ORIGINAL ARTICLE

Title: The use of probiotics as supplementary therapy in the treatment of patients with asthma: a pilot study and implications

Running title: Probiotics in asthmatic patients: pilot study

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Abstract

Objectives: Evaluate the use of probiotic as additional therapy in the treatment of children and adolescents with asthma in Belo Horizonte, MG-Brazil. **Methods:** A pilot longitudinal, experimental and non-randomized study with 30 patients from six to 17 years old from Belo Horizonte. In the basal appointment, all patients received beclomethasone and one group received a probiotic containing *Lactobacillus reuteri* (n=14). The patients were reassessed after 60 days at least, being submitted to the Asthma Control Test, spirometry and report the symptoms associated to asthma. **Results:** It was observed predominance of male sex (56.7%) and mean age of 10.6 years. The groups using probiotic did not differ regarding sex, age or atopy. In the longitudinal evaluation, it was observed an increase of the Asthma Control Test in probiotic group, as well as the reduction of the number of symptoms. There was an increase in the peak expiratory flow among those who used or not probiotic. **Conclusions:** This pilot study raise the hypothesis that the administration of probiotic as a supplementary therapy for the treatment of children and adolescents with asthma seems to have improved the patients' clinical condition. Further studies are needed to confirm the efficacy of probiotics in the asthma treatment.

Key words: Respiratory Hypersensitivity, Asthma, Public health, Respiratory Function Tests, Probiotics.

Introduction

Asthma is an airway inflammation that leads to a variable obstruction of intrapulmonary airflow, leading to recurrent episodes of breathlessness, wheeze, chest tightness and coughing (1,2). Asthma is the most common chronic disease in childhood (3), and it is estimated that up to 14% of children and 8.6% of adults worldwide have symptoms of this pathology (4). There is low mortality associated with asthma (3); however, it has a considerable financial and social cost, as well as being a prevalent cause of disability (5).

According to the guidelines of the Global Initiative for Asthma (2), the primary goal of asthma treatment is to obtain an optimal control of the disease, with minimum or absence of day and night symptoms, no limitation to physical activity, minimum need for medication for the relief of symptoms, normal pulmonary function or with a value close to normal, and the absence of exacerbations. All of this must be obtained with the use the lowest possible extent of medication, especially corticosteroids, according to a phased plan that takes into consideration the control of the disease, its severity and future risks (1,6). The use of probiotics arises as a possibility of additional therapy for treatment.

Probiotics are defined as "live microorganisms that when administered in adequate amounts, confer benefits to the host's health" (7,8). The use of these organisms could provide benefits to patient's immune system, leading to a better control of the disease with reduction of symptoms and an improvement in lung function of these patients. Besides, others action mechanisms of the probiotics includes the increase of epithelial barrier, adhesion to intestinal mucosa, inhibition of pathogen adhesion, exclusion of pathogenic microorganisms by competition and anti-microorganism substances production (7-9).

Several studies have investigated possible benefits of probiotics for prevention and/or treatment of asthma. A pilot study investigated the effects of probiotics in asthmatic children, conducted by Stockert et al. (10), found an improvement in lung function (peak of expiratory flow [PEF]), but no impact on patients' quality of life and use of asthma medications. Furthermore, Chen et al. (11) observed improvement in symptoms, lung function and immunological parameters in children who received probiotics. Liu et al. (12) suggests probiotic could enhance therapeutic effect of allergen specific immunotherapy in asthmatics. In studies performed in rats with allergic inflammation of the airways receiving injections of *Lactobacillus reuteri*,

Forsythe et al. (13) and Karimi et al. (14) observed a mitigation of inflammation and airway hyperresponsiveness in the group of animals that received the probiotic. However, there are some studies in which there was no evidence of beneficial effects of probiotics on asthmatics or allergics (15-20). These include the study by Giovannini et al. (15) in Italy with children suffering from allergic asthma and/or rhinitis from two to five years. The authors found that the administration of probiotics did not promote improvements among patients with asthma in relation to time free from symptoms or the number of episodes of the disease.

