Effectiveness of *Lactobacillus reuteri* for prevention and treatment of functional gastrointestinal disorders in infants, children and adolescents (Review)

Učinkovitost bakterije *Lactobacillus reuteri* pri preprečevanju in zdravljenju funkcionalnih gastro-intestinalnih motenj pri dojenčkih, otrocih in mladostnikih (pregled)

Rok Orel

University Medical Centre Ljubljana, Children's Hospital, Department of Gastroenterology, Hepatology and Nutrition

Univerity of Ljubljana, Medical Faculty; Chair of Pediatrics

University of Ljubljana, Biotechnical Faculty, Department of Food Technology and Nutrition

Institute for Probiotics and Functional Foods, Ltd.

Korespondenca/ Correspondence:
prof. Rok Orel, MD, PhD, Children's Hospital Ljubljana, Department of Gastroenterology, Hepatology and Nutrition, Bohoričeva 20, 1000 Ljubljana
Phone: +386 1 5229276

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Abstract

Functional gastrointestinal disorders (FGIDs) with their extremely high prevalence and important influence on patients' quality of life and health costs represent a major problem. Their etiopathogenesis is multifactorial and disturbances in a composition of intestinal microbiota as well as specific potentially pathogenic microorganisms seem to have crucial role in it. Probiotics with their broad spectrum of actions, including strengthening of colonisatiton resistance against pathogens, enhancement of barrier function, regulation of intestinal immune response, alleviation of inflammation, and both direct and indirect influence on gut motility or sensitivity, represent one of the most promising therapeutic strategies for these disorders. Numerous clinical studies revealed their efficacy in different FGIDs. However, the pathogenesis of different types of disorders is not similar, and neither are mechanisms of action of different probiotic strains. Several *Lactobacillus reuteri* strains exhibit various characteristics such as secretion of antimicrobial reuterin, production of short-chain fatty acids, down-regulation of inflammatory immune response, and direct influence on enteric nervous system among the others, which render them good candidates for prevention and treatment of various FGIDs. This paper reviews clinical studies on the effectiveness of *Lactobacillus reuteri* in the therapy of FGIDs in infants, children and adolescents. Results of multiple studies support its use for prevention and treatment of infant colic and improvement of delayed gastric emptying and regurgitation. In addition, individual studies suggest potential usefulness of specific *Lactobacillus reuteri* strains for the alleviation of constipation and functional abdominal pain.

Izvleček

Funkcionalne gastro-intestinalne motnje (FGIM) so pomembne zaradi svoje pogostosti, velikega vpliva na kakovost življenja bolnikov in visokih stroškov za zdravstveni sistem. Na njihov pojav vpliva veliko dejavnikov, med njimi pa sta izredno pomembna porušeno ravnotežje v sestavi črevesne mikrobiote in vloga potencialno patogenih mikroorganizmov. Probiotiki z vrsto različnih mehanizmov delovanja, kot so krepitev odpornosti proti kolonizaciji s patogenimi mikroorganizmi, krepitev funkcij črevesne pregrade, uravnavanje črevesnega imunskega odziva, umirjanje vnetja ter neposreden in posreden vpliv na črevesno gibljivost in občutljivost, so ena najbolj obetavnih možnosti za zdravljenje teh motenj. Številne klinične raziskave so dokazale njihovo učinkovitost pri zdravljenju različnih FGIM. Zavedati pa se je potrebno, da so mehanizmi patogeneze pri različnih FGIM med seboj razlikujejo, kot se razlikujejo med seboj tudi mehanizmi delovanja različnih specifičnih sevov probiotikov. Nekateri sevi bakterije *Lactobacillus reuteri* so zaradi svojih sposobnosti, kot so izločanje protimikrobne snovi reuterina, proizvodnja kratkoverižnih maščobnih kislin, zmanjšanje vnetnega imunskega odziva in neposredni vpliv na črevesni živčni sistem, dobri kandidati za preprečevanje in zdravljenje FGIM. Članek povzema izsledke kliničnih raziskav o učinkovitosti bakterije *Lactobacillus reuteri* pri zdravljenju FGIM pri dojenčkih otrocih in mladostnikih. Glede na rezultate teh raziskav lahko svetujemo uporabo bakterije *Lactobacillus reuteri* za preprečevanje in zdravljenje kolik dojenčkov, izboljšanje upočasnjenega praznenja želodca in regurgitacije (polivanja) dojenčkov. Posamezne raziskave pa kažejo tudi na uporabnost posameznih sevov bakterije *Lactobacillus reuteri* za lajšanje zaprtja in funkcionalnih trebušnih bolečin.

