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Effect of Lactobacillus reuteri LRE02-*Lactobacillus rhamnosus* LR04 combination and gastrointestinal functional disorders in an Emergency Department pediatric population

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Abstract. – OBJECTIVE: Probiotics are living microorganisms that, when administered per os in adequate amounts, may confer a health benefit on the host by the regularization of an unbalanced gastroenteric microbiota. The objective of this study was to evaluate treatment effectiveness, safety, and palatability of a probiotic's combination (*Lactobacillus reuteri* LRE02-DSM 23878 and *Lactobacillus rhamnosus* LR04-DSM 16605) in a pediatric Emergency Department setting with functional gastrointestinal disorders.

PATIENTS AND METHODS: Three groups were enrolled: children with functional abdominal pain; children with gastroenteritis; children with gas colic. Self-reporting sheets were delivered to each patient/parent after probiotics treatment. The primary outcome was to evaluate the evolution of clinical conditions in enrolled children.

RESULTS: The outcomes showed a statistical difference among children treated with probiotics and those who did not. In the functional abdominal pain group, 58.2% of patients had a moderate symptoms improvement and 33.5% had a complete disappearance of symptoms, while in the gas colic group, 68.2% of the infants had a moderate improvement and 23.2% had a complete resolution. In the gastroenteritis group, stool consistency and number of evacuations improved in children who took probiotic administration as well.

CONCLUSIONS: Probiotics therapy, at the recommended dosage of five drops per day for 15 days, is associated with symptoms improvement. Moreover, the use of probiotics led to a stool consistency's normalization in a shorter time, evaluated with BSS. A randomized trial is needed to confirm these results.

Key Words:
Children, Emergency Department, Gastrointestinal functional disorders, Probiotics.

Introduction

Probiotics are living microorganisms that, when administered *per os* in adequate amounts, may confer a health benefit on the host by the regularization of an unbalanced gastroenteric microbiota[1]. There are many different ways in which probiotics can help in resolve GI symptoms, such as the competitive exclusion of pathogenic microorganisms, inhibition of pathogen adhesion, production of anti-microbial substances, and modulation of the immune system[2-5]. As shown in numerous studies, gastrointestinal symptoms are common to all the children of the world. Pediatric gastrointestinal functional disorders (FGID) are disorders of the brain-intestine axis. They are the most common cause of chronic-recurrent abdominal pain in the pediatric population.

Epidemiological studies have shown that approximately 14-25% of infants, young children, and adolescents suffer from at least one gastrointestinal function disorder[6]. Functional abdominal pain, defined as a pain that appears at least four times per month and includes episodic or continuous abdominal pain that does not occur solely during physiologic events and which cannot be fully explained by another medical condition. Acute Gastroenteritis (AGE) is a very common

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disease in children. It accounts for millions of visits to primary care practices and to the Emergency Department. ESPGHAN has defined AGE as a decrease in consistency of stools and/or an increase in the frequency of evacuations, at least three in 24 hours, with or without fever or vomiting[7]. Infantile colic is a common problem affecting 10% to 30% of healthy, thriving infants. According to the Rome IV criteria, infantile colic may be diagnosed in an infant who is less than 5 months of age who present with recurrent and prolonged periods of crying, fussing or irritability that occur without an evident cause, and in whom there is no evidence of failure to thrive, fever or illness[8]. Probiotics seem to play an important role in the control of gastrointestinal symptoms in pediatric age.

Therefore, the aim of this study is to evaluate treatment effectiveness, safety, and palatability of a probiotic's combination (*Lactobacillus reuteri* LRE02- DSM 23878 and *Lactobacillus rhamnosus* LR04-DSM 16605) in a Pediatric Emergency Department (PED) setting with gastrointestinal symptoms.