Rose et al. (16) examining the impact of *Lactobacillus rhamnosus* GG ATCC 53103 in children aged 6 to 24 months with wheezing and family history of atopic diseases (in first-degree relatives) for six months also found no association between inhalation need and number of days free from symptoms among children who received or did not receive the probiotic.

Given the current controversy about the use of probiotic in children with asthma, the aim of this pilot study was to evaluate the effect of the including probiotic as a supplementary therapy to inhaled corticosteroids (ICS), dipropionate beclomethasone, in the treatment of children and adolescents with asthma in a Secondary Referral Unit (SRU) of Belo Horizonte, MG-Brazil. In particular, it investigated the effect of *Lactobacillus reuteri* since there are no previous Brazilian studies investigating its possible use.

Methods

Study design

This is a longitudinal, quasi-experimental pilot study with control group involving asthmatic children and adolescents aged between 6 and 17 years old treated in a SRU in Belo Horizonte between January 2015 and December 2015.

Study participants

Children and adolescents who were newly diagnosed with mild to moderate asthma (2) not previously prescribed an ICS or short-acting beta$_2$-agonist (SABA) were included. Patients suffering from other respiratory disorders such as bronchiolitis obliterans, interstitial pneumonia, sickle-cell disease, cystic fibrosis, sequels due to complicated pneumonia, tuberculosis or primary ciliary dyskinesia

were excluded. In addition, smokers, patients with cognitive impairment in the first attempt after initiation of the study were precluded.

During the baseline appointment, all children received the medicine and the prescription of conventional therapy beclomethasone (Clenil® 250 µg/shot - Laboratory Chiesi, Brazil), at a dose of 250 µg, twice daily hours (500µg/day) and had the term of consent signed. The Aerolin® (sulphate of salbutamol 120.5 mcg, norfluranoq.S.p. Laboratory Gsk, Brazil), at a dose of 100 µg, was prescribed in case of exacerbations. One group received the probiotic ProVance® (Laboratory Aché, Brazil) composed of 10^8 colony-forming units of *Lactobacillus reuteri* DSM 17938, at a dose of one capsule/day (8) and the other received a placebo (or no additional treatment for asthma). The allocation was performed in accordance with the final digit of the patient record, and the odd number ones received the probiotic.

Study outcomes

To evaluate the probiotics effects, the patients were reassessed in a minimum period of 60 days after the initiation of treatment. The outcomes of interest were the assessment of asthma control, pulmonary function and the frequency of main asthma symptoms.

At the two appointments, patients completed the asthma control test (ACT) (21), a spirometry with bronchodilator test and an assessment of the following symptoms: cough, wheezing, tiredness, chest pains, night-time symptoms (all symptoms cited before at night), limitations in physical activities and any school absenteeism. A skin allergy test was performed only in the first baseline appointment to identify atopic patients. A list of potential advent effects of probiotics were given to patient's and their caregivers, in order to be observed their occurrence during the treatment.

ACT

Nathan et al. (22) developed the Asthma Control Test to monitor responsiveness to clinical changes. It had been validated with internal consistency. The questionnaire has been validated for use in Brazil (21) in order to measure the ability to discriminate controlled from uncontrolled asthma, as well as the reproducibility and responsiveness of the questionnaire among Brazilian patients. ACT has five questions and assesses the frequency of shortness of breath and general asthma symptoms, use of rescue medications, the effect of asthma on daily

functioning, and overall self-assessment of asthma control in the last four weeks. Scores range from 5 (poor control of asthma) to 25 (complete control of asthma) and high scores reflect greater asthma control. Asthma is classified as controlled if the score is greater than 19.

