Gut microbiota changes in airway diseases: a systematic review

Alterações da microbiota intestinal em doenças das vias aéreas: uma revisão sistemática

Fabine Correia Passos1, Lucas Matheus Gonçalves de Oliveira2, Odilon Lobão Leal Neto2, Fabiola Ramos Jesus3, Michelle Miranda Lopes Falcão3, Margarida Célia Lima Costa Neves4, Antônio Carlos Moreira Lemos4, Gyselle Chrystina Baccan5

1Mestre em Alimentos, Nutrição e Saúde; 2Mestre em Bioquímica e Biologia Molecular pela Sociedade Brasileira de Bioquímica e Biologia Molecular; 3Graduado em Nutrição; 4Mestre em Imunologia; 5Doutora em Imunologia; 6Doutora em Medicina e Saúde, professora adjunto, UFBA; 7Doutor em Medicina e Saúde, Brazil, professor Associado, UFBA; 8Doutora em Bioquímica, Professor Associado UFBA

INTRODUCTION

Human gut microbiota (GM) has been extensively studied in recent years and has been recognized as a key player in the regulation of immune system. These studies have evidenced the presence of dysbiosis in some pathologies and have discussed the importance of these GM changes in the development and outcome of diseases. In this context, evidences have indicated the existence of the complex relationships between GM and the lung, with implications for both lung and intestinal diseases. Airway diseases (AD) are characterized by some changes in pulmonary function, usually with chronic inflammation, impairment of the life quality, without effective treatments that lead to the cure. In this group are cystic fibrosis (CF), asthma, pulmonary hypertension (PH) and chronic ob-

INTRODUÇÃO

Estudos têm destacado a importância da microbiota intestinal (MI) para as defesas imunológicas do hospedeiro, influenciando o desenvolvimento e a fisiologia do hospedeiro. Mudanças na composição e diversidade da GM foram detectadas em algumas doenças e podem estar implicadas nos mecanismos fisiopatológicos delas. Objetivo: o objetivo desta revisão foi avaliar estudos sobre a microbiota intestinal (MI) de pacientes com doenças das vias aéreas (DA). Metodologia: esta pesquisa bibliográfica foi realizada em quatro bases de dados, utilizando a combinação dos descritores: “Microbiota Gastrointestinal”, “Microbiota Intestinal”, “Microbiota Intestinal”, “Fibrose Cística” (CF), “Asma”, “Hipertensão Pulmonar” (HP), “Doença Pulmonar Obstrutiva Crônica” (DPOC). Resultados: quinze estudos foram incluídos: dez de CF e cinco de asma. Nenhum estudo sobre outra DA correspondeu aos critérios de inclusão. Em todos os estudos sobre FC, foram detectadas alterações na MI, particularmente alterações microbianas qualitativas e quantitativas. Para a asma, os dados mostraram mudanças na MI, incluindo também uma redução da quantidade, uniformidade e diversidade microbiana e na razão Bacteroidetes/Firmicutes. Conclusão: os dados atuais indicam a existência de alterações na MI nas DA. No entanto, devido aos poucos estudos para asma e à falta de investigações para HP e DPOC, não foi possível confirmar se essas alterações na MI são observadas em outras DA também. Além disso, esta revisão mostra a necessidade de mais estudos nessa área para caracterizar a disbiose e quais alterações são mais frequentes em pacientes com DA.

Palavras-chave: Microbiota Intestinal. Doenças das Vias Aéreas. Fibrose Cística. Asma. Hipertensão Pulmonar. Doença Pulmonar Obstrutiva Crônica.
Structural pulmonary disease (COPD). Mechanisms through which GM could influence the lung immune responses are not well understood, but some evidences point to a crosstalk between these organs, which is mediated by GM, specially through toll-like receptors (TLR) signaling and cellular homing.

The aim of this systematic review was to show an overview of the current knowledge about the GM changes in the main ADs, to evaluate whether there is dysbiosis and to determine what are the main alterations observed in these diseases.

RESULTS

In the electronic search conducted in the databases, it was found 1912 articles. 1101 duplicated articles were excluded. After the analysis of titles and/or abstracts, 64 articles were selected. Only 15 articles met the inclusion criteria of this review (fig 1).