Patients and Methods

Study Design

This is a monocentric survey on a cohort of children recruited from patients admitted to the PED of the "A. Gemelli" Hospital in Rome between January 2019 and December 2019. Children aged between 1 month and 18 years with a diagnosis of functional abdominal pain, according to Rome IV criteria (Table I), (Group A), gastroenteritis (Group B), and gas colic (Group C) were recruited. Functional patients were subclassified among the various classes of disorders. Besides, Group A patients who had met the inclusion criteria and who in the second phase of the study did not maintain adherence to these criteria, but for whom the requirements for abdominal pain not otherwise specified (FAP-nos) are met have been included as such, while patients who had met the inclusion criteria but who in the second phase of the study did not maintain adherence to these criteria and for which the requirements for FAP-nos were not met were excluded from the functional sample.

Table I. Rome IV diagnostic criteria for functional abdominal pain disorders[9].

Rome IV diagnostic criteria for functional abdominal pain disorders (AP-FGIDs)
Irritable bowel syndrome The criteria must be fulfilled for at least 2 months and include all of the following. • Abdominal pain at least 4 days per month associated with defecation and/or a change in the frequency of stool and/or a change in the appearance of stool • Abdominal pain does not resolve with resolution of constipation (children in whom the pain resolves have functional constipation, not irritable bowel syndrome) • After appropriate evaluation, the symptoms cannot be fully explained by another medical condition
Functional dyspepsia The criteria must be fulfilled for at least 2 months before diagnosis and must include one or more of the following bothersome symptoms at least 4 days per month. • Postprandial fullness. • Early satiation. • Epigastric pain or burning not associated with defecation. • After appropriate evaluation, the symptoms cannot be fully explained by another medical condition.
Abdominal migraine The criteria must be fulfilled for at least 6 months before diagnosis and include all of the following occurring at least twice. • Paroxysmal episodes of intense, acute periumbilical, midline or diffuse abdominal pain lasting 1 hour or more (should be the most severe and distressing symptom). • Episodes are separated by weeks to months; the pain is incapacitating and interferes with normal activities; stereotypical pattern and symptoms in the individual patient. • The pain is associated with two or more of the following: anorexia, nausea, vomiting, headache, photophobia or pallor. • After appropriate evaluation, the symptoms cannot be fully explained by another medical condition. Functional abdominal pain not otherwise specified. The criteria must be fulfilled for at least 2 months before diagnosis and at least four times per month and include all of the following. • Episodic or continuous abdominal pain that does not occur solely during physiological events (for example, eating and menses). • Insufficient criteria for irritable bowel syndrome, functional dyspepsia or abdominal migraine. • After appropriate evaluation, the abdominal pain cannot be fully explained by another medical condition.

All patients included in the study were randomized by an electronic system to receive or not a probiotic combination [*Lactobacillus reuteri* LRE02 (DSM 23878, 2×10^8 CFU daily) and *Lactobacillus rhamnosus* LR04 (DSM 16605) 1×10^9 CFU daily] for 15 days. This treatment was added to prescriptions, frequently represented by antibiotics, deemed necessary by the physician. 5 days after the end of the treatment, parents of the enrolled patients were contacted to be subjected to a telephone interview.

Based on this interview, Medical Doctors (MDs) evaluated both the treatment effectiveness in determining remission of the symptoms, both safety and palatability of the drug, and the possible occurrence of adverse effects (AEs). To evaluate outcomes in children with acute gastroenteritis (Group B), a validated scale (the Modified Vesikari Scale, Table II) was used[9-11], while in Group A and C a clinical outcome reported by parents as "no improvement", "moderate improvement" or "complete disappearance of symptoms" was used. For Group A and B, modifications of diarrhea and stool consistency were also evaluated using Bristol Stool Scale (BBS).

The primary outcome of this study was to evaluate the evolution of clinical conditions in enrolled children.

Secondary outcomes were: (1) assessment of stool solidity using the Bristol Stool Scale (BSS)[12]; (2) evaluation of the prevalence of AEs between the groups.

Written informed consent was obtained from the parents of children enrolled in the study, according to the principles outlined in the Declaration of Helsinki.

Statistical Analysis

We performed statistical analysis with GraphPad Prism version 8.01 for Windows (GraphPad Software®, San Diego, USA, www.graphpad.com). The tests used to control distributions normality (or non-normality) were: Shapiro-Wilk normality test, D'Agostino and Pearson omnibus normality test, Kolmogorov-Smirnov normality test. We applied the Mann-Whitney test for non-parametric distribution. Differently, we performed the standard Student's *t*-test. To match the prevalence between the groups we applied the *Chi*-squared test. We considered statistically significant a *p-value* <0.05.