Spirometry

The spirometry test was performed using the VMI ATS (Clement Clarke, EC0120, United Kingdom) in standing patients, positioning the mouthpiece of a disposable spirometer in the mouth and blocking the nostrils with a nose clip. The test was administered by an accredited technician. The device was calibrated daily prior of testing. The patients underwent a maximum inspiration followed by a maximum forced exhalation. This procedure was repeated three times before and 15-20 minutes after inhalation of a fast acting bronchodilator spray - Aerolin® (sulphate of salbutamol 120.5 mcg, norfluranoq.S.p. Laboratory Gsk, Brazil). Measurements were performed of forced vital capacity (FVC), forced expiratory volume in one second (FEV_1), PEF and the Tiffeneau index (FEV_1/FVC). The predicted values were obtained from the equations of Koopman et al. (23).

Evaluation of adverse effects

The caregivers were questioned about the occurrence of the following symptoms that could indicate any adverse reactions of probiotics. These included diarrhea, vomiting, nausea, abdominal discomfort, burning sensation in the stomach and/ or a bad taste in their mouth not related to the patients' food habits.

Data analysis

This is a pilot study designed for an exploratory analysis and as a proof of concept study about the inclusion of probiotics as supplementary treatment of asthmatics. There was no calculation of sample size. The findings would be used to calculate the sample size for a larger study.

The categorical variables were presented as frequencies, and the numerical as mean ± standard deviation (sd), in the case of normality (assessed via the Shapiro-Wilk test), or median ± inter-quartile range (IQR), in the case of non-normal distribution. To evaluate the association among categorical variables the Fisher exact test was used for independent samples and McNemar chi-square test for longitudinal analysis. For the comparison of numerical variables between two groups, Student-t

and Wilcoxon tests have been adopted in their versions for independent samples and paired ones. The analyzes were performed using the free program R version 3.3.2. The interpretation of p-values was only descriptive, at 5% of significance level.

Ethical approval

This study was approved by the Research Ethics Committee of the University Hospital São José/University Medical Sciences - MG under the number CAAE 36416714.4.0000.5134.

Results

During the study, 37 patients met the inclusion criteria, four of them were excluded due to being present at only one appointment (one probiotic group) and three for having been at the second appointment with less than 60 days in between (one in the probiotic group). The sample was composed of 30 children and adolescents, 14 received the probiotic. There was a predominance of males (56.7%), the mean age was 10.6 ± 2.5 years and the mean body mass index (BMI) was 18.8 ± 3 kg/m^2. The median time between the baseline and the second appointment was 63 ± 7 days, 63.3% of the patients had already been hospitalized, 80% had already sought emergency care and 70% were classified as atopic subjects in allergy tests at baseline. Among the patients using probiotics, 50% were boys, the mean age was 11 ± 2.5 years, the mean BMI 19.9 ± 3.4 kg/m^2, 42.9% had already been hospitalized, 64.3% had already been in emergency care, 71.4% were atopic. There were no significant differences in patient characteristics between the control and intervention group (Table 1).

In the longitudinal evaluation, there was a significant increase in the ACT score among patients who used probiotics, (difference mean ± sd 3.85 ± 6.7, p=0.049) (Figure 1) and a significant reduction in the number of symptoms (difference mean ± sd -2.64 ± 3.48, p=0.023). In addition, there was a reduction in the number of patients who reported wheezing (78.6% to 21.4%, p=0.046). There were no significative differences in control group (Table 2).

Regarding the parameters measured by pre-bronchodilator spirometry, it was an increase of PEF among patients who did not (difference mean ± sd 12.47 ±

12.33%, p=0.005) and who consumed the probiotic (difference mean ± sd 17.89 ± 17.54, p=0.005) (Table 3).

The occurrences of the signs of possible adverse effects did not differ between patients who received or not the probiotic.