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Introduction

Functional gastrointestinal disorders (FGIDs) are characterized by typical combinations of defined chronic or recurrent symptoms, such as abdominal pain or discomfort, regurgitation, vomiting, diarrhea, constipation etc., that cannot be explained by known structural or tissue abnormalities. Some of them are age dependent and are classified into defined clinical entities with respect to patients' age and specific combinations of symptoms.[1,2] The prevalence of FGIDs is extremely high and it is estimated that up to a quarter of population suffers from at least one of these frequent conditions.[3-6] Moreover, several studies revealed that, at least in developed countries, they can be attributed to an underlying organic disease in less than 10 % of children with chronic gastrointestinal symptoms such as recurrent abdominal pain or chronic constipation.[7,8] Despite their benign nature, FGIDs have a significant impact on the quality of life of both children and their family members, with effects on physical functioning, general development, temperament and moods, school performance and parents' absence from work.[9,10] Interestingly, self-reported quality of life by children with functional abdominal pain was found to be lower not only in comparison with healthy children but also with children with inflammatory bowel disease, and their parents perception of the disorder's impact on their children's lives was even worse than that of the children themselves.[11]

The pathogenesis of FGIDs is multifactorial and not fully understood.[12] Traditionally, visceral hypersensitivity and dysmotility of the gut were regarded as main ethiologycal factors. With advances of functional brain imaging techniques and discovery of the important role of neurotransmitters and certain hormones for the functioning of the gastrointestinal system, a new model of disturbed brain-gut axis was developed. This model linked FGIDs with observations of numerous psychological factors, such as early life traumatic events and chronic stress, playing a role in the modulation of the development of disorder behavior. Despite our perception of a firm dividing line between the organic and the functional, there is a growing evidence of the crucial role of low-grade intestinal inflammation in the development of many if not all FGIDs. Increased concentrations of fecal calprotectin and elevated levels of pro-inflammatory cytokines, such as TNF-α, Il-1β, Il-6 and Il-8, both in the gut and peripheral blood of patients with FGIDs, clearly point out the importance of inflammatory mechanisms.[13,14] Both lymphocytes and mast cells were found in close proximity of enteric nerve cells. They can produce and release a wide array of cytokines that are capable of affecting enteric nerve functions and muscle contractility.[12] Moreover, there is substantial evidence that infectious agents and alimentary allergens can both trigger and perpetuate such inflammatory processes that lead to the development of typical symptoms of FGIDs.[15,16]

Several observations implicate intestinal microbiota as an important factor in FGIDs pathogenesis. Studies revealed that acute gastroenteritis, particularly bacterial, is a common environmental trigger for irritable bowel syndrome (IBS). Two large population based surveys showed that an episode of acute gastroenteritis within previous 2 years increased a risk for the development of IBS three- to five-fold.[17,18] In addition, in patients with different FGIDs alterations in the microbiota composition have been demonstrated, which may create a framework for the development of low-grade inflammation and aberrant intestinal metabolism of bile salts, short-chain fatty acids, and gas, resulting in symptoms such as abdominal pain, diarrhea, constipation or bloating.[19] In early studies using culture-based techniques, a depletion of lactobacilli and bifidobacteria together with a greater instability of the intestinal microbiota, was observed.[20,21] More recently, the use of new molecular genetics methods revealed much more complex alterations in microbiome. As an example, the analyses of 71 samples from 22 children with IBS showed a dramatic increase of the class γ-*proteobacteria* with *Haemofilus parainfluenzae* being most prominent component of this group. Moreover, they found a novel

