The Use of *Lactobacillus reuteri* as an Adjunct in the Treatment of Children with Newly Diagnosed Asthma in a Tertiary Hospital in the Philippines

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ABSTRACT

Rationale: Probiotics are live microorganisms that exert beneficial effects on the host, including a reduction of allergic disease symptoms. *Lactobacillus reuteri* in particular was shown to attenuate the allergic airway, and when used as an adjunct in the treatment of asthma in children, resulted in decreased fractional exhaled nitric oxide and interleukin levels when compared to placebo. However, insufficient information is available regarding the significance of *L. reuteri* as an adjunct in the treatment of allergic disorders, particularly in allergic airway disease.

Objectives: The objective of the study was to determine the efficacy of *L. reuteri* as an adjunct for the control of newly diagnosed asthma in children.

Methodology and Population: In this analytical, experimental, prospective, randomized controlled trial, 44 asthmatic patients aged 6–18 years were recruited from the University of Santo Tomas Hospital outpatient department, for a study period of three months. Baseline clinical assessment included skin prick test to aeroallergens, spirometry, and Childhood Asthma Control Test. Asthma severity and level of control was based on the National Asthma Education and Prevention Program's Expert Panel 3 (EPR-3) and the Global Initiative for Asthma Guidelines 2018, respectively. Patients were randomized and half of them received *L. reuteri* 26.5 mg/chewable tab (at least 100 million colony forming units) once daily for 30 days, while the other half did not. Spirometry and C-ACT test were conducted at the start of intervention as baseline, after one month, two months, and three months post intervention. The results of patients from the experimental group were compared to results of patients in the control group.

Results: The FEV_1 of patients in the *L. reuteri* group was significantly higher than those in the control group ($p = 0.045$). The median FVC of the two groups significantly differed from each other through time ($p = 0.007$), with the *L. reuteri* group having significantly higher FVC than the control group. There were statistically significant improvements in ACT scores between patients in the *L. reuteri* and control groups, particularly at two months and three months of treatment. Among polysensitized patients, there were significant improvements in the FEV_1, $FEF_{25–75}$, and FVC between the *L. reuteri* and control group.

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Academic editor: Raymond L. Rosales

Submitted date: December 2, 2019

Accepted date: March 30, 2023

Conclusion: The use of *L. reuteri* as an adjunct was associated with significant lung function improvement and asthma symptom control amongst newly diagnosed asthmatic children.

Key Words Lactobacillus reuteri, Probiotics, Asthma, Children

INTRODUCTION

Asthma is a heterogeneous disease, usually characterized by chronic airway inflammation resulting in episodic airflow obstruction.[1,2] Worldwide, childhood asthma appears to be increasing in prevalence, despite considerable improvements in our management and pharmaceutical options to treat asthma. One of the explanations for increased prevalence of these allergic type of diseases lies in the "hygiene hypothesis".[3] This hypothesis involves immunologic mechanisms in which the dysregulation of cellular immunity leads to allergic conditions owing to an imbalance of T-helper (TH) cell type 1 to TH2. A relative lack of exposure to microorganisms during infancy or early childhood can lead to a predominant TH2 response.[4] Because of modern public health practices, the hygiene hypothesis postulates that individuals living in the industrialized world develop a relative deficiency in immune stimulation by microbes, rendering them vulnerable to the development of allergic hypersensitivities and associated diseases.[5]

Probiotics are defined as live microorganisms, that when administered in adequate amounts, can confer a health benefit to the host.[6,7] Probiotics could exert a beneficial effect on prevention as well as treatment of allergic diseases through modification of the immune system of the host via the gut ecosystem.[3] The mechanisms of action of probiotics can be quite vast and divergent. They modulate the permeability of epithelial barriers, alter the inflammatory potential of epithelial cells, compete with pathogens for mucosal colonization, or directly modify the activity of immune cells.[8] Clinical trials have also suggested that exposure to microbes through the gastrointestinal tract powerfully shapes the immune function. Consumption of probiotics helps stimulate intestinal microbiota and suppress the TH2 response, leading to improvements in the balance between TH1 and TH2.[4] Systemic effects include enhancing monocyte and immunoglobulin A (IgA) activity in enterocytes and other tissues, such as that of the respiratory tract.[5] This immunologic switch to a T-helper 1 from a T-helper 2 type of T-cell milieu can be protective to the atopic individual, by lessening the atopic interleukins secreted by these type of cells, and can function to decrease hypersensitive reactions, such as bronchial asthma.