Results

We enrolled 457 patients from 1 month to 18 years old, with a mean age (±SD) of 28±21 months. Group A, (children with functional abdominal pain), included 176 patients (38.51%),

Table II. Modified Vesikari Scale*.

Scale component	Score on the Vesikari Scale			
	0 Points	1 Point	2 Points	3 Points
Duration of diarrhea (hr)	0	1-96	97-120	≥ 121
Maximum no. of watery stools per 24 hr	0	1-3	4-5	≥ 6
Duration of vomiting (hr)	0	1-24	25-48	≥ 49
Maximum no. of vomiting episodes per 24 hr	0	1	2-4	≥ 5
Maximum recorded rectal temperature (°C)†	< 37.0	37.1-38.4	38.5-38.9	≥ 39.0
Unscheduled health care visit	None	NA	Primary care	Emergency department
Treatment	None	Rehydration with intravenous fluids	Hospitalization	NA

*In the modified Vesikari scale score, one variable (percent dehydration) in the original score was replaced with the variable of unscheduled health care visits to better measure the effect of acute gastroenteritis in outpatients, given that the ability to perform frequent in-person assessments in an outpatient cohort of children can be challenging. Scores range from 0 to 20, with higher scores indicating more severe disease. Children with a score of 9 or more were considered to have moderate-to-severe gastroenteritis. NA denotes not applicable. †Temperatures were adjusted for the location of measurement: 1.1°C was added to axillary temperatures and 0.6°C was added to oral temperatures. Modified from: Schnadower D, Tarr PI, Casper TC, Gorelick MH, Dean JM, O'Connell KJ, Mahajan P, Levine AC, Bhatt SR, Roskind CG, Powell EC, Rogers AJ, Vance C, Sapien RE, Olsen CS, Metheney M, Dickey VP, Hall-Moore C, Freedman SB. Lactobacillus rhamnosus GG versus placebo for acute gastroenteritis in children. N Engl J Med 2018; 379: 2002-2014.

Table III. Overall distribution of the infections among the enrolled patients.

Infections	Prevalence
Upper Respiratory Tract Infections (URTI)	57 (59.3%)
Lower Respiratory Tract Infections (LRTI)	21 (21.8%)
Urinary Tract Infections (UTI)	8 (8.4%)
Otitis (OT)	6 (6.3%)
Other	4 (4.2%)

group B (children with gastroenteritis), 249 patients (54.49%), and group C (children with gas colic) 32 patients (7.0%). 96 children (21%) also had other infections that required antibiotic therapy (Table III).

Group A (Functional Abdominal Pain)

As for group A, during the study, 1130 children with abdominal pain were admitted to the PER: 493 had organic pain, 461 were doubtful cases, and 176 had functional abdominal pain, as shown in Figure 1.

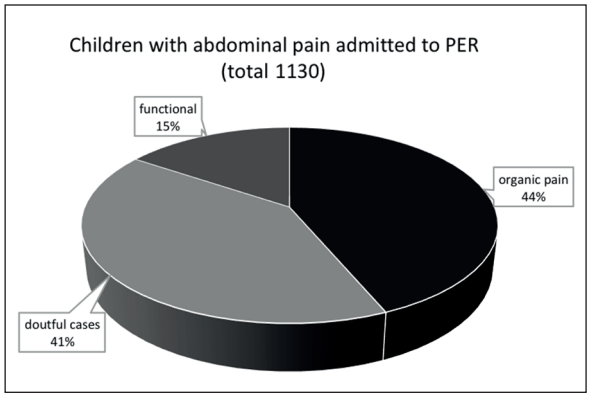

Figure 1. Distribution of Group A population.

Children with functional abdominal pain were included in the study. As shown in Figure 2, the main age was between 7 and 15 years; 56.4% of the patient were female.

Of these 176 patients, 35 were hospitalized, 107 were referred to outpatient facilities and the remaining 34 were sent home. The main diagnoses in this group were functional dyspepsia (38 cases, 21.6%), IBS (21 cases, 11.9%), abdominal migraine (15 cases, 8.5%), and FAP (27 cases, 15.3%) (Figure 3).

