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**ANÁLISE DA MICROBIOTA FECAL DE CRIANÇAS ALÉRGICAS
E NÃO ALÉRGICAS ÀS PROTEÍNAS DO LEITE DE VACA**

Tese apresentada ao Programa de Pós-graduação em Microbiologia do Instituto de Ciências Biomédicas da Universidade de São Paulo, para obtenção do Título de Doutor em Ciências.

Área de Concentração: Microbiologia

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(*in memoriam*)

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RESUMO

CARDOSO, N. S. **Análise da microbiota fecal de crianças alérgicas e não alérgicas às proteínas do leite de vaca.** 2019. 92 f. Dissertação (Doutorado em Microbiologia) Instituto de Ciências Biomédicas, Universidade de São Paulo, 2019.

A microbiota intestinal está envolvida na regulação de várias funções fisiológicas, incluindo a degradação de substâncias não digeríveis, produção de ácidos graxos de cadeia curta, redução da colonização de patógenos por conta da competição pelos sítios de adesão e permeabilidade intestinal. É composta predominantemente por bactérias anaeróbias sendo os filos *Bacteroidetes* e *Firmicutes* os mais prevalentes. A composição da microbiota intestinal e fecal pode variar em quantidade e especificidade nos sítios anatômicos. Neste estudo foi analisada a composição bacteriana das microbiotas fecais em crianças com e sem alergia às proteínas do leite de vaca. Foi realizado o isolamento bacteriano para os gêneros *Lactobacillus*, *Bifidobacterium*, *Bacteroides/Parabacteroides* e *Clostridium*, análise quantitativa das fezes por PCR em Tempo Real e a filogenia por AP-PCR dos isolados de *Bifidobacterium* e *Lactobacillus* de ambos os grupos estudados. Das 25 fezes de crianças com alergia, 14 (56%) delas apresentaram espécies de *Lactobacillus*, 19 (76%) de *Bifidobacterium*, 15 (60%) de *Clostridium*, 24 (96%) de *Bacteroides* e/ou de *Parabacteroides*. Já no grupo de crianças não alérgicas 20 (80%) foram positivas para *Lactobacillus*, 23 (92%) para *Bifidobacterium*, 9 (36%) para *Clostridium*, 22 (88%) para *Bacteroides* e/ou *Parabacteroides*. Em ambos os grupos na faixa etária de 6 a 10 anos de idade, as amostras fecais apresentaram maior diversidade de espécies, observando-se a presença de *Lactobacillus* spp., *L. rhamnosus*, *L. casei*, *Bifidobacterium* spp., *B. infantis*, *Parabacteroides distasonis*, *Bacteroides fragilis*, *B. vulgatus*, *C. innocuum*, e *C. septicum*. A análise quantitativa dos microrganismos realizada nas fezes mostrou diferenças estatisticamente significativas para *Clostridium perfringens* ($p = 0,037$), *Clostridium* Cluster I ($p = 0,005$), *Escherichia coli* ($p = 0,001$) e *Bacteroidetes* ($p = 0,051$). Os dendrogramas obtidos com as espécies de *Lactobacillus* e *Bifidobacterium* de ambos os grupos de crianças analisados, mostraram uma diversidade genética heterogênea e a presença de clones

bacterianos, pertencentes na maioria das vezes, por bactérias isoladas no mesmo paciente. Na literatura mundial se observam escassos estudos sobre a composição da microbiota intestinal e/ou fecal de crianças com alergia ao leite de vaca. Certamente, este estudo comparativo poderá servir como base para pesquisas mais profundas nesta linha de investigação.

Palavras chave: Microbiota intestinal; Microbiota fecal; Alergia alimentar; Alergia as proteínas do leite de vaca; Crianças alérgicas.

ABSTRACT

CARDOSO, N. S. **Fecal microbiota analysis of children allergic and non-allergic to cow's milk proteins.** 2019. 92 p. Dissertação (Doutorado em Microbiologia) Instituto de Ciências Biomédicas, Universidade de São Paulo, 2019.

