% sequence identity,[22] PyNAST aligned against Greengenes 16S rRNA marker-gene sequences (v13.8)[23] and assigned according to the RDP Classifier Tool (v 2.2).[24] In the negative control, several taxa were discovered, but no single taxon dominated. Singletons and OTUs representing possible human sequences, Archaea, Cyanobacteria, unassigned OTUs, and those detected in the negative control background were filtered out and considered as contaminating sequences before downstream analysis. The obtained dataset was converted to a quality-filtered OTU table with normalized absolute counts or relative abundance.

RESULTS

Two of the 165 patients admitted to the hospital due to lower respiratory tract infection included in the study presented with CF diagnosis. The demographic and clinical characteristics of the two participants who provided sputum samples are included in Table 1.

We investigated the bacterial community structure in the sputum samples from the CF patients at E1 and E2 to gain insight into whether the pulmonary exacerbation affected the respiratory microbiome in CF patients, even after the adjustment of the influence of antibiotics treatment.

The sputum samples showed evidence of the presence of both aerobic and anaerobic bacteria. Detected genera with high abundance in the E1-related sputum sample of the first patient included Achromobacter, Streptococcus, Prevotella, Haemophillus. At E2, Achromobacter, Staphylococcus,

Table 1. The demographic information and clinical features of the two participants diagnosed with CF

Participant Descriptions	Patient_1	Patient_2
Age, year	13	10
Gender	Female	Female
BMI, kg/m^2	20.2	21.0
FEV_1% predicted* at the E1	46.7	49.4
FEV_1% predicted* at the E2	54.1	NA
Traditional culture results	No growth	No growth
Treatment	Piperacillin/Tazobactam as empiric (IV: 240 to 400 mg piperacillin/kg/day divided every 8 hours), then Amoxicillin/ Clavulanic acid after the culture result	Piperacillin/Tazobactam as empiric (IV: 240 to 400 mg piperacillin/kg/day divided every 8 hours), then Amoxicillin/ Clavulanic acid after the culture result

* The normal percent is 80% or greater
Abbreviations: "BMI: Body Mass Index; FEV1: Forced Expiratory Volume in the 1st second; E1: exacerbation event; E2: 14 days after the exacerbation event and the appropriate treatment"

Stenotrophomonas, Streptococcus, Pseudomonas were the highest abundant genera.

Regarding the second patient, Veillonella, Streptococcus, Lactobacillus, Pseudomonas, Prevotella, Rothia, Actinomyces were the highest abundant genera at E1. Whereas, Streptococcus, Veillonella, Lactobacillales, Rothia. Actinomyces, Prevotella, Pseudomonas were the highest abundant genera.

Figure 1 shows the bacterial genera distribution of sputum samples from CF-paired patients at the E1 versus E2. The changes in the lung microbiome were not uniform, and the relative abundance of the principal genera underwent observed shifts due to this change.

Taxon names that end in "unclassified" are shown under the category of the lowest known taxon level because it is