Clinical characteristics and psychosocial factors associated with functional abdominal pain are reported in Figure 4.

In this group stool consistency was evaluated at the beginning of the treatment (T0) and after 15 days (T1). The results, reported in Table III, show statistical differences between T0 and T1.

The telephone interview performed at the end of the treatment showed that 58.2% of patients with functional abdominal pain had a moderate symptoms improvement, 33.5% had a complete disappearance of symptoms, and the remaining 8.3% showed no improvement at the end of the therapy.

Group B (Children with Gastroenteritis)

In Group B, 249 patients were enrolled. 203 (81.5%) of them had viral gastroenteritis, 42 (16.9%) bacterial gastroenteritis, and 4 (1.6%) parasitic gastroenteritis. As for population characteristics, 66.7% were female and the most affected age group was between 9 and 35 months of age. Of these 249 patients, 75 were hospitalized, 120 were referred to outpatient facilities, and the remaining 54 were sent home. Stool consistency results after the treatment are reported in Figure 5. At T0, the mean value of the BSS (Bristol Stool Scale) score was 4.5±1.5 (median 4, IQR

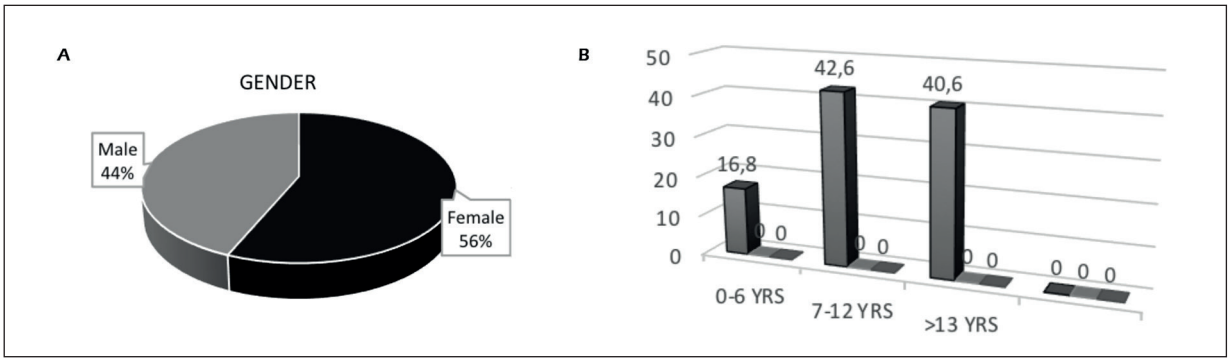

Figure 2. A-B, Gender and age distribution in Group A.

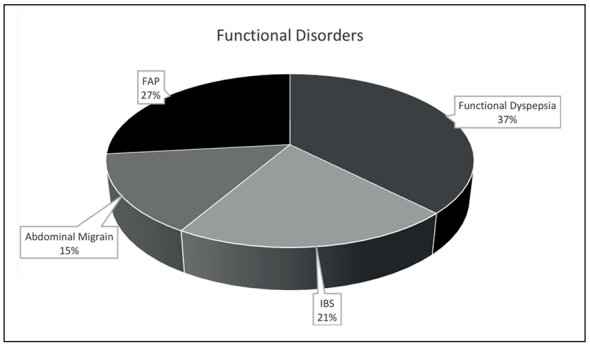

Figure 3. Types of functional disorders in Group A.

3-5), while fifteen days after the treatment (T1), the mean value of BBS was 3.7±1.2 (median 4, IQR 3-4), (median 4, IQR 3-5) (p<0.0001). No moderate or severe BBS score are reported by the parents of treated children.

In this group, diarrhea improved after 1.7 days from the beginning of the treatment and the number of evacuations per day was 3.5±1.8 without no report of significant weight loss.