Regarding the studies comparing GM from individuals with CF and their HI, we found 363 articles. Of these articles, 211 were duplicated studies. After reading titles and/or abstracts only 28 were selected. Outcomes of interest were identified in ten articles (fig 1).

About asthma, we initially found 1341 articles that compared the GM of patients with HI; of these, 789 were excluded because they were duplicates. After performing the analysis of titles and/or abstracts, 32 were screened. Only five articles presented all inclusion criteria (fig 1).

About the systematic search using the descriptors for PH, 35 articles were found and 12 duplicated articles were excluded. No articles compared the GM of the individuals with PH and HI (fig 1).

In the systematic search for COPD, it was used the additional descriptors “pulmonary emphysema” and “bronchitis”, although these expressions are no longer used to define COPD. For PE, only two articles were found, and none of them addressed the outcomes of interest (fig 1). About the systematic search using the descriptors “chronic obstructive pulmonary disease”, 131 articles were found. After reading the titles and/or abstracts of the remaining, no articles were found that addressed the outcomes of interest (fig 1).

Major GM changes in airway diseases

The overall studies found alterations in the GM; however the taxonomic groups differed among the articles (Table 1). The data showed evidence of reduction of the microbial richness and/or diversity in CF patients. Only three studies associated the GM species diversity with the age, two of them showed reduction of the species diversity with the increase in age. Despite the few studies about GM and asthma, the results indicate some alterations. Only one study evaluated the species diversity in asthma patients. A reduction of the microbial richness and diversity with no changes in alpha-diversity was described in one study.

The main phyla evaluated in articles were Firmicutes, Bacteroidetes and Actinobacteria. Regarding the CF, the increase in the relative abundance of Firmicutes was detected in two studies. On the other hand, two other studies described a decrease in this phylum. A reduction of the abundance of Bacteroidetes was detected in three studies and an increase in this phylum was observed in one study. Only two studies showed reduction of the levels of Bacteroides in asthma patients, also a reduction of Bacteroidetes/Firmicutes ratio in one study on asthma.

Regarding CF, studies have showed a decrease in the beneficial intestinal bacteria that could be considered markers of gut health. In two studies with the genus Roseburia, there was a decrease in those bacteria. In four studies, the authors found a reduced level of Faecalibacterium prausnitzii. The data showed one study describing the reduction of F. prausnitzii in asthma and other one showed increase.

Regarding the Actinobacteria, it was found a reduction in two studies and other two articles found an increase.
in the levels of this phylum in CF patients. Low levels of Bifidobacterium spp. were detected by many authors. Only one study found that the order Lactobacillales was significantly enriched in CF patients. Regarding asthma, a reduction in the levels of Bifidobacterium, B. longum, B. breve and B. bifidum, and increase level of Bifidobacterium, B. adolescentis was detected. This genus was negatively correlated with the disease duration and positively with the IgE levels in asthma patients. Regarding Lactobacillus, one study identified higher levels of this genus in asthma patients, but other showed a reduction of these bacteria.

Regarding CF, an increase in the level of Proteobacteria was observed in some studies while a reduction was described by others authors. The increased abundance of Escherichia coli was found in two studies that assessed the GM of children. In contrast, in one study, authors detected a reduction in the levels of this bacterial species.

In asthma patient only one study identified higher levels of the E. coli. A reduction in the abundance of Clostridium clusters XIVa and of Cl. coccoides. Other studies observed an increase in the level of the, Cl. difficile, Cl. clostridioforme and Cl. nexile in CF patient. But a reduction in the abundance of Cl. leptum sub group was showed in asthma patients.

The relationship of the GM composition and the lung function was evaluated only in three articles for CF. The forced expiratory volume in 1s (FEV1) and the forced vital capacity (FVC) did not show any correlation with the GM composition. One article showed positive relation between FEV1 and GM diversity. When studying the GM of children with CF, it was observed that the alteration in GM leads to changes in functional capacities of the microbiome. Metabolomic analyses suggests that the changes in the GM could be involved with pathways of unsaturated fatty acids biosynthesis and xenobiotic metabolism.

Figure 1 – Flowchart of identification and selection of articles about composition of GM of patients with Asthma, CF, HP, PE, Bronchitis and COPD.