Once the immune system is stimulated by gut bacteria after birth, it begins to generate more T cells (including regulatory T cells) and dendritic cells, and thus is able to mount stronger antibody responses. The presence of this stimulation skews the immune system away from a more TH2 dominated response towards a more balanced TH1 immune profile.[8] The compounding mechanism of these effects ultimately results in achieving tolerance.[5]

Lactobacilli, one of the most widely used probiotics, are considered to induce reactions involving TH1 cells and improve the outcome of allergic diseases.[9,10] *Lactobacillus reuteri (L. reuteri)*, in particular, has been shown to provide multiple benefits such as prevention as well as improvement of numerous disorders.[6] Initially isolated in 1962, it is a Gram-positive, non-sporulating, facultative anaerobic bacteria that normally colonizes the gastrointestinal tract of humans. It has been known to exhibit numerous beneficial properties such as inhibition of pathogenic microorganisms,[11] secretion of antimicrobial intermediaries and even modulating host immune responses by reducing the production of proinflammatory cytokines while promoting regulatory T cell development and function.[6] In a study by Forsythe, the use of *L. reuteri* in murine models resulted in a significant attenuation of the allergic airway, as well as reduction of interleukins 5 and 13 (IL-5, IL-13) in the bronchoalveolar lavage fluid.[12] In a study by Miraglia del Giudice, *L. reuteri* plus Vitamin D3, when used as an adjunct for the treatment of asthma in children resulted in decreased fractional exhaled nitric oxide and interleukin levels when compared to placebo.[13,14] *L. reuteri* was able to significantly reduce bronchial inflammation and increase interleukin 10 (IL-10) in these asthmatic children.[13] However, in a study by Abrahamsson, the effect of *L. reuteri* on sensitization and immunoglobulin E (IgE)–associated eczema in infancy did not lead to lower prevalence of respiratory allergic disease in school age children.

[15] In a meta-analysis of randomized controlled trials by Ceon Kang, prenatal and postnatal Lactobacillus supplementation did not prevent atopic disease.[16] Since there have been only a few clinical trials, insufficient information is available regarding the significance of *L. reuteri* as an adjunct in the treatment of allergic disorders, specifically in allergic airway diseases.[3]

Objectives

The objective of the study was to determine the efficacy of *L. reuteri* as an adjunct for the control of newly diagnosed asthmatic children.

The study also aimed to compare spirometry parameters between newly diagnosed asthmatic children given *L. reuteri* versus those not receiving the adjunct. Asthma Control Test (ACT) or Childhood Asthma Control Test (C-ACT) results were to be compared between newly diagnosed asthmatic children given *L. reuteri* versus those not receiving the adjunct. The study additionally aimed to compare spirometry parameters of aeroallergen monosensitized and polysensitized asthmatic children given *L. reuteri* versus those not receiving the adjunct.

METHODOLOGY

Study Design

This study was an analytical, experimental, prospective, randomized controlled trial approved by the Research Ethics Committee (REC) of the University of Santo Tomas Hospital (USTH) conducted in the Children's Asthma Unit of the Department of Pediatrics at the USTH between September 2019 and December 2019.

Patients

Forty-four patients aged 6–18 years old with newly diagnosed bronchial asthma were included in the study. Patients were excluded if they were currently or previously on aeroallergen immunotherapy, current therapy with inhaled corticosteroids in the previous eight weeks, and suffering from other respiratory, cardiovascular or systemic diseases.

Bronchial asthma was classified as intermittent, mild persistent, moderate persistent or severe persistent based on the National Asthma Education and Prevention Program: EPR 3: Guidelines for the diagnosis and management of asthma, summary report 2007 (Appendix A). Participants were also classified as: well controlled, partly controlled or uncontrolled, based on the Global Initiative for Asthma (GINA) 2018 guidelines (Appendix B). Age-appropriate informed consent from the parents and verbal assent of the child were both obtained and documented.

All patients underwent skin prick test (SPT) to aeroallergens (Appendix C), spirometry (Appendix D), and the ACT for children 12 years old and above, or the C-ACT for children aged 4–11 years (Appendix E, F) upon enrollment into the study. Patients were then randomized into two groups: Group A (experimental group) was given *L. reuteri* DSM 17938 (one chewable tablet containing at least 100 million colony forming units or 26.5 mg), to be taken once daily in the morning, for 30 days, and Group B (control group) who received no additional intervention. The other treatment protocols were based on the stepwise approach of the GINA 2018 guidelines. The participants followed up, and were assessed monthly using spirometry and the ACT or C-ACT from the time of enrollment and as follows: one month, two months and three months from the start of intervention.