Ruminococcus-like microbe associated with IBS and that an increased abundance of several bacterial taxa from the genus *Alistipes* could be associated with a greater frequency of pain.[22] It has been postulated that specific changes in microbiome can lead to the development of different disturbances in bowel function, as lower numbers of *Lactobacilli* were found only in patients with diarrhea-predominant but not constipation-predominant IBS, while in the latter an abundance in *Veillonela* spp. was discovered.[23] Although the research of complex mechanisms implicating intestinal microorganisms in the pathogenesis of FGIDs is still in its pioneer age, it may well serve as a basis for the development of new rational therapeutic approaches to these disorders.

Potential mechanisms of action of probiotics in FGIDs

Multiple mechanisms of action enable probiotics to act on many factors that seem to be important in the pathogenesis of FGIDs. They can interfere with growth of potentially pathogenic microorganisms and their ability to adhere to the intestinal epithelium. Bifidobacteria, Lactobacilli, including *Lactobacillus reuteri*, and several other commensals metabolize non-digestible carbohydrates into short-chain fatty acids which decrease pH of the intestinal milieu to a level that is unfavorable to most pathogenic bacteria. They produce a number of bacteriostatic compounds, such as hydrogen peroxide, hydrogen sulphide and bacteriocins.[24] *Lactobacillus reuteri* produces a broad-spectrum antimicrobial substance, reuterin.[25,26] Moreover, some probiotics are capable to degrade toxic substances and gas produced by other intestinal bacteria.

Probiotics can enhance epithelial barrier function by inducing the production of mucus and defensins, straightening of tight junctions, and accelerating epithelial repair. In addition, by the interaction with different pattern-recognition receptors they can influence epithelial cells, M cells, dendritic cells and other antigen-presenting cells to process and present luminal antigens to the underlying mucosal immune system.[27] As both intestinal microorganisms and food antigens may be implicated in triggering and maintaining low-grade inflammation frequently observed in patients with FGIDs, the way of handling luminal antigens may play an important role.

Many probiotics act through the regulation of the immune response. This effect differs in relation to the specific strain, and to put it simply, with respect to their influence on the immune system, probiotics can be classified into two groups: the first with predominantly immune-stimulating and the second with anti-inflammatory properties.[28] *Lactobacillus reuteri* seems to poses strong anti-inflammatory properties since anti-inflammatory cytokines, such as TGF-β2 and IL-10 were found in the colostrum of women supplemented with *L. reuteri* during pregnancy, resulting in reduced sensitization to allergens in their infants.[29] Moreover, in a randomized clinical study of efficacy of *L. reuteri* enema for the therapy of active ulcerative colitis in children, a significant decrease in pro-inflammatory IL-1β, TNF-α and IL-8, and a simultaneous increase in anti-inflammatory IL-10, were detected in the probiotic but not in the placebo group.[30]

In addition to the capability of many probiotics to influence intestinal hypersensitivity and motility by the modulation of intestinal microbiota, metabolism and inflammation, few specific strains also exerted direct action on the enteric nervous system. *Lactobacillus acidophilus* can induce the expression of μ-opioid and cannabinoid receptors in intestinal epithelial cells and mediates analgesic functions in the gut similar to the effects of morphine.[31] On the other hand, *Lactobacillus reuteri* can affect gut motility, contractility and pain perception through the inhibition of calcium-dependent potassium channel in enteric nerves.[32–34] Several animal studies highlighted a direct influence of intestinal microorganisms on neurochemical mechanisms in the brain and consequently central responses to stress and animal behavior.[35,36] Probiotics can affect brain functions by several mechanisms, with the reduction of inflammatory cytokines leading to exaggerated response of hypothalamic-pituitary-adrenal axis to

stress and dysfunction of the brain-gut axis being probably the most important.[37] Moreover, some probiotic strains are potentially capable to induce an increase in plasma concentrations of tryptophan, a precursor to serotonin, which is a key transmitter within the brain-gut axis, and modify concentrations of several other neurotransmitters in the frontal and amygdaloid cortex, parts of the brain deemed to by importantly implicated in the pathogenesis of FGIDs.[38]