Fonte: Autoria própria
Table 1 – Studies about gut microbiota changes and airway diseases.

| Reference                  | Disease    | Sample size          | Age range      | Technique for Analysis | Results: patients compared with healthy individuals |
|----------------------------|------------|----------------------|----------------|------------------------|---------------------------------------------------|
| Burke et al. 2017<sup>a</sup> | Cystic Fibrosis | 43 patients 69 HI   | 21-40 years   | NGS<sup>a</sup>        | ↓Bacteroidetes, Proteobacteria, Cyanobacteria and Verrucomicrobia  
↑Firmicutes and Actinobacteria  
↑Alcaligenaceae, Prevotellaceae, Bifidobacteriaceae and Peptococcaeace  
↑Enterococcus, Bacteroides, Leunonoto  
↓Roseburia, Prevotella, Odoribacter, Faecalibacterium and Bifidobacterium |
| Fouhy et al. 2017<sup>b</sup> | Cystic Fibrosis | 6 patients 6 HI     | 20-71 years    | NGS qPCR<sup>b</sup>   | ↑Firmicutes  
↑Actinobacteria, Bacteroidetes and Proteobacteria  
↑Bifidobacterium, B. Longum and Faecalibacterium prausnitzii  
↑Enterococcus faecalis, Clostridium and Ruminococcus gravis |
| Miragoli et al. 2017<sup>c</sup> | Cystic Fibrosis | 30 patients 8 HI    | 10-22 years    | PCR-DGGE<sup>c</sup> qPCR | ↓Escherichia coli/Shigella, Blautia spp., Faecalibacterium prausnitzii, Collinsella aerofaciens, Dialister invisus, Eubacterium rectale and Bifidobacterium adolescentis, Ruminococcus gravis, Bifidobacterium spp., Cl. cocoides group, and Ruminococcaece family, Ba. vulgatus and Ba. uniformis  
↓Butyrate-producing bacteria and acetogens  
↓Prevalence and abundance of Sulfate-Reducing Bacteria |
| Debyser et al. 2016<sup>d</sup> | Cystic Fibrosis | 15 patients 11 HI   | 1.6-15.6 years | Gel electrophoresis LC-MS/MS<sup>d</sup> | ↑Firmicutes  
↑Proteobacteria and Bacteroidetes  
↓Spectral count of proteins from the genera Faecalibacterium, Eubacterium, Roseburia and Ruminococcus gravis  
↓Spectral count of proteins from the Blautia and Clostridium species  
↑Burkholderiales and Enterobacteriaceae, Clostriidiales and genus Clostridium (Clostridium clostridioforme and Clostridium nexile) |
| Manor et al. 2016<sup>e</sup> | Cystic Fibrosis | 14 patients 12 HI   | < 3 years      | NGS                    | ↓α – diversity (Shannon index)  
↑Proteobacteria (E. Coli) and Actinobacteria  
↓Firmicutes, Bacteroidetes and Verrucomicrobia  
↑Lactobacillales, Veillonella  
↓Clostridiales but ↑Cl. difficile |
| Nielsen et al. 2016<sup>f</sup> | Cystic Fibrosis | 23 patients 35 HI   | 0-18 years     | NGS                    | ↓Number and diversity  
↑Bacteroides, Genera Streptococcus and Fallovinafractor  
No difference in Clostridium abundance but # tendencies with age|
| Duytschaever et al. 2013<sup>g</sup> | Cystic Fibrosis | 21 patients 24 HI   | 8 months – 5, 6 years | DGGE CE<sup>g</sup> qPCR | ↓Bifidobacterium longum, Bifidobacterium catenulatum, Bifidobacterium pseudocatenulatum and Bifidobacterium adolescentis  
↓Clostridium XIV |
| Hoffman et al. 2013<sup>h</sup> | Cystic Fibrosis | 12 patients 12 HI   | 15 days – 3,6 years | NGS                    | ↑E. coli |
| Scanlan et al. 2012<sup>i</sup> | Cystic Fibrosis | 4 patients 4 HI     | 3-72 years     | PhyloChip<sup>i</sup>   | ↓Taxonomic richness, evenness and diversity  
↑Bifidobacterium sp.  
↑Inter-individual variation |
| Duytschaever et al. 2011<sup>j</sup> | Cystic Fibrosis | 21 patients 24 HI   | 9 months –15 years | Bacterial culture DGGE | ↓Clostridia, Bifidobacterium spp., Veillonella spp., and Bacteroides and Prevotella spp  
↑Enterobacterial counts |
| Ishaq et al. 2018<sup>k</sup>  | Asthma | 15 patients 5 HI     | 30-45 years    | PCR-DGGE Real-time PCR CE | ↓Bifidobacterium, Lactobacillus and Clostridium leptum sub group |
| Wang et al. 2018<sup>l</sup>  | Asthma | 36 patients 185 HI   | 15 years       | NGS                    | ↓Faecalibacterium prausnitzii, Sutterella wadsworthensis and Bacteroides stercoris  
↓Microbial richness, evenness and diversity |
| Begley et al. 2018<sup>m</sup>  | Asthma | 24 patients 8 HI    | 18-69 years    | NGS                    | ↓Bacteroidetes/Firmicutes ratio  
↑Bacteroides, Enterobacteraceae  
↑Bifidobacterium, Lachnospiraceae |
| Okba et al. 2018<sup>n</sup>  | Asthma | 80 patients 40 HI   | 18-45 years    | Bacterial Culture ↑Lactobacillus and E. coli |
| Hevia et al. 2016<sup;o</sup>  | Asthma | 21 patients 22 HI   | 28-50 years    | NGS                    | ↑Faecalibacterium, Bifidobacterium adolescentis  
↑Bifidobacterium longum, Bifidobacterium breve and Bifidobacterium bifidum |