Group C (Infants with Gas Colic)

In this group, 32 patients were enrolled. The characteristics of this group were: 72.6% of the patients were male and the most affected age group was between 3 and 5 months of age. None

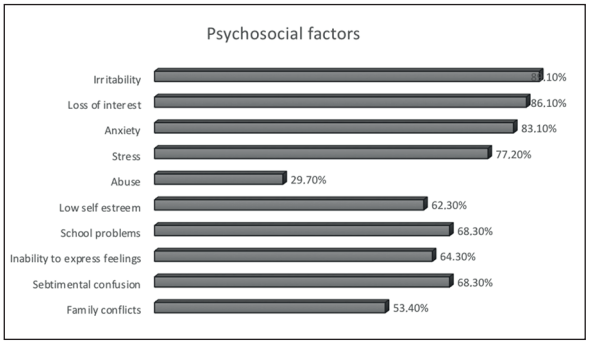

Figure 4. Characteristics and psychosocial factors.

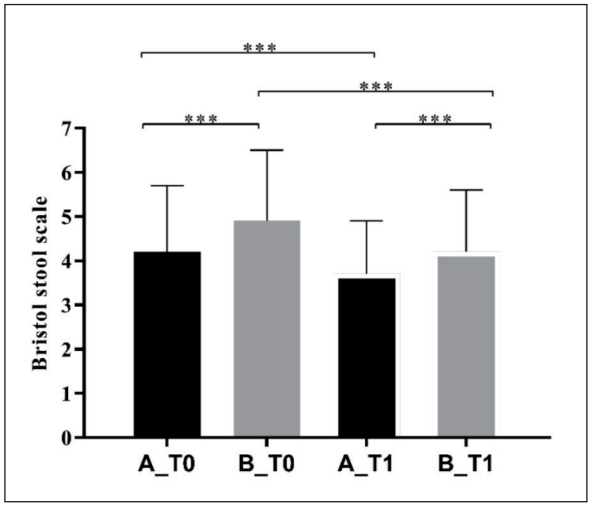

Figure 5. Bristol Stool Scale (BSS) score for evaluation of stool consistency in group B patients at the beginning (T0) and 15 days after the treatment (T1). (***p-value: < 0.001).

of these infants were hospitalized but all were subsequently referred to outpatient facilities. The telephone interview performed at the end of the treatment reported that 68.2% of the infants had a moderate improvement, 23.2% had a complete disappearance of symptoms and the remaining 8.6% showed no improvement at the end of the therapy. No side effects were reported during and after the treatment with probiotics.

Discussion

The word probiotic (from the latin pro and the Greek βιοσ literally meaning "for life") was introduced by the German scientist Werner Kollath in 1953 to designate "active substances that are essential for a healthy development of life[13].

Since then, the scientific world has started to increase knowledge about probiotics and their health applications, both for intestinal and extra-intestinal disorders[14], especially in children[15-18].

A recent recommendation of Working Group on Probiotics and Prebiotics of the European

Table IV. Comparison of stool consistency (Bristol Stool Scale) in patients with functional abdominal pain at the beginning of the treatment (T0) and 15 days after (T1).

	Time	T0	T1	*p*-value
Bristol Stool Scale	T0	6.3 ± 0.5	6.3 ± 0.5	> 0.05
	T1	3.7 ± 1.3	4.4 ± 1.4	**< 0.001**
p-value		**< 0.001**	**< 0.001**	

Society of Gastroenterology, Hepatology, and Pediatric Nutrition (ESPGHAN) promotes the use of some probiotic strains for the prevention of antibiotic-associated diarrhea (AAD), in particular *Lactobacillus rhamnosus*[18,19]. The rationale for the use of these products relies on the hypothesis that AAD is caused by dysbiosis, an imbalance between intestinal microbiota and the host[18,19], re-established by the capability of probiotics to modulate the immune system, induce of anti-inflammatory and anti-oxidant responses, compete for pathogen exclusion, and produce of anti-microbial substances.

The effects of probiotics are strain- and dose-dependent. The production processes can affect the characteristics of the probiotic, causing differences in concentration and presence of any contaminants in the various products on the market[18,20,21]. Anyway, probiotics carry out their action with different mechanisms: maintenance of the integrity of the gut barrier, modulation of the content of intestine microbiota through competitive pathogen exclusion, local immune response by the gut-associated immune system, prevention of bacterial translocation, induction of anti-inflammatory and antioxidant responses, as well as the production of anti-microbial substances[22].