In conclusion, probiotics can target multiple mechanisms in dysfunctional "microbiome-gut-brain axis". However, it should not be forgotten that the mechanisms of action of probiotics are species- and even strain-specific, and no strain can exhibit all mentioned functions. Moreover, pathologic mechanisms behind diverse functional disturbances probably differ so much that specific probiotic strains should be selected for each individual functional disorder separately with respect to their specific mechanisms of action. *Lactobacillus reuteri* strains with their specific properties acting on different levels and by several mechanisms are good candidates for the treatment of FGIDs. Nevertheless, the most important guidance for a proper practical use of probiotics is based on the results of high-quality clinical trials. The continuation of this article is intended to review the clinical evidence of the efficacy of *Lactobacillus reuteri* strains for the therapy of different FGIDs.

Infantile colic

Infantile colics are defined as paroxysms of irritability, fussing or crying that start and stop without obvious cause, with episodes lasting 3 or more hours per day and occur at least 3 days per week for at least one week in an infant from birth to 4 months of age with no failure to thrive.[2] The exact mechanisms for the occurrence of infantile colic remain unexplained, but painful contractions of the gut, abundance of gas in the gastrointestinal tract either because of aerophagia or extensive gas production by microbiota, food hypersensitivity or intolerance and parental misinterpretation of normal crying have been all proposed as possible causative factors.[39] Lower Lactobacilli counts were observed in infants with colic in comparison with non-colicky infants.[40,41] The potential effectiveness of *Lactobacillus reuteri* in the treatment of infant colic was evaluated in several clinical trials.

In an early study by Savino et al. the efficacy of two different therapeutic approaches was compared. Ninety breastfed infants with colic were randomized into two groups, the first receiving *Lactobacillus reuteri* ATCC 55730 (10^8 live bacteria per day) and the second simethicone (60 mg per day) for 28 days.[42] To avoid the possible influence of cow's milk protein allergy, all lactating mothers were ordered to follow cow's milk-free diet throughout the study period. Parents were asked to record the daily average crying time and number of colic episodes as well as the observed adverse events. The primary outcome of the study was a reduction in the daily average crying time from the beginning to the end of treatment period. The secondary outcome was the number of responders in each of the two groups, with children who experienced a decrease in daily crying time for more than 50 % during study period declared as responders. Seven patients were excluded from final analysis for different reasons. No infant was withdrawn because of any trial-relatd adverse event. The average crying times per day were significantly shorter in the group treated with probiotic compared to simethicone-treated group at follow-up on days 7, 14, 21 and 28 ($P < 0.001$). At the beginning of the study, the median crying time per day was similar for probiotic and simethicone group (197 minutes/day; range: 180–276 minutes/day; vs. 197 minutes/day; range: 180–278 minutes/day) but was reduced to 51 minutes/day (range: 26–105 minutes/day) in the probiotic group compared to 145 minutes/day (range: 70–191 minutes/day). At the end of the study 39 (95 %) patients treated with *L. reuteri* but only 3 (7 %) of those treated with simethicone were proclaimed responders. The differences between probiotic and simethicon group remain similar when analyzed separately for children with and without family history of atopy. The authors concluded that *Lactobacillus reuteri* improved colicky

symptoms in breastfed infants within 1 week of treatment compared to simethicon group.