<sup>a</sup>Next Generation Sequencing (NGS), <sup>b</sup>Real-time Polymerase chain reaction (qPCR), <sup>c</sup>Denaturing Gradient Gel Electrophoresis (DGGE), <sup>d</sup>Liquid Chromatography – Mass spectrometry/Mass spectrometry (LC-MS/MS), <sup>e</sup>Capillary Electrophoresis (CE), <sup>f</sup>High-density phylogenetic microarray (PhyloChip).
DISCUSSION

Cystic fibrosis, asthma, PH and COPD are chronic diseases that affect the lungs and the life quality of patients, with hundreds of millions of people suffering every day from these illnesses. Recently, the role of the GM in the regulation of the immune response has been recognized while studies have showed the existence of GM changes during some pathologies. In this review, we bring information on what currently is known regarding the GM alterations during some AD.

Few studies were found that match the inclusion/exclusion criterion of this review. Most of them were about CF. That highlights the need for further studies to detect the existence of GM changes in AD and to determine its importance in the pathology of these diseases. We included only studies without any intervention (probiotics, prebiotics or antibiotics) considering the effect on GM. Only data from human GM was analyzed because of limitations of some experimental models and because animals have a distinct GM. Exclusion criterions such as respiratory and intestinal infection were used to avoid bias in the interpretation of results. The rigor inclusion / exclusion criteria were necessary to provide the most relevant evidence related to the aim. The predominance of studies that evaluate the GM in CF could be reflected by the plenty of articles about lung microbiome for this disease. Although lung microbiome changes have been described to asthma and COPD as well, the volume of data has not been so wide as the one for CF. PH is a less prevalent disease than is CF, asthma and COPD, and this situation can explain the lack of studies about GM changes during the disease.

The development of the more advanced techniques of molecular biology has enabled a more accurate analysis of the GM. These new techniques have not only facilitated the identification of non-cultivated microorganisms but also improved some experimental limitations such as the low sensitivity and the low reproducibility of the traditional techniques, allowing the identification of a larger number of species and better understanding about the complexity of the intestinal ecosystem. In our review, identification and classification of bacteria from the GM were predominantly performed through culture-independent techniques.

Despite the large number of data indicating the importance of the GM changes during some diseases, there is little knowledge about the existence of GM alterations in AD. According to the data, patients with CF showed a reduction in the GM diversity and richness. For asthma, the available data still do not avail a conclusion about the GM changes, even because one of study used stool bacteria culture for the analysis of the composition of GM, which prevents wider conclusions. Microbial diversity decreased with age in CF patients, what is expected in healthy individuals also. In addition, the dysfunction in the Transmembrane Conductance Regulator (CFTR), associated with the CF treatments, could affect the microbial diversity. CFTR gene variants were related to the shifts in fecal microbiota profiles in CF patients. Individuals with low bacterial richness are characterized as showing more adiposity, insulin resistance and dyslipidaemia and a more inflammatory phenotype. Another alteration detected in the studies that evaluated CF patients concerned the relative abundance of the Firmicutes, Bacteroidetes and Actinobacteria phyla. Changes in the Firmicute/Bacteroidete ratio have been described in some pathological conditions. Changes in the Firmicutes/Bacteroidetes ratio have been described in infants and adults and in some pathological conditions such as obesity and IBD. Studies in experimental models of obesity, where mice have been feed with high-fat diet, and in ob/ob mice, have shown that the increase of the Firmicutes/Bacteroidetes ratio has been implicated in the increment of the efficiency of the energy harvest in addition to elevation of the endotoxin levels and induction of the colonic inflammation.