Discussion

The groups were similar and comparable. There was no significant difference between the groups of patients who used or not the *Lactobacillus reuteri.* In the group who received the supplement, an increase in ACT score and a decrease in the number of symptoms, in particular the occurrence of wheezing, was seen with no significant changes in the control group. The spirometry results were common to the two groups (longitudinal increase of pre- bronchodilator PFE), which implies that probiotics had no impact on patients' spirometry tests. The possible adverse effects evaluated were not associated to the use of the supplement.

The longitudinal increase of PEF pre-bronchodilator indicates a possible improvement in relation to the obstruction of the lower airways, by decreasing the bronchial hyperresponsiveness in all patients. These results may have been influenced by the insertion of the standard treatment (beclomethasone in patients who were not previous users of both ICS and SABA), by the guidelines about the allergic markers and also by climatic changes between the evaluations. The increase in PEF in the sample, for example, was expected with the insertion of the regular treatment by ICS for a period of two months (2). Another possible explanation would be a better performance of patients in the second test due to training they had when they performed it previously. This is notable in PEF, as this is a functional parameter effort-dependent (24). FEV_1 is the most important parameter of follow-up of patients with asthma, since its fall increases the risk of exacerbations, regardless of the presentation of the symptoms (25).

The findings of this pilot study suggest that the use of probiotics may have promoted an attenuation of clinical symptoms, since the ACT evaluates in four of five questions aspects related to the symptoms. However, this needs to be evaluated further before any definitive statements can be made due to the small sample size. These findings are similar to those of Chen et al. (11) who showed a decrease in asthma/allergic rhinitis symptoms, and improvements in FEV_1, FVC and FEV_1/FVC measured in the spirometry and PEF measured daily for patients who received

probiotic. The authors also reported significant reduction of immunological parameters measured in peripheral blood mononuclear cells (TNF-α, IFN-γ, IL-12 and IL-13). However, we did not see an improvement in lung function in the probiotic group in our study. This difference could be due to variations in the method used to measure lung function.

In addition, Stockert et al. (10) found a reduction of the variation of PEF in patients who used a probiotic, however, not differences were identified in the assessment of FEV_1, quality of life and use of extra medications. The findings from Stockert et al. (10) resembles the present study with respect to improvements in lung function, evaluated, in this case by daily variation of PEF measured with a portable measuring instrument. The differences observed in the results between the two studies could potentially be explained by the use of different strains of probiotics promoting different benefits to the host (8).

Prescott and Bjorksten (26) reported that the effects of probiotics on allergic diseases can be influenced by factors such as genetic differences in microbial response, microbial composition, individual microbiota, diet, allergic predisposition and use of antibiotics which could also account for the different results seen. However, in our study we did not find association between the probiotics effect and allergic profile.

As limitations, we have the quasi-experimental characteristics (non-randomization and not blinding of participants and researchers). The use of Koopman's equations for the predicted values of spirometry parameters has been derived from a sample that includes the children and adolescents' age range. There are no national equations recently published derived from a sample of Brazilian children and adolescents up to the time of initiation of this study. The ACT was developed for adolescents of at least 12 years old. The application of the ACT to sample of patients younger than 12 years old in this study is mitigated due to the fact that children below 12 years were helped by their caregivers in completing the questionnaire.

The results of this pilot study suggests a trend towards improvement of the clinical profile of asthmatic patients who had the *Lactobacillus reuteri* as a supplementary therapy.

This is one of the first investigations on the efficacy of probiotics carried out with Brazilian children and adolescents suffering from asthma. However, given conflicting results in the literature, the known variability on effects of probiotics for

each strain, and currently little knowledge about the mechanism of the potential benefits of probiotics in patients with asthma, we believe more studies are necessary, especially randomized controlled clinical trials and prospective cohort studies, to gather more evidence and knowledge about the possible potential benefits of probiotics to asthmatic patients before their routine use in children can be advocated.