Safety and Ethical Considerations

Safety monitoring included adverse events. Safety assessments continued for 60 days after the active treatment period. This study was conducted in compliance with ethical principles of the Declaration of Helsinki 2015 on Ethical Principles for Medical Research Involving Human Subjects, adopted by the General Assembly of the World Medical Association. This study was also in compliance with the Philippine National Ethical Guidelines for Health and Health-Related Research of 2017, in research involving minors and children. The primary investigator and co-author are certified with Good Clinical Practice (GCP). An approval from the Review Ethics Committee (REC) of the University of Santo Tomas Hospital was obtained prior to starting the study.

Statistical Analysis

Univariate analysis

Descriptive statistics were used to summarize the general and clinical characteristics of participants. Frequency and proportion was used for nominal variables, median and range for ordinal variables, and mean and standard deviation for interval/ratio variables.

Bivariate analysis

Independent T test, Mann-Whitney U test and Fisher's Exact/Chi-square test were used to determine the difference of mean, median and frequency between *L. reuteri* and placebo groups, respectively.

Repeated measures

Repeated measures of analysis of variance (ANOVA) were used to determine the difference between *L. reuteri* and placebo groups over time.

All valid data was included in the analysis. Missing variables were neither replaced nor estimated. Null hypothesis was rejected at 0.05α-level of significance. STATA 15.0 was used for data analysis.

Framework Design

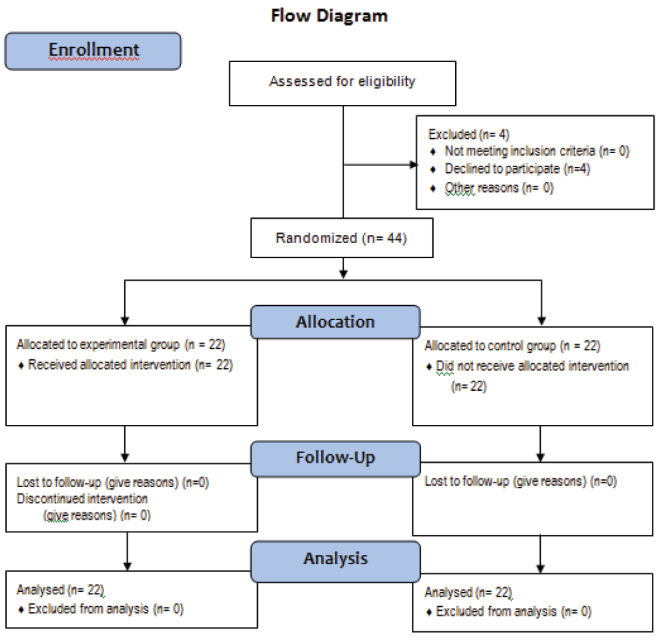

Figure 1 Flowchart

RESULTS

A total of 44 participants were included in the study. Most participants (75%) were between 6 to 11 years old. There were 28 male participants (64%) and 16 female participants (36%). There were 22 patients given *L. reuteri*, and 22 patients not given *L. reuteri* (Table 1).

The clinical profile showed that 55% of these patients had mild persistent asthma, 61% were partially controlled, 43% had concomitant mild persistent allergic rhinitis, and 50% had a family history of atopy. The participants were mostly exposed to pets (59%), cigarette smoke (52%), and cockroach (45%), with 55% considered polysensitized. In terms of asthma, allergic rhinitis, atopy profile, environmental exposures, and aeroallergens, there was insufficient evidence to state a difference between the two patient groups (Table 2).

The FEV_1 performance of patients in the *L. reuteri* group was consistently higher than those in the control group. Significant differences in FEV_1 were observed between the two groups across the observation period (p = 0.045) (Table 3). The $FEF_{25–75}$ of the two groups did not significantly differ through time (p = 0.489). The median FVC of the two groups significantly differed from each other through time (p = 0.007), with the *L. reuteri* group having consistently higher FVC than the control group. The FEV_1 to FVC ratios of the two groups did not differ significantly throughout the observation period (p = 0.795). Peak expiratory flow rate (PEFR) increased steadily over time for both groups, but there was no sufficient evidence to prove that they significantly differed (p = 0.399). No significant differences were found between *L. reuteri* and the control groups for reversibility measurements involved.

There were statistically significant differences in ACT scores between patients in the *L. reuteri* and control groups, particularly at 2 months and 3 months of treatment (p = 0.027) (Table 4). No statistically significant differences in C-ACT scores were observed between patients in the *L. reuteri* and control groups (p = 0.365).

There were no significant differences in the spirometry between monosensitized patients in the *L. reuteri* and control groups across time (Table 5).

Table 1 Demographic profile of 44 participants grouped into those given *L. reuteri* and those who were not given *L. reuteri*. Data reported as n (%) or mean ± SD.