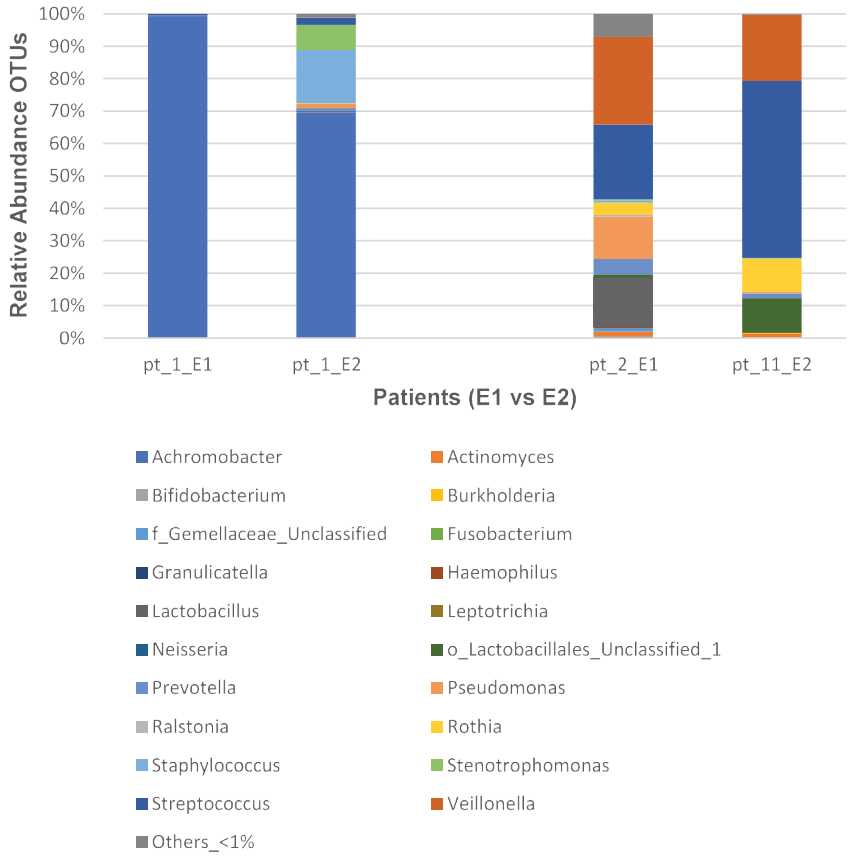

Figure 1. Bacterial taxa distribution (genera-level) of sputum samples from the two CF-paired patients at the exacerbation (E1) versus two weeks following the exacerbation (E2)

possible that they have not been assigned to the genus level. Abbreviations: CF: cystic fibrosis; E1: exacerbation; E2: two weeks after the exacerbation.

DISCUSSION

This study aimed to investigate the respiratory microbiome in CF, given that there is no previous study with this aim due to the rare CF diagnosis in Jordan. Using the 16S rRNA marker-gene sequencing, we investigated the bacterial communities in sputa during and after pulmonary exacerbation to determine what changes occurred.

The NGS Illumina MiSeq was utilized, thereby comprehensively revealing the lung microbiome composition and aiding the diagnosis of less frequent bacteria linked with culture difficulties and, therefore, untargeted by conventional antimicrobial therapy. Utilizing Illumina MiSeq's high-throughput sequencing technology, our work revealed an extreme bacterial diversity in sputum. Complex microbial communities exist in the lungs of CF patients, as evidenced by the detection of a wide variety of species in high abundance. Innovative molecular techniques are used to characterize the microbiota of CF patients. Therefore, the current study, which relies on NGS Illumina MiSeq, provides novel information regarding sputum microbiota in CF patients during pulmonary exacerbation and 14 days following the exacerbation. Observed differences were identified between the analyzed samples.

Our findings show that airway microbial populations can be subdivided into two categories, as observed in other CF studies.[25-28] One category comprises a relatively small number of taxa dominating the niche. Among our patients with CF, the aerobic genera predominated the communities: Pseudomonas,

Streptococcus, Achromobacter, and Staphylococcus, in addition to the anaerobic genus Prevotella and Veillonella. The second group encompassed less encountered taxa with low abundance accounting for most of the observed community richness.

It was further observed that patients with CF, in our study, had distinctive microbiomes. The airway composition of the microbial communities varied from one patient to another. It is worth noting that the patient-specific community structures that showed a dominance of a single or a few genera were consistent with a previous CF study.[25] This study has shown a unique profile that is maintained over a span of time.

In a previous study, anaerobic Prevotella was highly detected in CF patients,[29] and it was considered a core member in CF patients[26] that can facilitate the growth of other bacteria.[30] This suggests that some Prevotella species may potentially contribute to the pathogenicity of lung disease in CF patients. However, in our study, the Prevotella genus was more abundant during the exacerbation related samples compared with the paired samples two weeks after CF exacerbation for the second patient. In contrast, the opposite was observed for the first patient.