We suppose that this alteration could be implicated in the increase in the colonic and systemic inflammation. We suppose that this alteration could be implicated in the increase in the colonic and systemic inflammation. The current data do not allow determining whether changes in microbial diversity and richness are consequences or factors that contribute to the development of AD, but they show the existence of a lung-gut axis with possible implications for AD.

Similar to CF and other pathologies, asthma patients exhibit GM alterations that characterize dysbiosis: reduction of microbial richness and diversity. Under representation of F. prausnitzii and Roseburia spp. are characteristics of dysbiosis and was detected. These results are similar to those observed in other pathologies. F. prausnitzii and Roseburia spp. metabolize dietary components and produce short-chain fatty acids (SCFAs). Low amounts of these primary energy sources for the colonocytes may result in decreased intestinal barrier integrity. The SCFAs play a protective role against intestinal inflammation and is likely to be important in both health and in specific disease states. Caco-2 cells treated with F. prausnitzii and its extracellular vesicles produced lower levels of inflammatory and higher levels of the anti-inflammatory cytokines. Roseburia intestinalis suppressed intestinal inflammation by increasing Treg cell numbers and expression of the anti-inflammatory cytokines TSLP, TGF-8, and interleukin-10 (P < 0.05) in lipopolysaccharide-treated Caco-2 cells. The decrease of F. prausnitzii and Roseburia spp. from GM might contribute to an increase of pulmonary inflammation during CF.

Bifidobacteria, beneficial bacteria for the intestine, are also altered in CF patients and asthma. This has been described for other pathological conditions. Some studies detected a reduction of bifidobacteria or lactobacilli and others showed an increase of these genus in asthma patient. These bacteria have been used in the treatment of some pathologies because of their probiotic...
effect, and some authors have discussed the beneficial effect of this supplementation for the asthma patients 46. The supplementation with a mixture of L. acidophilus, L. bulgaricus, B. bifidum, S. thermophilus (Bio-plus, Supherb, Israel) reduced pulmonary exacerbations in patients with CF 47. In children with CF, the administration of L. rhamnosus GG (LGG) restored the composition of the GM, reduced the intestinal inflammation and contributed to its beneficial clinical effects 48. Also, the supplementation with probiotics used commercially contains 10^9 CFU bacteria: L. casei, L. rhamnosus, S. thermophilus, B. breve, L. acidophilus, B. infantis and L. bulgaricus improve quality of life and pulmonary exacerbation 49. These data suggest that supplementation with the probiotics improved the respiratory function.

Data also show an alteration in the abundance of E. coli in patients with CF and asthma. The expansion of E. coli in the CF gut may be due to the decreased presence of antimicrobial SCFAs, the increased oxygenation of GI mucosal surfaces as a result of inflammation and the higher availability of glycerol from fat malabsorption 50. E. coli levels were increased in homozygous-F508del, the most common CFTR mutation, and in severe CF patients, while beneficial species (Faecalibacterium prausnitzii, Bifidobacterium spp., and Eubacterium limosum) were reduced 51. Similar results found to those were present investigation in a study involving children with CF in the use of antibiotic therapy 52. The elevation of the E. coli levels of asthma patients was analyzed by bacterial culture 18 using different methodologies, which could explain in part the opposite data obtained by groups 52.

The FEV₁ is a marker of the lung function and has been used to monitor the evolution of the ADs. Cystic Fibrosis patients with severe lung dysfunction (FEV₁ ≤ 40%) had reduced α-diversity when compared with those with mild or moderate lung dysfunction 53. However, no correlation was found in the fecal microbiota with the FEV₁. The analysis of the relation of bacteria groups from GM with the function parameters may improve the understanding about the impact of the intestinal bacteria in the development and outcome of the disease.