The intestinal microbiota is involved in regulating of several physiologic functions, including the degradation of substances not digestible, production of fat acids of short chain, reduction of the colonization of pathogens due to the competition by the sites of adhesion and intestinal permeability. It is composed predominantly of anaerobic bacteria being the most prevalent phylum *Bacteroidetes* and *Firmicutes*. The composition of the intestinal and fecal microbiota may vary in quantity and specificity in anatomical sites. In this study we analyzed the bacterial composition of fecal microbiotas in children with and without allergy to cow's milk proteins. Bacterial isolation was performed for the genera *Lactobacillus*, *Bifidobacterium*, *Bacteroides/Parabacteroides* and *Clostridium*, quantitative analysis of feces by Real-time PCR and phylogeny by AP-PCR of *Bifidobacterium* and *Lactobacillus* isolates from both groups studied. Of the 25 feces of children with allergies, 14 (56%) of them presented *Lactobacillus* species, 19 (76%) of *Bifidobacterium*, 15 (60%) of *Clostridium*, 24 (96 %) of *Bacteroides* and/or *Parabacteroides*. In the group of non-allergic children 20 (80%) were positive for *Lactobacillus*, 23 (92%) for *Bifidobacterium*, 9 (36%) for *Clostridium*, 22 (88%) for *Bacteroides* and/or *Parabacteroides*. In both groups aged 6 to 10 years, fecal samples showed a greater diversity of species, observing the presence of *Lactobacillus* spp., *L. rhamnosus*, *L. casei*, *Bifidobacterium* spp., *B. infantis*, *Parabacteroides distasonis*, *Bacteroides fragilis*, *B. vulgatus*, *C. innocuum*, e *C. septicum*. The quantitative analysis of the microorganisms performed in the feces showed statistically significant differences for *Clostridium perfringens* ($p = 0,037$), *Clostridium* Cluster I ($p = 0,005$), *Escherichia coli* ($p = 0,001$) e *Bacteroidetes* ($p = 0,051$). The dendrograms obtained with the species of *Lactobacillus* and *Bifidobacterium* from both groups of children analyzed, showed heterogeneous genetic diversity and the presence of bacterial clones, belonging most of the time, by isolated bacteria from the same patient. In the worldwide literature, there are scarce

studies on the composition of intestinal and/or fecal microbiota of children with cow's milk allergy. Certainly, this comparative study could serve as a basis for deeper research in this line of research.

Keywords: *Intestinal microbiota, Faecal microbiota, Food Allergy, Allergy to the proteins of cow's milk, Allergic children.*

1. INTRODUÇÃO

A alergia ou hipersensibilidade alimentar constitui-se uma resposta imunológica adversa às proteínas presentes nos alimentos, causando vários sintomas ao paciente (WASERMAN e WATSON, 2011). Existem outras reações adversas aos alimentos que podem ocorrer sem o envolvimento do sistema imune do hospedeiro, tais como: deficiências enzimáticas digestivas (ex.: intolerância à lactose) e presença de toxinas (ex.: intoxicação alimentar por estafilococos), entre outras (FERREIRA et al., 2007).

Embora a alergia alimentar possa ser desencadeada por praticamente a maioria dos alimentos, os mais significativos são: leite de vaca, ovo, amendoim, mariscos, peixes, trigo e soja (RONA et al., 2007; TAYLOR-BLACK e WANG, 2012). Dentre eles, a alergia ao leite de vaca é a que apresenta maior prevalência, por ser o primeiro alimento introduzido na dieta em idade pediátrica, e pelo seu elevado potencial alergênico. As reações alérgicas causadas pelas proteínas presentes no leite de vaca (LV) podem ou não ser mediadas pela imunoglobulina E (IgE), a qual produz sintomas característicos em cada tipo de reação alérgica (HEINE et al., 2002; BRILL et al., 2008; JEONG et al., 2017). Também fatores genéticos, assim como a quantidade e frequência de exposição aos alérgenos alimentares podem influenciar uma resposta alérgica observada nos primeiros anos de vida.