Thus, probiotics positive supplementation outcomes are not limited to the gut; indeed, they also play positive effects at distant sites and organs, such as bones, skin, brain, and heart[16,23].

Children dysbiosis can cause long-term effects, being a risk factor for obesity[24], functional gastrointestinal disorders[25], impaired neurocognitive outcome[16]. Besides, dysbiosis seems to be a contributing factor of the pathophysiological mechanisms of colic and functional abdominal pain disorders. Weerth et al[26] revealed asserted that infants with colic had slower colonization, lower diversity and stability of gut microorganism, and decreased the concentration of *Lactobacillus*, *Bifidobacterium* and other butyrate-producing bacteria.

In addition, the assumption that change in intestinal microbiota represents a predisposing factor for the development of functional abdominal pain[27] encouraged us to examine if probiotics have some positive impacts in terms of evolution of clinical conditions and stool consistency in these groups of patients.

Probiotics are mostly composed of *Bifidobacterium* and *Lactobacillus*. These ones represent a core group of well-studied species likely to impart some general benefits[1]. In particular, *Lactobacillus rhamnosus* is the preferable probiotic strain for preventing AAD, as demonstrated by a Cochrane review published in 2015[10], because it causes fewer adverse events and the NNT is quite low. *L. reuteri* has been even extensively studied in several intestinal conditions, and its therapeutic and preventive effects have been documented[28]. Moreover, current evidence shows that the recommended product serving for daily consumption shall contain a quantity of 10^9 live cells of at least one of the strains[20,29,30].

In our study, we used a combination of encapsulated probiotics (*Limosilactibacillus reuteri* LRE02-DSM 23878, 2×10^8 CFU, and *Lacticaseibacillus rhamnosus* LR04-DSM 16605, 1×10^9 CFU), reaching both the effective numbers of CFU and biological activity; microencapsulation indeed increases the resistance of probiotic microorganisms during the gastro-duodenal transit.

The outcomes showed a statistical difference among children treated with probiotics and those who did not. In group A (functional abdominal pain), 58.2% of patients had a moderate symptoms improvement, 33.5% had a complete disappearance of symptoms, while in group C (infants with gas colic), 68.2% of the infants had a moderate improvement, 23.2% had a complete resolution. In group B, stool consistency and number of evacuations improved in children who took probiotic administration as well; fifteen days after the treatment (T1), the mean value of BBS was statistically improved (3.7±1.2, instead of 4.5±1.5 at T0), and diarrhea improved after 1.7 days from the beginning of the treatment. The number of evacuations per day was 3.5±1.8 without any report of significant weight loss. Therefore, this is an important outcome in order to avoid dehydration, which is a frequent cause of hospitalization in patients with this condition.

Interesting data comes from Group A, which was composed of children with different conditions, such as functional dyspepsia, IBS, abdominal migraine, and FAP: all patients, regardless of the diagnosis and the source of the disorder, benefited from probiotic treatment. Likewise, Group B patients improved their symptoms and stool consistency both in case of viral gastroenteritis and in case of bacterial or parasitic gastroenteritis.

Moreover, no moderate or severe side effects were reported during and after the treatment with probiotics in any group of our analysis. We specify that some children, especially in Group B, were taking antibiotic therapy at the same time, but in the evaluations, the enrolled cohort was not stratified by any eventual type of antibiotics used.

Examining our data, we can support the use of probiotics association to reduce symptoms and stool consistency. Even though symptoms have been reported by parents, the utilization of an international scale (BSS) reduced possible biases. Moreover, the large number of patients included strengthens the achieved results.

Conclusions

The probiotic mixture of *L. reuteri* LRE02-*L. rhamnosus* LR04, at the recommended dosage of five drops per day for 15 days, reduce symptoms and stool consistency without an increase in adverse events in patients aged between 1 month and 18 years with FGID, gastroenteritis, and gas colic. Improvement of symptoms and better stool consistency have been demonstrated regardless of the type of diagnosis and source of the disorder.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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