Subsequently, the same group performed a randomized, double-blind study comparing the efficacy of *Lactobacillus reuteri* against placebo.[43] They applied novel daughter *L. reuteri* strain DSM 17938 instead of previously used ATCC 55730 strain, because the latter had been found to carry potentially transferable resistance genes for tetracycline and lincomycin on the plasmid DNA.[44] Fifty exclusively breastfed colicky infants were randomized to receive either *L. reuteri* DSM 17938 (10^8 CFU/day) or placebo for 21 days. Parents were requested to record daily crying time, stool characteristics and frequency, and adverse events. Primary outcome was defined as a reduction in average daily crying time, and the secondary outcome as the number of responders defined as 50 % reduction in crying time from the baseline. In addition, fecal microbiota before and at the end of treatment was analyzed by fluorescence in situ hybridization (FISH). All 25 patients from probiotic and 21 patients from placebo group completed the study. At the beginning there was no difference in median crying times between the two groups. Analyses revealed a significant reduction in daily crying time in *L. reuteri* group compared to placebo group (35 minutes/day vs. 90min/day; P = 0.022), and a significantly higher number of responders in the probiotic group compared to placebo on days 7 (20 vs. 8; P = 0.006), 14 (24 vs. 13; P = 0.007) and 21 (24 vs. 18; P = 0.049) respectively. There were no treatment-related adverse events. Microbiological analysis of fecal samples revealed a significant increase in Lactobacilli (P = 0.002) and a reduction in *E. coli* (P = 0.001) in *Lactobacillus reuteri* group but not in placebo group. The authors concluded that daily administration of *L. reuteri* DSM 17938 improved symptoms compared to placebo and that this effect might be related to induced changes in the intestinal microbiota.

In a recent double-blind, randomized trial with similar design, Szajewska et al. compared the efficacy of *L. reuteri* DSM 17938 (10^8 CFU/day)with placebo.[45] Eighty exclusively or predominantly breastfed infants were randomly assigned to probiotic or placebo group therapy for 21 days. The primary outcome measures were the treatment success, defined as the percentage of children achieving a reduction in daily crying time ≥ 50 % during the study, and the duration of crying in minutes per day at 7, 14, 21, and 28 days after randomization. The secondary outcome measures were persistence of colics, parental perception of colic severity, and family quality of life as indicated by parental scoring. The percentage of responders was significantly higher (P < 0.001) and the mean daily crying time was significantly reduced (P < 0.0001) in probiotic group compared to placebo at all follow-up visits. In addition, parental perception of colic severity and family quality of life were significantly better in the *L. reuteri* group (both P < 0.0001). Therefore, the results of this study confirmed that breastfed infants with colic benefit from the administration of *Lactobacillus reuteri* DSM 17938. Future studies should clarify the effectiveness of this strain in formula-fed infants with colic. Excellent safety profile of probiotics in otherwise healthy children and relative lack of other effective therapies favor such therapeutic approach.

A large multicenter double-blind, placebo-controlled study involving 8 pediatric and neonatology centers in Italy was performed to evaluate the effectiveness of supplementation with *Lactobacillus reuteri* DSM 17938 in the prevention of different FGIDs in newborn infants.[46] From 589 enrolled infants, both breastfed or formula-fed, 468 completed the study. Infants were randomized at the 3rd day of life to receive either *Lactobacillus reuteri* 10^8 CFU/day or placebo. Parents were instructed to record colic, regurgitation, defecation and crying time. In the probiotic group, compared to the placebo group, the mean time of daily crying was reduced (45.07 ± 12.34 min vs. 96.36 ± 34.67 min; P < 0.01) and the number of daily stool evacuations increased (4.01 ± 1.1 vs. 2.8 ± 0.6; P < 0.01), while the number of regurgitations per day was only insignificantly lower (2.7 ± 1.5 vs. 3.3 ± 2.3; NS). The authors concluded that supplementation with *Lac-*

tobacillus reuteri reduced the onset of colic and constipation in the first month of life.

Infant regurgitation

Infant regurgitation is a FGID defined as regurgitation two or more times per day for 3 or more weeks without retching, hematemesis, aspiration, apnea, failure to thrive, feeding or swallowing difficulties, or abnormal posturing in otherwise healthy infants 3 weeks to 12 months of age.[2] Several mechanisms favor gastroesophageal reflux, the material basis of regurgitation, in infants compared to adults, including undeveloped anti-regurgitation functions of gastroesophageal junction, nutrition with predominantly liquid food and a great deal of time spent in recumbent position. As reflux episodes predominantly occur postprandially when stomach is filled with food, decreased gastric emptying rate may also predispose such infants to regurgitation. Therefore, the effect of *Lactobacillus reuteri* on gastric emptying as well as on regurgitation was studied in several clinical trials.