	Total (n=44)	**With *L. reuteri* (n=22)**	**Without *L. reuteri* (n=22)**	**p**
	Frequency (%); Mean ± SD			
Age, years				0.728*
6-11	33 (75.00)	17 (77.27)	16 (72.73)	
12-18	11 (25.00)	5 (22.73)	6 (27.27)	
Sex				0.531*
Male	28 (63.64)	15 (68.18)	13 (59.09)	
Female	16 (36.36)	7 (31.82)	9 (40.91)	
Weight, kg	25.5 (16-65)	29.5 (16-65)	19 (16-61)	0.132†
Height, cm	122 (100-172)	125 (100-153)	112.5 (102-172)	0.136†
BMI				0.604‡
<18.5	31 (70.45)	14 (63.64)	17 (77.27)	
18.5-24.9	10 (22.73)	6 (27.27)	4 (18.18)	
25-29.9	3 (6.82)	2 (9.09)	1 (4.55)	
Residential Areas				
Urban	44 (100)	22 (100)	22 (100)	
Rural	0	0	0	

Statistical tests used: * Chi-square test; † Mann-Whitney U test; ‡ Fisher's Exact test

Table 2 Clinical profile of 44 participants grouped into those given *L. reuteri* and those who were not given *L. reuteri*. Data reported as n (%) or mean ± SD.

	Total (n=44)	**With *L. reuteri* (n=22)**	**Without *L. reuteri* (n=22)**	**p**
	Frequency (%); Mean ± SD			
Asthma classification				0.334‡
Intermittent	4 (9.09)	2 (9.09)	2 (9.09)	
Mild persistent	24 (54.55)	15 (68.18)	9 (40.91)	
Moderate persistent	12 (27.27)	4 (18.18)	8 (36.36)	
Severe persistent	4 (9.09)	1 (4.55)	3 (13.64)	
Asthma control				0.577‡
Well-controlled	6 (13.64)	3 (13.64)	3 (13.64)	
Partially-controlled	27 (61.36)	12 (54.55)	15 (68.18)	
Uncontrolled	11 (25.00)	7 (31.82)	4 (18.18)	
Allergic Rhinitis				1.000‡
Mild intermittent	15 (34.09)	8 (36.36)	7 (31.82)	
Moderate intermittent	1 (2.27)	0	1 (4.55)	
Mild persistent	19 (43.18)	10 (45.45)	9 (40.91)	
Moderate/severe persistent	9 (20.45)	4 (18.18)	5 (22.73)	
Family History of Atopic Disease	22 (50.00)	12 (54.55)	17 (77.27)	0.546*
Allergic rhinitis	24 (54.55)	10 (45.45)	14 (63.64)	
Atopic dermatitis	1 (2.27)	0	1 (4.55)	
Allergic asthma	22 (50.00)	12 (54.55)	10 (45.45)	

(Continued)

Table 2 Clinical profile of 44 participants grouped into those given *L. reuteri* and those who were not given *L. reuteri*. Data reported as n (%) or mean ± SD.(Continued)

	Total (n=44)	With *L. reuteri* (n=22)	Without *L. reuteri* (n=22)	p
	Frequency (%); Mean ± SD			
Environmental exposures				
Flooding	6 (13.64)	4 (18.18)	2 (9.09)	0.664‡
Cigarette smoking	23 (52.27)	14 (63.64)	9 (40.91)	0.131*
Cockroach	20 (45.45)	13 (59.09)	7 (31.82)	0.069*
Pets	26 (59.09)	14 (63.64)	12 (54.55)	0.540*
Automotive smoke	17 (38.64)	8 (36.36)	9 (40.91)	0.757*
Aeroallergens				0.545*
Monosensitized	20 (45.45)	9 (40.91)	11 (50.00)	
Polysensitized	24 (54.55)	13 (59.09)	11 (50.00)	
Aeroallergen Sensitivity (wheals ≥ 3 mm)				
D. farina	44 (100)	22 (100)	22 (100)	-
D. pteronyssinus	44 (100)	22 (100)	22 (100)	-
Cat pelt	5 (11.36)	2 (9.09)	3 (13.64)	1.000‡
Horse hair	1 (2.27)	0	1 (4.55)	1.000‡
Cockroach	15 (34.09)	7 (3.18)	8 (36.36)	0.750*
Mosquito	21 (47.73)	11 (50.00)	10 (45.45)	0.763*
Vital Signs				
Systolic blood pressure	100 (90 – 120)	100 (90 – 100)	90 (90 – 120)	0.198†
Diastolic blood pressure	60 (60 – 70)	70 (60 – 70)	60 (60 – 70)	<0.001†
Heart rate	90.34 ± 8.66	89.45 ± 6.76	91.23 ± 10.31	0.504§
Respiration rate	25.39 ± 3.18	25.09 ± 3.29	25.68 ± 3.11	0.544§
Temperature	36.67 ± 0.16	36.66 ± 0.13	36.70 ± 0.18	0.444§
O2 saturation, %	98.55 ± 0.01	98.36 ± 0.01	98.73 ± 0.01	0.209§
Skin (dry)	6	2	4	0.664‡
Nose (congested)	19	10	9	0.128*
Lungs				
Wheezes	11 (25.00)	6 (27.27)	5 (22.73)	0.472*
Rhonchi	10 (22.73)	5 (22.73)	5 (22.73)	0.498*