Identifying the prevalence of certain bacteria in the lower respiratory tract is not straightforward, which stems primarily from the difficulties associated with acquiring samples not contaminated by upper airway secretions.[31,32] One way to avoid this was using bronchoscopy, as it is notable that investigations depending on sputum to detect bacterial infection reflect greater levels of bacterial isolation when compared with the bronchoscopy studies.[31,33,34] Although the possibility of contamination of sputum from sources external to the lung (e.g., sinuses, pharynx, and mouth) is a serious one, it should be recognised that some CF studies have indicated that the contamination of sputum by bacterial species inside the oral cavity is not considerable.[35] Furthermore, it was demonstrated that anaerobic bacteria cultured from CF sputum samples were obtained not from the oral cavity but from the lungs.[32]

The main limitations of this study were the relatively small sample size for the matched patients and including only a single sampling time point during each event. As such, the statistical comparison results may not represent a larger population. However, the findings should add valuable information, given the lack of previous CF reports in Jordan with a particular reference to the respiratory microbiome. Such an association needs to be assessed by a large-scale study because this finding may have important implications for pulmonary exacerbation management. A greater understanding of this relationship should provide a more targeted use of antibiotics, particularly during exacerbations, hence improving clinical efficacy and patient outcomes.

CONCLUSIONS

This is the first study, based in Jordan, to report and describe the respiratory microbiome during and after exacerbation of CF patients. This study suggests that pulmonary exacerbation in CF patients can result in alterations in their respiratory microbiome. The findings may have important implications for pulmonary exacerbation management. A better knowledge of this link may make it possible to employ antibiotics in a more focused manner, particularly during exacerbations. This has the potential to improve clinical efficacy and outcomes for CF patients.

SUPPLEMENTARY MATERIALS

Not applicable

AUTHOR CONTRIBUTIONS

Conceptualization, A.R.A.; methodology, A.R.A. and R.A.A.; investigation, A.R.A.; data curation, A.R.A.; writing—original draft preparation, A.R.A. and M.A.; writing—review and editing, A.R.A., A.A., M.Z., M.S.A, and M.A.; visualization, A.A.; supervision, A.R.A. All authors have read and agreed to the published version of the manuscript."

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INSTITUTIONAL REVIEW BOARD STATEMENT

The study was conducted in accordance with the Declaration of Helsinki, and approved by the Institutional Review Board (or Ethics Committee) of Islamic hospital in Amman, Jordan (IRB: 101/2021/1053)).

INFORMED CONSENT STATEMENT

Informed consent was obtained from all subjects involved in the study.

DATA AVAILABILITY STATEMENT

Data are contained within the article.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