In the first study by Indrio et al., the influence of *Lactobacillus reuteri* on regurgitation, vomiting, defecation and daily crying time as well as on gastric emptying was studied.[47] Twenty formula-fed healthy preterm newborns were randomly assigned to *Lactobacillus reuteri* ATCC 55730 10^8 CFU a day or placebo for 30 days. For comparison, 10 exclusively breastfed newborns not receiving any supplementation were included. The number of regurgitations, vomiting, stool evacuations per day and minutes of crying per day were recorded by nurses during hospital stay and by parents after discharge. In addition, electric gastric activity and gastric emptying were assessed by electrogastrography (EGG) and gastric ultrasonography at baseline and at the end of the study period. The breastfed newborns and those receiving *L. reuteri* had significantly less regurgitation episodes a day than infants in the placebo group (1.6 ± 0.3, 2.1 ± 0.9, 4.2 ± 1.1, respectively; $P_{\text{breast milk vs. placebo}} < 0.01$, $P_{\text{L.reuteri vs. placebo}} < 0.01$), significantly more evacuations per day (4.8 ± 0.2, 3.7 ± 0.5, 2.1 ± 0.4, respectively; and significant shorter daily crying time (66 ± 11 min, 32 ± 6 min, 88 ± 16 min, respectively; $P_{\text{breast milk vs. placebo}} < 0.05$, $P_{\text{L.reuteri vs. placebo}} < 0.01$). None of the EGG variables showed any differences between the three groups, but gastric emptying rate was significantly faster, and fasting antral area significantly smaller in breast-fed and *L. reuteri* groups than in the placebo group (gastric emptying rate: $P_{\text{breast milk vs. placebo}} < 0.04$, $P_{\text{L.reuteri vs. placebo}} < 0.01$; antral area: $P_{\text{breast milk vs. placebo}} < 0.03$, $P_{\text{L.reuteri vs. placebo}} < 0.01$). Formula-fed newborns supplemented with *Lactobacillus reuteri* had motility patterns similar to those of breastfed newborns. The authors concluded that supplementation with *Lactobacillus reuteri* improved feeding tolerance and gut function in formula-fed preterm newborns.

In the second randomized study an influence of three different infant formulas or breastfeeding on gastrointestinal motility was evaluated.[48] Seventeen infants were breastfed, while the others were randomly assigned to standard formula ($n = 12$), the same formula with addition of probiotic *L. reuteri* 10^8 CFU/day ($n = 10$), and the same formula supplemented with prebiotics (0.8 g/dl of a mixture from galactooligosaccharides (GOS) and fructooligosaccharides (FOS) in a ratio 9:1) ($n = 10$). Gastric EMG and ultrasound were performed at the beginning and after 30 days. At baseline, EGG and gastric emptying data were similar in all four groups. At the end of the study period, a significantly higher percentage of propagation electric activity was found in breastfed, probiotic- and prebiotic-supplemented groups compared to regular formula-fed controls ($P < 0.05$). Regarding gastric emptying rate, again the half-emptying time was significantly shorter in the breast-fed, probiotic- and prebiotic-supplemented groups in comparison with the control group ($P < 0.05$). Although the assessment of clinical symptoms was not performed, the study results gave new insights into upper gastrointestinal tract physiology and the effects of probiotic *L. reuteri* and prebiotics on its function in newborn infants. Feeding with infant formula supplemented with *L. reuteri* or prebiotics (GOS + FOS) stimulate gastric

emptying mimicking the effect of breast milk.

The same group of authors performed a double-blind placebo-controlled interventional trial regarding the efficacy of *Lactobacillus reuteri* DSM 17938 strain in infants with regurgitation.[49] Forty-two consecutive infants, younger than 4 months, with uncomplicated regurgitation were randomized to *L. reuteri* 10^8 CFU/day or placebo for 30 days. Parents were asked to record regurgitation episodes, and gastric emptying was recorded by ultrasound at the beginning and at the end of the study. Nineteen infants from the probiotic and 15 infants from the control group completed the study. At baseline, all parameters were similar for both groups. The number of regurgitations per day significantly decreased in probiotic group compared to controls (1.0 [1.0–2.0] vs. 4.0 [3.0–5.0]; $P < 0.001$). In addition, in the probiotic group gastric emptying rate was significantly increased (+11.7 [-3.9 to + 24.0] % vs. +8.4 [-27.0 to + 23.5] %; $P = 0.01$) and fasting antral area reduced (3.0 [2.0–4.2] cm^2 vs. 4.0 [2.4–5.9] cm^2; $P = 0.01$), confirming one of the possible physiological mechanisms responsible for the clinical effect.