Os indivíduos alérgicos às proteínas do leite de vaca são submetidos a uma dieta de exclusão do leite e derivados, a fim de eliminar ou amenizar os sintomas. A eliminação de alguns componentes do LV na dieta de crianças, como por exemplo, o cálcio e a lactose, podem levar à deficiência de crescimento da criança e à disbiose da microbiota intestinal, aumentando o risco de desenvolver futuramente outras patologias (ISOLAURI et al., 1998; TURNBAUGH et al., 2007).

A microbiota intestinal é o conjunto de microrganismos presentes no trato gastrointestinal de humanos e animais, e o seu desequilíbrio tem sido associado às doenças intestinais, tais como: doenças inflamatórias do intestino, síndrome do intestino irritável, doença celíaca e alergias (GAREAU et al., 2010).

Alguns pesquisadores relacionaram o papel da microbiota intestinal com a indução à tolerância oral envolvida na patogênese da alergia alimentar, devido ao estímulo e desenvolvimento do sistema imunológico; além de também estar envolvida com a resistência às infecções produzidas por diversos patógenos (CONWAY, 2005; O'HARA et al., 2006; ARRIETA et al., 2015). Sabe-se que, no trato intestinal, existe um estado de constante modulação, pois ao mesmo tempo em que o sistema imunológico está pronto para agir contra bactérias patogênicas, também é capaz de se manter tolerante em relação à microbiota residente (WINKLER et al., 2007; AITORO et al., 2017).

Determinados microrganismos vivos, quando ingeridos em certas quantidades podem trazer benefícios à saúde, são os denominados probióticos, como por exemplo, algumas espécies de *Bifidobacterium* e *Lactobacillus*. Estudos demonstraram a capacidade de *Bifidobacterium* spp. utilizar oligossacarídeos fucosilados do leite humano, e favorecer seu crescimento em relação a outras bactérias que fazem parte da microbiota intestinal, tais como *Escherichia coli* e *Clostridium perfringens* (DETHLEFSEN e RELMAN, 2011; MARCOBAI et al., 2011).

Espécies de *Bacteroides* também podem utilizar oligossacarídeos fucosilados como fonte de carbono, o que sugere que a colonização do intestino é auxiliada pelas propriedades do leite, realçando a importância da dieta na determinação da composição da microbiota intestinal humana (MARCOBAI et al., 2011).

Existem evidências de que indivíduos portadores de enfermidades, como doença intestinal inflamatória ou alergias, apresentam microbiota intestinal diferente dos indivíduos saudáveis, com baixa diversidade microbiana. Porém, na maioria dos casos, não é possível afirmar se essa diferença na composição microbiana é característica de algum determinado quadro clínico. Isto sugere maiores estudos para definir as diferenças qualitativas e quantitativas da composição desta microbiota (BISGAARD et al., 2011; AITORO et al., 2017; VAN DEN ELSEN et al., 2019).

Na literatura mundial, são escassos os estudos sobre a composição da microbiota intestinal de crianças com alergia ao leite de vaca. Assim, a identificação da composição dessa microbiota residente poderá fornecer o

suporte microbiológico necessário para melhor conhecer o impacto que a exclusão do leite de vaca pode provocar.

7. CONCLUSÕES

Com os dados obtidos neste estudo podemos concluir que:

1. As crianças com alergia ao leite de vaca apresentaram maior número de espécies do gênero *Clostridium*;
2. Pela análise quantitativa, as crianças não alérgicas apresentaram maior número de bactérias pertencentes aos gêneros: *Bifidobacterium*, e *Clostridium* Cluster I; aos filos: *Firmicutes* e *Bacteroidetes* e a espécie *Escherichia coli*; e
3. As espécies de *Lactobacillus* e *Bifidobacterium* de ambos os grupos de crianças mostraram diversidade genética heterogênea.

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