Statistical tests used: * Chi-square test; † Mann-Whitney U test; ‡ Fisher's Exact test; § Independent T test

Table 3 Effects of treatment on FEV_1, FEF_{25-75}, FVC, FEV_1/FVC, PEFR, and reversibility measurements on FEV_1, FEF_{25-75}, and PEFR on participants grouped into those given *L. reuteri* and those who were not given *L. reuteri*.

	Baseline	1 month	2 months	3 months	p-value
FEV_1					
With *L. reuteri*	91.5 (67–113)	93 (74–110)	95.5 (83–105)	95.5 (85–100)	**0.045**
Without *L. reuteri*	89.5 (49–107)	88.5 (52–107)	92 (70–99)	93 (81–99)	
FEF_{25-75}					
With *L. reuteri*	76 (33–130)	82 (45–121)	85 (53–112)	86.5 (65–101)	0.489
Without *L. reuteri*	79 (26–113)	83.5 (33–109)	87 (41–107)	89.5 (49–102)	
FVC					
With *L. reuteri*	89 (81–116)	95 (87–115)	99 (90–111)	99 (92–109)	**0.007**
Without *L. reuteri*	85 (60–100)	89.5 (69–103)	95 (75–112)	96.5 (87–107)	

(Continued)

Table 3 Effects of treatment on FEV_1, $FEF_{25–75}$, FVC, FEV_1/FVC, PEFR, and reversibility measurements on FEV_1, $FEF_{25–75}$, and PEFR on participants grouped into those given *L. reuteri* and those who were not given *L. reuteri*. (Continued)

	Baseline	1 month	2 months	3 months	p-value
FEV_1/FVC					
With *L. reuteri*	101 (77–112)	97 (82–110)	95 (91–99)	95 (91–99)	0.795
Without *L. reuteri*	101 (71–113)	98 (67–112.5)	95 (85–105)	96 (87–99)	
PEFR					
With *L. reuteri*	94 (77–110)	95 (83–112)	97 (87–110)	98 (89–105)	0.399
Without *L. reuteri*	90.5 (76–120)	93 (70–115)	95 (82–110)	95 (86–115)	
Reversibility of FEV_1					
With *L. reuteri*	13 (2–26)	9 (0–21)	5.5 (1–15)	4 (2–6)	0.156
Without *L. reuteri*	13 (5–36)	10.5 (3–25)	6 (2–17)	4 (2–14)	
Reversibility of $FEF_{25–75}$					
With *L. reuteri*	35.5 (-9–66)	23.5 (2–49)	17 (3–29)	11 (3–27)	0.250
Without *L. reuteri*	36.5 (13–100)	22.5 (12–59)	17 (5–43)	13.5 (3–28)	
Reversibility of PEFR					
With *L. reuteri*	19.5 (6–24)	12.5 (5–17)	8 (5–14)	6.5 (2–13)	0.082
Without *L. reuteri*	20.5 (3–25)	15.5 (7–21)	10 (5–17)	7 (1–17)	

Table 4 Effect of treatment on ACT and C-ACT scores on participants grouped into those given L. reuteri and those who were not given L. reuteri.

	Baseline	1 month	2 months	3 months	p-value
ACT					
With *L. reuteri*	16.5 (16–20)	19 (18–21)	23 (22–24)	24 (23–25)	**0.027**
Without *L. reuteri*	17 (15–18)	19 (17–21)	21 (19–23)	22.5 (19–24)	
C-ACT					
With *L. reuteri*	17 (15–20)	19.5 (17–21)	21 (20–25)	26 (24–27)	0.365
Without *L. reuteri*	18 (15–20)	19.5 (17–22)	21.5 (19–23)	24 (18–26)	

Table 5 Spirometry results of monosensitized patients (n = 24) grouped into those given *L. reuteri* and those who were not given *L. reuteri*.