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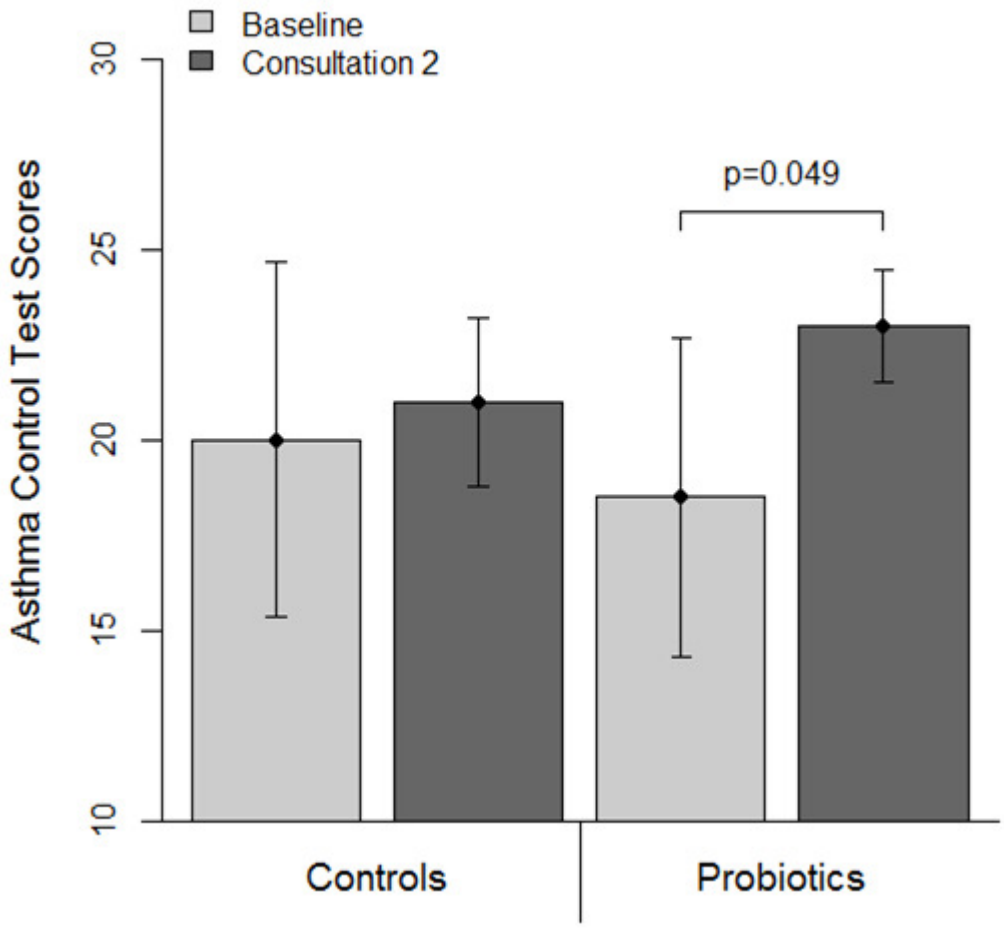
Legend of Figure

Figure 1. Asthma Control Test Scores for probiotic use



Tables

Table 1. Clinical and sociodemographic characteristics of the asthmatic children and adolescents at the baseline. Belo Horizonte, 2015.

Variables	Controls *(n=16)*	Probiotics *(n=14)*	P-value
Male gender	10 (62.5%)	7 (50%)	0.713[F]
Follow-up time (days)	63 ± 14	63.5 ± 7	0.836[W]
Age (years)	10.2 ± 2.5	11 ± 2.5	0.383[T]
Body mass index (kg/m^2)	17.8 ± 2.2	19.9 ± 3.4	0.059[T]

Hospitalization	13 (81.3%)	6 (42.9%)	0.057[F]
Emergency care	15 (93.8%)	9 (64.3%)	0.072[F]
Atopic allergy test	11 (68.8%)	10 (71.4%)	1.000[F]

P-values refers to: [F] Fisher exact, [T] Student-t and [W] Wilcoxon Mann-Whitney for independent samples.