The current data suggest the presence of the intestinal dysbiosis in individuals with CF and asthma. However, it is not possible to affirm that there is dysbiosis in other AD, due to the insufficient number of publications. The main alterations in the GM of CF and asthma patients are similar to the GM changes observed in other pathologic conditions such as alterations in Firmicutes and Bacteroidetes phyla, in the microbial diversity and richness and in the levels of beneficial bacteria as bifidobacteria. This panorama reveals the necessity of more studies that could bring information about the GM changes and the relevance of dysbiosis for the AD. This information could show similarities of GM changes in the distinct AD and bring new possibilities of studies or therapies for the patients.

Support statement

LMG de Oliveira is funded by a fellowship from the Fundação de Amparo à Pesquisa do Estado da Bahia.

REFERENCES

1. NOVERR, M. C.; HUFFNAGLE, G. B. Does the microbiota regulate immune responses outside the gut? Trends Microbiol., Cambridge, v. 12, n. 12, p.562-568, Dez. 2004.
2. ICHINOHE, T. et al. Microbiota regulates immune defense against respiratory tract influenza A virus infection. Proc. Natl. Acad. Sci., U.S. A., Washington, v. 108, n. 13, p.5354-5359, 14 Mar. 2011.
3. MATEER, S. W. et al. Potential mechanisms regulating pulmonary pathology in inflammatory bowel disease. J. Leukoc. Biol., New York, v. 98, n. 5, p.727-737, 25 Ago. 2015.
4. ABREU, M. T. Toll-like receptor signalling in the intestinal epithelium: how bacterial recognition shapes intestinal function. Nat. Rev. Immunol., London, v. 10, n. 2, p.131-144, Feb. 2010.
5. MIRAGOLI, F. et al. Impact of cystic fibrosis disease on archaea and bacteria composition of gut microbiota. FEMS Microbiol. Ecol., Oxford, v. 93, n. 2, p.93-95, 2 nov. 2016.
6. SCANLAN, P. D. et al. Gut dysbiosis in cystic fibrosis. J. Cyst. Fibros., Amsterdam, v. 11, n. 5, p.454-455, 2012.
7. NIELSEN, S. et al. Disrupted progression of the intestinal microbiota with age in children with cystic fibrosis. Scientific Reports, London, v. 6, n. 1, p.24857-24867, 4 May 2016.
8. MANOR, O. et al. Metagenomic evidence for taxonomic dysbiosis and functional imbalance in the gastrointestinal tracts of children with cystic fibrosis. Scientific Reports, London, v. 6, n. 1, p.22493-22502, Mar. 2016.
9. BURKE, D. G. et al. The altered gut microbiota in adults with cystic fibrosis. BMC Microbiol., London, v. 17, n. 1, p.58-68, 9 Mar. 2017.
10. WANG, Q. et al. A metagenome-wide association study of gut microbiota in asthma in UK adults. BMC Microbiol., London, v. 18, n. 1, p.114-120, 12 Sept. 2018.
11. FOUHY, F. et al. A pilot study demonstrating the altered gut microbiota functionality in stable adults with Cystic Fibrosis. Scientific Reports, London, v. 7, n. 1, p.6685-6696, 27 July 2017.
12. DEBYSER, G. et al. Faecal proteomics: A tool to investigate dysbiosis and inflammation in patients with cystic fibrosis. J. Cyst. Fibros., Amsterdam, v. 15, n. 2, p.242-250, Mar. 2016.
13. BEGLEY, L. et al. Gut microbiota relationships to lung function and adult asthma phenotype: a pilot study. Bmj Open Respiratory Research, London, v. 5, n. 1, p.000324-000330, Sept. 2018.
14. HEVIA, A. et al. Allergic patients with long-term asthma display low levels of bifidobacterium adolescents. PLOS One, San Francisco, v. 11, n. 2, p.0147809-0147820, 3 Feb. 2016.
15. DUYTSCHAEVER, G. et al. Dysbiosis of bifidobacteria and Clostridium cluster XIVa in the cystic fibrosis fecal microbiota. J. Cyst. Fibros., Amsterdam, v. 12, n. 3, p.206-215, May 2013.
16. DUYTSCHAEVER, G. et al. Cross-Sectional and longitudinal comparisons of the predominant fecal microbiota compositions of a group of pediatric patients with cystic fibrosis and their healthy siblings. Applied And Environmental Microbiology, Washington, v. 77, n. 22, p.8015-8024, 16 Sept. 2011.
17. ISHAQ, H. M. et al. Gut Microbe analysis between asthma patients
and healthy Volunteers in Shaanxi Province, Xian, China. *Pakistan Journal Of Zoology*, Pakistan, v. 50, n. 1, p.165-173, Jan. 2018.