	Baseline	1 month	2 months	3 months	p
With *L. reuteri* (n=13)					
FEV_1	89 (67–99)	92 (74–99)	92 (83–99)	94 (85–99)	0.791
FEF_{25-75}	75 (33–110)	79 (45–107)	81 (53–104)	84 (65–101)	0.909
FVC	87 (81–100)	91 (87–99)	97 (90–103)	98 (92–107)	0.087
FEV_1/FVC	100 (77–112)	97 (82–106)	95 (91–99)	95 (91–98)	1.000
PEFR	89 (85–110)	93 (87–112)	97 (90–110)	98 (91–105)	0.200
Without *L. reuteri* (n=11)					
FEV_1	96 (60–107)	93 (69–107)	96 (75–99)	96 (84–99)	
$FEF_{25–75}$	85 (46–113)	92 (61–109)	95 (69–107)	94 (74–102)	
FVC	88 (65–100)	92 (75–103)	98 (88–112)	98 (90–107)	
FEV_1/FVC	104 (92–113)	99 (92–113)	95 (85–99)	96 (92–99)	
PEFR	92 (78–120)	94 (70–115)	95 (82–110)	96 (86–115)	

Table 6 Spirometry results of polysensitized patients (n = 20) grouped into those given *L. reuteri* and those who were not given *L. reuteri*.

	Baseline	1 month	2 months	3 months	p
With *L. reuteri* (n=9)					
FEV_1	92 (87–113)	93 (88–110)	96 (91–105)	96 (91–100)	**0.010**
FEF_{25-75}	77 (57–130)	83 (71–121)	85 (77–112)	87 (79–97)	**0.020**
FVC	90 (85–116)	97 (90–115)	99 (96–111)	100 (96–109)	**0.001**
FEV_1/FVC	101 (89–112)	97 (90–110)	96 (93–99)	95 (92–99)	0.336
PEFR	94 (77–100)	96 (83–103)	97 (87–100)	98 (89–103)	0.201
Without *L. reuteri* (n=11)					
FEV_1	81 (49–95)	83 (52–98)	88 (70–98)	92 (81–98)	
FEF_{25-75}	77 (26–82)	81 (33–86)	86 (41–89)	86 (49–92)	
FVC	84 (60–92)	89 (69–96)	92 (75–99)	95 (87–105)	
FEV_1/FVC	99 (71–113)	97 (68–110)	96 (92–105)	96 (87–99)	
PEFR	89 (76–100)	92 (83–100)	93 (88–100)	95 (90–100)	

However, among polysensitized patients, there were significant differences in the FEV_1, FEF_{25-75}, and FVC between the *L. reuteri* and control groups (Table 6).

DISCUSSION

This study was the first to evaluate *L. reuteri* as an adjunct in the treatment of asthma. Results showed that subjects given *L. reuteri* achieved significant improvements in FEV_1, FVC and ACT than those observed in the control group. Notably, the effect was more prominent among polysensitized patients.

Probiotics, through restoration of gut microflora, from a state of dysbiosis, to one of eubiosis[5] have been linked to the prevention and even improvement of allergic diseases, such as atopic dermatitis, allergic rhinitis and asthma.[17] While both the experimental and control group in this study showed significant improvement of asthma control over time, there were statistically significant improvements in the objective and subjective control of asthma between those that received *L. reuteri* versus those that did not. The FEV_1 and FVC of subjects that took the *L. reuteri* were significantly higher when compared to those subjects that did not. In a study by Chen, it was observed that there were significant improvements in pulmonary function test results, such as FEV_1 and FVC, of patients that were given a probiotic compared to those that were not,[3] which was similar to the result of this study. It was suggested that this might be due to the ability of probiotics to decrease bronchial hyperreactivity in asthmatic children.[3] *L. reuteri* induces a TH1 reaction with a subsequent decrease in TH2 cytokines such as IL-4, IL-5 and IL-13, as well as an increase of the regulatory interleukin IL-10 in asthma,[13] and this could have contributed to significant improvement of pulmonary function test results of the subjects who were given the probiotic.

There was improvement of the PEFR over time between subjects given *L. reuteri* and those that were not. However, it was not statistically significant. It should be noted however, that the PEFR in this study was taken only once in every follow-up. In the study of Chen, *Lactobacillus gasseri* A5 as an adjunct to the treatment of asthma in children significantly improved the PEFR of children who were given the probiotic as compared to those who were not. [3] However, it was noted that the daytime PEFR between the two groups did not differ significantly, and this data seems to be compatible with the results of this study as well, as the PEFR of subjects were only taken during the daytime. The exact reason or mechanism why this occurred is yet to be elucidated at this time.