References

1. O'Sullivan BP, Freedman SD. Cystic fibrosis. Lancet. 2009;373(9678):1891-1904. https://doi.org/10.1016/s0140-6736(09)60327-60335
2. Alsayed AR, Abed A, Khader HA, et al. Molecular Accounting and Profiling of Human Respiratory Microbial Communities: Toward Precision Medicine by Targeting the Respiratory Microbiome for Disease Diagnosis and Treatment. Int J Mol Sci. 2023;24(4):4086. https://doi.org/10.3390/ijms24044086
3. Lipuma JJ. The changing microbial epidemiology in cystic fibrosis. Clin Microbiol Rev. 2010;23(2):299-323. https://doi.org/10.1128/cmr.00068-09
4. Surette MG. The cystic fibrosis lung microbiome. Ann Am Thorac Soc. 2014;11(Suppl 1):S61-S65. https://doi.org/10.1513/AnnalsATS.201306-159MG
5. Sanders DB, Bittner RC, Rosenfeld M, et al. Failure to recover to baseline pulmonary function after cystic fibrosis pulmonary exacerbation. Am J Respir Crit Care Med. 2010;182(5):627-32. https://doi.org/10.1164/rccm.200909-1421OC
6. Klepac-Ceraj V, Lemon KP, Martin TR, et al. Relationship between cystic fibrosis respiratory tract bacterial communities and age, genotype, antibiotics and Pseudomonas aeruginosa. Environmental Microbiology. 2010;12(5):1293-1303. https://doi.org/10.1111/j.1462-2920.2010.02173.x
7. Zhao J, Schloss PD, Kalikin LM, et al. Decade-long bacterial community dynamics in cystic fibrosis airways. Proceedings of the National Academy of Sciences. 2012;109(15):5809-5814. https://doi.org/10.1073/pnas.1120577109
8. Flanagan J, Brodie E, Weng L, et al. Loss of bacterial diversity during antibiotic treatment of intubated patients colonized with Pseudomonas aeruginosa. Journal of Clinical Microbiology. 2007;45(6):1954-1962. https://doi.org/10.1128/JCM.02187-06
9. Cox MJ, Allgaier M, Taylor B, et al. Airway microbiota and pathogen abundance in age-stratified cystic fibrosis patients. PloS One. 2010;5(6):e11044. https://doi.org/10.1371/journal.pone.0011044
10. Rawashdeh M, Manal H. Cystic fibrosis in Arabs: a prototype from Jordan. Ann Trop Paediatr. 2000;20(4):283-286. https://doi.org/10.1080/02724936.2000.11748148
11. Nazer HM. Early diagnosis of cystic fibrosis in Jordanian children. J Trop Pediatr. 1992;38(3):113-115. https://doi.org/10.1093/tropej/38.3.113
12. Comeau AM, Accurso FJ, White TB, et al. Guidelines for implementation of cystic fibrosis newborn screening programs: Cystic Fibrosis Foundation workshop report. Pediatrics. 2007;119(2):e495-e518. https://doi.org/10.1542/peds.2006-1993
13. Farrell PM, Rosenstein BJ, White TB, et al. Guidelines for diagnosis of cystic fibrosis in newborns through older adults: Cystic Fibrosis Foundation consensus report. The Journal of Pediatrics. 2008;153(2):S4-s14. https://doi.org/10.1016/j.jpeds.2008.05.005
14. De Boeck K, Wilschanski M, Castellani C, et al. Cystic fibrosis: terminology and diagnostic algorithms. Thorax. 2006;61(7):627-635. https://doi.org/10.1136/thx.2005.043539
15. Flume PA, Mogayzel Jr PJ, Robinson KA, et al. Cystic fibrosis pulmonary guidelines: treatment of pulmonary exacerbations. American Journal of Respiratory and Critical Care Medicine. 2009;180(9):802-808. https://doi.org/10.1164/rccm.200812-1845PP
16. Al-Dulaimi A, Alsayed AR, Maqbali MA, et al. Investigating the human rhinovirus co-infection in patients with asthma exacerbations and COVID-19. Pharm Pract (Granada). 2022;20(2):2665. https://doi.org/10.18549/PharmPract.2022.2.2665
17. Alsayed AR, Talib W, Al-Dulaimi A, et al. The first detection of Pneumocystis jirovecii in asthmatic patients post-COVID-19 in Jordan. Bosn J Basic Med Sci. 2022;22(5):784-790. https://doi.org/10.17305/bjbms.2022.7335
18. Alsayed AR, Hasoun L, Khader HA, et al. Co-infection of COVID-19 patients with atypical bacteria: A study based in Jordan. Pharmacy Practice. 2023;21(1):1-5. https://doi.org/10.18549/PharmPract.2023.1.2753
19. Alsayed A, Al-Doori A, Al-Dulaimi A, et al. Influences of bovine colostrum on nasal swab microbiome and viral upper respiratory tract infections - A case report. Respir Med Case Rep. 2020;31:101189. https://doi.org/10.1016/j.rmcr.2020.101189
20. Alsayed AR, Abed A, Jarrar YB, et al. Alteration of the Respiratory Microbiome in Hospitalized Patients with Asthma–COPD Overlap during and after an Exacerbation. Journal of Clinical Medicine. 2023;12(6):2118. https://doi.org/10.3390/jcm12062118
21. Caporaso JG, Kuczynski J, Stombaugh J, et al. QIIME allows analysis of high-throughput community sequencing data. Nature methods. 2010;7(5):335-336. https://doi.org/10.1038/nmeth.f.303
22. Edgar RC, Haas BJ, Clemente JC, et al. UCHIME improves sensitivity and speed of chimera detection. Bioinformatics. 2011;27(16):2194-2200. https://doi.org/10.1093/bioinformatics/btr381
23. Caporaso JG, Bittinger K, Bushman FD, et al. PyNAST: a flexible tool for aligning sequences to a template alignment. Bioinformatics. 2009;26(2):266-267. https://doi.org/10.1093/bioinformatics/btp636
24. Wang Q, Garrity GM, Tiedje JM, et al. Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Applied and Environmental Microbiology. 2007;73(16):5261-5267. https://doi.org/10.1128/AEM.00062-07
25. Fodor AA, Klem ER, Gilpin DF, et al. The adult cystic fibrosis airway microbiota is stable over time and infection type, and highly resilient to antibiotic treatment of exacerbations. PLoS One. 2012;7(9):e45001. https://doi.org/10.1371/journal.pone.0045001
26. van der Gast CJ, Walker AW, Stressmann FA, et al. Partitioning core and satellite taxa from within cystic fibrosis lung bacterial