18. OKBA, A. M. et al. Fecal microbiota profile in atopic asthmatic adult patients. *Eur. Ann. Allergy Clin. Immunol.*, Paris, v. 50, n. 03, p.117-124, Feb. 2018.

19. HOFFMAN, L. R. et al. *Escherichia coli* dysbiosis correlates with gastrointestinal dysfunction in children with cystic fibrosis. *Clin. Infect. Dis.*, Chicago, v. 58, n. 3, p.396-399, 30 out. 2013

20. LEVY, M. n. et al. Dysbiosis and the immune system. *Nat. Rev. Immunol.*, London, v. 17, n. 4, p.219-232, 6 Mar. 2017.

21. ERICSSON, A. C. "The use of non-rabid model species in microbiota studies", *Laboratory Animals*, London, v. 53, n. 3, p. 259–270, Jun. 2019.

22. ACOSTA, N. et al. Sputum microbiota is predictive of long-term clinical outcomes in young adults with cystic fibrosis. *Thorax*, London, v. 73, n. 11, p.1016–1025, 22 Ago. 2018.

23. DIAO, W. et al. Symptom-related sputum microbiota in stable chronic obstructive pulmonary disease. *Int. J. Chronic Obst. Pulm. Dis.*, Auckland, v. 13, p.2289-2299, July 2018.

24. MATHIEU, E. et al. Paradigms of lung microbiota functions in health and disease, particularly, in Asthma. *Front. Physiol.*, Lausanne, v. 9, p.116-124, 11 Ago. 2018.

25. FRAHER, M. H. *Firmicutes/Bacteroidetes ratio in an adult Ukrainian population*, *PLoS ONE*, San Francisco, v. 7, n. 6, p.20944-20955, 9 June. 2011.

26. BENVUS, R. F. et al. Probiotic Effects of wheat arabinoxylan related to the increase in *bifidobacteria, roseburia and bacteroides* in dietary fibre in colonic fermentation in healthy human subjects. *Front. Physiol.*, Lausanne, v. 5, p.165, 2017.

27. CLAESSON, M. J. et al. Gut microbiota composition correlates with diet and health in the elderly. *Nature*, London, v. 488, n. 7410, p.178-184, 13 July 2012.

28. SCHIPPA, S. et al. Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) allelic variants relate to shifts in faecal microbiota of cystic fibrosis patients. *PLoS One*, San Francisco, v. 8, n. 4, p.61176-61187, 17 Abr. 2013.

29. CHATELIER, E. L. et al. Richness of human gut microbiome correlates with metabolic markers. *Nature*, London, v. 500, n. 7464, p.541-546, Ago. 2013.

30. JEFFERY, I. B. et al. An irritable bowel syndrome subtype defined by species-specific alterations in faecal microbiota. *Gut*, London, v. 61, n. 7, p.997-1006, Dec. 2012.

31. MOUZAKI, M. et al. Intestinal microbiota in patients with nonalcoholic fatty liver disease. *Hepatology*, Baltimore, v. 58, n. 1, p.120-127, 14 May 2013.

32. MARIAT, D., FIRMESSE, O., LEVENEZ, F., et al. "The firmicutes:bacteroidetes ratio of the human microbiota changes with age", *BMC Microbiology*, London, v. 9, p. 1-6, Jun. 2009.

33. BERVOETS, L. et al. Differences in gut microbiota composition between obese and lean children: a cross-sectional study. *Gut pathogens*, London, v. 5, n. 1, p. 10, 2013.

34. KOLIADA, A. et al. "Association between body mass index and Firmicutes:Bacteroidetes ratio in an adult Ukrainian population", *BMC Microbiology*, London, v. 17, n. 1, p. 4-9, May. 2017.