In terms of subjective measures of the control of asthma, there was a significant improvement of ACT scores of subjects that were given *L. reuteri* compared to those that were not, particularly at the second and third month post intervention. While gradual objective improvement of lung function can be detected by spirometry, subjective control may not be immediately felt in some asthmatic patients unless inflammation was completely reversed,[18] and this could be the reason for delayed improvement of ACT scores in these subjects.

For the C-ACT scores however, although there was significant improvement in both groups over time, there was no statistically significant difference observed between those that were given the probiotic and those that were not. Some studies that employed the use of probiotics for asthma showed no statistical difference of C-ACT scores between those that were given probiotics and those that were not as well, which was consistent with the results of this study.[3,13] While the results were not statistically significant, it can be seen in this study that the values of C-ACT scores by the third month post treatment had begun to increase for the probiotic group over the group that was not given the probiotic. If this linear trend were to continue, it might be possible to see a more significant difference in results if the groups were followed up over a longer period of time.

Fifty-four percent of patients included in this study were polysensitized (54%), and it was in this group that significant differences in FEV_1, FEF_{25-75}, and FVC between the *L. reuteri* and placebo groups were noted. Those who were polysensitized to aeroallergens tended to have a more severe clinical picture of disease and more impaired quality of life when compared to monosensitized individuals. It had also been noted that polysensitized individuals with allergic rhinitis were more frequently associated with symptoms of asthma.[19] Asthmatics who were polysensitized to aeroallergens were continuously exposed to a variety of perennial environmental allergens, and this resulted in the continuous stimulation of mast cells leading to persistent release of mediators that could lead to chronic bronchoconstrictive effects on the airways. Polysensitized individuals have been observed to have lower IL-10 and interferon gamma (IFN-γ), leading to bronchial impairment;[20] thus, the ability of probiotics to regulate IL-10[13] could be the reason for improved FEV_1, FVC and FEF_{25-75} among those given *L. reuteri* compared to those who were not given the probiotic.

There were no safety concerns and reported adverse effects among patients in the study.

CONCLUSION

The results of this study showed that the use of *L. reuteri* DSM 17938 as an adjunct in the treatment of asthma had a significant impact on FEV_1, FVC and ACT results of children newly diagnosed with asthma. Children who were polysensitized to aeroallergens may receive the most benefit from this probiotic.

Recommendations

The limitations of this study included the small sample size and short duration of the observation period. However, given the consistency of effects across different end points, the results justify longer studies powered to examine the clinical efficacy of *L. reuteri*. It can also be recommended to maximize the dose of *L. reuteri* to one tablet twice a day, and for the probiotic to be given for a longer duration than one month, to see if there will be a greater therapeutic effect on the subjects. Other tests, such as the fractional concentration of exhaled nitric oxide (FeNO), as well as the quantification of serum immunoglobulins and interleukins over time will also add to the objectivity of the study results. It is also recommended to perform the study with the use of a double-blind, placebo-controlled trial to decrease the bias of the investigator.

Competing Interests/Conflict of Interests

The probiotic used in this study (*L. reuteri* 26.5 mg/ chewable tablets) was funded and provided by the primary investigator to the study participants. The research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