communities. Isme J. 2011;5(5):780-791. https://doi.org/10.1038/ismej.2010.175
27. Han MK, Huang YJ, Lipuma JJ, et al. Significance of the microbiome in obstructive lung disease. Thorax. May 2012;67(5):456-463. https://doi.org/10.1136/thoraxjnl-2011-201183
28. Madan JC, Koestler DC, Stanton BA, 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(4): e00251-12. https://doi.org/10.1128/mBio.00251-12
29. O'Neill K, Bradley JM, Johnston E, et al. Reduced bacterial colony count of anaerobic bacteria is associated with a worsening in lung clearance index and inflammation in cystic fibrosis. PloS One. 2015;10(5):e0126980. https://doi.org/10.1371/journal.pone.0126980
30. Sherrard LJ, Graham KA, McGrath SJ, et al. Antibiotic resistance in Prevotella species isolated from patients with cystic fibrosis. Journal of Antimicrobial Chemotherapy. 2013;68(10):2369-2374. https://doi.org/10.1093/jac/dkt191
31. Hurst JR, Wilkinson TM, Perera WR, et al. Relationships among bacteria, upper airway, lower airway, and systemic inflammation in COPD. CHEST Journal. 2005;127(4):1219-1226. https://doi.org/10.1378/chest.127.4.1219
32. Rogers G, Carroll M, Serisier D, et al. Use of 16S rRNA gene profiling by terminal restriction fragment length polymorphism analysis to compare bacterial communities in sputum and mouthwash samples from patients with cystic fibrosis. Journal of Clinical Microbiology. 2006;44(7):2601-2604. https://doi.org/10.1128/JCM.02282-05
33. Patel I, Seemungal T, Wilks M, et al. Relationship between bacterial colonisation and the frequency, character, and severity of COPD exacerbations. Thorax. 2002;57(9):759-764. https://doi.org/10.1136/thorax.57.9.759
34. Wilkinson TM, Patel IS, Wilks M, et al. Airway bacterial load and FEV1 decline in patients with chronic obstructive pulmonary disease. American Journal of Respiratory and Critical Care Medicine. 2003;167(8):1090-1095. https://doi.org/10.1164/rccm.200210-1179OC
35. Tunney MM, Field TR, Moriarty TF, et al. Detection of anaerobic bacteria in high numbers in sputum from patients with cystic fibrosis. American Journal of Respiratory and Critical Care Medicine. 2008;177(9):995-1001. https://doi.org/10.1164/rccm.200708-1151oc