35. MURPHY, E. F. et al. "Composition and energy harvesting capacity of the gut microbiota: relationship to diet, obesity and time in mouse models", *Gut*, London, v. 59, n. 12, p. 1635–1642, Dec. 2010.

36. KIM, K. A. et al. "High Fat Diet-Induced Gut Microbiota Exacerbates Inflammation and Obesity in Mice via the TLR4 Signaling Pathway", *PLoS ONE*, San Francisco, v. 7, n. 10, Oct. 2012.

37. ZHANG, D. et al. "The cross-talk between gut microbiota and lungs in common lung diseases", *Frontiers in microbiology*, [s.l], v. 11, n., p. 1-14, Feb. 2020.

38. MACHIELS, K. et al. A decrease of the butyrate-producing species *Roseburia hominis* and *Faecalibacterium prausnitzii* defines dysbiosis in patients with ulcerative colitis. *Gut*, London, v. 63, n. 8, p.1275-1283, 10 Sept. 2014.

39. JIANG, S. et al. A reduction in the butyrate producing species *Roseburia spp.* and *Faecalibacterium prausnitzii* is associated with chronic kidney disease progression. *Antonie Leeuwenhoek*, Wageningen, v. 109, n. 10, p.1389-1396, 18 July 2016.

40. NEYRINCK, A. M. et al. *Prebiotic Effects of wheat arabinoxylan related to the increase in *bifidobacteria, roseburia and bacteroides* in dietary fibre in colonic fermentation in healthy human subjects. Br J. Nutr.*, London, v. 104, n. 5, p.693-700, 29 Mar. 2010.

41. RABIEJ, N. et al. "Induction effects of Faecalibacterium prausnitzii and its extracellular vesicles on toll-like receptor signaling pathway gene expression and cytokine level in human intestinal epithelial cells", *Cytokine*, San Diego, v. 121, n. May, p. 154718, 2019.

42. SHEN, Z. et al. "Insights into Roseburia intestinalis which alleviates experimental colitis pathology by inducing anti-inflammatory responses", *J. Gastroenterol. Hepatol.*, Australia, v. 33, n. 10, p. 1751-1760, Mar. 2018.

43. KERCKHOFFS, A. P. M. et al. *Lower Bifidobacteria counts in both duodenal mucosa-associated and fecal microbiota in irritable bowel syndrome patients. World J. Gastroenterol.*, Beijing, v. 15, n. 23, p. 2887, 2009.

44. MENNINI, M. et al. *Probiotics in asthma and allergy prevention. Front. Pediatr.*, Lausanne, v. 5, p. 165, 2017.

45. WEISS, B. et al. Probiotic supplementation affects pulmonary exacerbations in patients with cystic fibrosis: a pilot study. *Pediatr. pulmonol.*, Philadelphia, v. 45, n. 6, p. 536-540, 2010.

46. BRUZZESE, E. et al. Disrupted intestinal microbiota and intestinal inflammation in children with cystic fibrosis and its restoration with *lactobacillus GG*: a randomised clinical trial. *PLoS One*, San Francisco, v. 9, n. 2, p.87796-87807, 19 Feb. 2014.

47. JAFARI, S. A. et al. Effects of probiotics on quality of life in children with cystic fibrosis; a randomized controlled trial. *Iranian journal of pediatrics*, Tehran, v. 23, n. 6, p. 669, 2013.

48. MATAMOUROS, S. et al. Adaptation of commensal proliferating *Escherichia coli* to the intestinal tract of young children with cystic fibrosis. *Proc. Natl. Acad. Sci., U.S.A.*, Washington, v. 115, n. 7, p.1605-1610, 29 Jan. 2018.

49. DE FREITAS, M. B. et al. "Altered intestinal microbiota composition, antibiotic therapy and intestinal inflammation in children and adolescents with cystic fibrosis", *PLoS ONE*, San Francisco, v. 13, n. 6, p. 1-14, Jun. 2018.
52. PANEK, M. et al. Methodology challenges in studying human gut microbiota – effects of collection, storage, DNA extraction and next generation sequencing technologies. *Scientific Reports*, London, v. 8, n. 1, p.5143-5156, 23 Mar. 2018.

*Submetido em:* 03/11/2019  
*Aceito em:* 01/04/2020