Table 2. Symptoms history of the asthmatic children and adolescents according to the use of probiotic. Belo Horizonte, 2015.

Variables	Measures	Controls *(n=16)*	Probiotics *(n=14)*	P-value
Number of symptoms	Baseline	2 ± 5.5	4 ± 3	0.054[W]
	2nd consultation	1 ± 2.5	1 ± 3	0.669[W]
	P-value	0.251[Wp]	0.023[Wp]	
Cough	Baseline	7 (43.8%)	13 (92.9%)	0.007[F]
	2nd consultation	6 (37.5%)	7 (50%)	0.713[F]
	P-value	1.000[Mc]	-	
Wheezing	Baseline	5 (31.2%)	11 (78.6%)	0.014[F]
	2nd consultation	1 (6.2%)	3 (21.4%)	0.316[F]
	P-value	0.371[Mc]	0.046[Mc]	

Tiredness	Baseline	5 (31.2%)	10 (71.4%)	0.066[F]
	2nd consultation	5 (31.2%)	4 (28.6%)	1.000[F]
	P-value	1.000[Mc]	0.077[Mc]	
Chest pain	Baseline	4 (25%)	5 (35.7%)	0.694[F]
	2nd consultation	-	2 (14.3%)	0.209[F]
	P-value	-	0.371[Mc]	
Nighttime symptoms	Baseline	10 (62.5%)	10 (71.4%)	0.709[F]
	2nd consultation	4 (25%)	5 (35.7%)	0.694[F]
	P-value	0.131[Mc]	0.131[Mc]	
Limitation of physical activities	Baseline	4 (25%)	5 (35.7%)	0.694[F]
	2nd consultation	2 (12.5%)	4 (28.6%)	0.378[F]
	P-value	1.000[Mc]	1.000[Mc]	
Absent from school	Baseline	7 (43.8%)	7 (50%)	1.000[F]
	2nd consultation	4 (25%)	3 (21.4%)	1.000 [F]
	P-value	0.617[Mc]	0.221[Mc]	

P-values refers to: [F] Fisher exact, [Mc] McNemar Chi-Square test, [Wp] Wilcoxon for paired samples and [W] Wilcoxon Mann-Whitney for independent samples

Table 3. Pre-salbutamol spirometry parameters, as percentage of predicted values, of the asthmatic children and adolescents according to the use of probiotic. Belo Horizonte, 2015

Variables		**Controls** *(n=16)*	**Probiotics** *(n=14)*	**P-value**
FEV_1(L)				
Baseline		77.6 ± 16.9	79.4 ± 14	0.667[W]
2nd consultation		81.5 ± 8.4	85.5 ± 8.1	0.242[W]
	P-value	0.301[Wp]	0.147[Wp]	

FVC (L)				
Baseline		54.1 ± 15.3	61.5 ± 26.8	0.381[T]
2nd consultation		57.4 ± 20.5	68.1 ± 25.7	0.254[T]
	P-value	0.947[Tp]	0.396[Tp]	
***FEV$_1$/FVC* (%)**				
Baseline		104 ± 15.1	97 ± 21.6	0.910[W]
2nd consultation		106.8 ± 10.3	106.9 ± 14.1	0.920[W]
	P-value	0.476[Wp]	0.306[Wp]	
PEF (L/min)				
Baseline		75.5 ± 19.5	74.9 ± 17.3	0.928[T]
2nd consultation		85.7 ± 20.2	86.8 ± 16.5	0.871[T]
	P-value	0.005[Tp]	0.005[Tp]	

P-values refers to the tests: [F] Fisher exact, [T] Student-t e [W] Wilcoxon Mann-Whitney for independent samples,[Tp] Student-t e [Wp]Wilcoxon for paired samples
FEV_1 = forced expiratory volume in one second; FVC = forced vital capacity, PEF = peak expiratory flow