REFERENCES

1. Global Initiative for Asthma. Global Strategy for Asthma Management and Prevention, 2018. Available from: www.ginasthma.org
2. Kliegman R, Stanton B, Geme JW, Schor NF, Behrman RE. Nelson textbook of pediatrics. 20th Ed. Philadelphia, PA: Elsevier; 2016.
3. Chen Y-S, Jan R-L, Lin Y-L, Chen H-H, Wang J-Y. Randomized placebo-controlled trial of lactobacillus on asthmatic children with allergic rhinitis: Effects of lactobacillus on childhood asthma and allergic rhinitis. *Pediatr Pulmonol* [Internet]. 2010;45(11):1111–20. Available from: http://dx.doi.org/10.1002/ppul.21296
4. Kim S-O, Ah Y-M, Yu YM, Choi KH, Shin W-G, Lee J-Y. Effects of probiotics for the treatment of atopic dermatitis: a meta-analysis of randomized controlled trials. *Ann Allergy Asthma Immunol* [Internet]. 2014;113(2):217–26. Available from: http://dx.doi.org/10.1016/j.anai.2014.05.021
5. Fiocchi A, Fierro V, La Marra F, Dahdah LA. The custom clearance of pro- and prebiotics in allergy prevention. *Ann Allergy Asthma Immunol* [Internet]. 2016;117(5):465–7. Available from: http://dx.doi.org/10.1016/j.anai.2016.05.008
6. Mu Q, Tavella VJ, Luo XM. Role of Lactobacillus reuteri in human health and diseases. *Front Microbiol* [Internet]. 2018;9:757. Available from: http://dx.doi.org/10.3389/fmicb.2018.00757
7. Vliagoftis H, Kouranos VD, Betsi GI, Falagas ME. Probiotics for the treatment of allergic rhinitis and asthma: systematic review of randomized controlled trials. *Ann Allergy Asthma Immunol* [Internet]. 2008;101(6):570–9. Available from: http://dx.doi.org/10.1016/S1081-1206(10)60219-0
8. Dongarra ML, Rizzello V, Muccio L, Fries W, Cascio A, Bonaccorsi I, et al. Mucosal immunity and probiotics. *Curr Allergy Asthma Rep*. 2013;13:19–26.
9. Cross ML, Stevenson LM, Gill HS. Anti-allergy properties of fermented foods: an important immunoregulatory mechanism of lactic acid bacteria? *Int Immunopharmacol* [Internet]. 2001;1(5):891–901. Available from: http://dx.doi.org/10.1016/s1567-5769(01)00025-x
10. Kirjavainen PV, Arvola T, Salminen SJ, Isolauri E. Aberrant composition of gut microbiota of allergic infants: a target of bifidobacterial therapy at weaning? *Gut* [Internet]. 2002;51(1):51–5. Available from: http://dx.doi.org/10.1136/gut.51.1.51
11. Valeur N, Engel P, Carbajal N, Connolly E, Ladefoged K. Colonization and immunomodulation by Lactobacillus reuteri ATCC 55730 in the human gastrointestinal tract. *Appl Environ Microbiol* [Internet]. 2004;70(2):1176–81. Available from: http://dx.doi.org/10.1128/AEM.70.2.1176-1181.2004
12. Forsythe P, Inman MD, Bienenstock J. Oral treatment with live Lactobacillus reuteri inhibits the allergic airway response in mice. *Am J Respir Crit Care Med* [Internet]. 2007;175(6):561–9. Available from: http://dx.doi.org/10.1164/rccm.200606-821OC
13. Del Giudice M, Maiello M, Decimo N, Fusco F, Agostino D, Sullo B, et al. Airways allergic inflammation and L. reuterii treatment in asthmatic children. *Journal of Biological Regulators & Homeostatic Agents*. 2012;26(1):35–40.
14. Del Giudice M, Maiello M, Allegorico N, Iavarazzo A, Capasso L, Capristo M. Lactobacillus reuterii DSM 17938 plus Vitamin D3 as an ancillary treatment in allergic children with asthma. *Ann Allergy Asthma Immunol*. 2016;117:703–7.
15. Abrahamsson TR, Jakobsson T, Björkstén B, Oldaeus G, Jenmalm MC. No effect of probiotics on respiratory allergies: a seven-year follow-up of a randomized controlled trial in infancy. *Pediatr Allergy Immunol* [Internet]. 2013;24(6):556–61. Available from: http://dx.doi.org/10.1111/pai.12104
16. Kang C, Kang Sim DE. Lactobacillus for prevention of pediatric atopic disorders: A meta-analysis of randomized controlled trials. *J Allergy Clin Immunol* [Internet]. 2019;143(2):AB280. Available from: http://dx.doi.org/10.1016/j.jaci.2018.12.857
17. Prescott SL, Björkstén B. Probiotics for the prevention or treatment of allergic diseases. *J Allergy Clin Immunol* [Internet]. 2007;120(2):255–62. Available from: http://dx.doi.org/10.1016/j.jaci.2007.04.027
18. Bora M, Alpaydin AO, Yorgancioglu A, Akkas G, Isisag A, Coskun AS, et al. Does asthma control as assessed by the asthma control test reflect airway inflammation? *Multidiscip Respir Med* [Internet]. 2011;6(5):291–8. Available from: http://dx.doi.org/10.1186/2049-6958-6-5-291
19. Ciprandi G, Cirillo I. Monosensitization and polysensitization in allergic rhinitis. *Eur J Intern Med* [Internet]. 2011;22(6):e75-9. Available from: http://dx.doi.org/10.1016/j.ejim.2011.05.009
20. Ha EK, Baek JH, Lee S-Y, Park YM, Kim WK, Sheen YH, et al. Association of polysensitization, allergic multimorbidity, and allergy severity: A cross-sectional study of school children. *Int Arch Allergy Immunol* [Internet]. 2016;171(3–4):251–60. Available from: http://dx.doi.org/10.1159/000453034