Pain-related FGIDs

Childhood functional abdominal pain is characterized by episodic or continuous abdominal pain at least once per week for at least 2 months with no evidence of an inflammatory, anatomic, metabolic, or neoplastic process that explains the subject's symptoms. In addition, there should be no other important symptoms, such as changed bowel habits, regurgitation or vomiting, implicating other forms of FGIDs. When abdominal pain is accompanied by headache, limb pain, or sleeping difficulty for at least 25 % of the time and causes some loss of child's daily functioning, this disorder can be classified as functional abdominal pain syndrome. Functional dyspepsia includes persistent or recurrent pain or discomfort centered in the upper abdomen that is not relieved by defecation or associated with the onset of a change in stool frequency or stool form (i.e., not irritable bowel syndrome), while in irritable bowel syndrome pain or discomfort should be associated with changes in the frequency or form (appearance) of stool or improvement with defecation.[1]

Several systematic reviews and meta-analyses that were published on probiotic effectiveness in pain-related FGIDs were mainly focused on irritable bowel syndrome. Some of them included studies in adults only,[50,51] children only,[52] or both children and adults.[53,54] The majority of these meta-analyses, with the exception of the one including children only, in which only two studies had been found eligible for the analysis, showed a beneficial impact of probiotics on global symptoms as well as on specific symptoms such as abdominal pain or bloating. Although meta-analyses have a strength of statistically aggregating the data from numerous trials and are therefore considered the gold standard to support evidence of the efficacy of a defined therapeutic approach, they also have several limitations. They aggregate results of studies using different probiotics, doses, patient populations etc.[55] It has been well established that the effects of probiotics are both strain specific and dose dependent. Moreover, effective mechanisms of probiotic action to achieve beneficial effects in diverse subgroups of disorders (for example diarrhea-predominant vs. constipation-predominant IBS) can be completely different. These and a relatively small number of uniform study subjects may well be the reasons why a double-blind, placebo-controlled, randomized study of the efficacy of *Lactobacillus reuteri* ATCC 55730 in adult patients with IBS revealed marginally significant differences only for constipation and passing gases but not for general scores between probiotic and placebo therapy.[56]

The only double-blind, placebo-controlled, randomized trial using *Lactobacillus reuteri* in children with functional abdominal pain was published by Romano et al.[57] They randomized 60 children between 6 and 16 years of age into two groups, receiving either *L. reuteri* DSM 17938 10^8 CFU twice daily or placebo for 4 weeks. Patients were followed-up for additional 4 weeks after stopping the medication. They were requested to record frequency and intensity of pain

(by using Wong-Baker scale), use of drugs and any other symptoms. Two children from each group were lost to follow up due to poor compliance. Pain frequency decreased significantly in both groups ($P < 0.05$), revealing the importance of placebo effect in FGIDs. However, pain intensity decreased significantly with time only in *L. reuteri* group ($P < 0.001$) but not in the placebo group ($P > 0.05$), and was significantly lower in the probiotic than in the placebo group at both 4 and 8 weeks ($P < 0.05$). Moreover, the intensity of pain continued to decrease in patients treated with *L. reuteri* even after stopping the supplementation. This study provided an excellent evidence of efficacy of *Lactobacillus reuteri* DSM 17938 strain for the treatment of functional abdominal pain in children, which may be very important from clinical point of view, as a vast majority of therapeutic strategies were demonstrated as ineffective in this particular FGID.

Functional constipation

Functional constipation in infants, children and adolescents is diagnosed when at least two of the following are met in the period of one month: two or fewer defecations per week, at least one episode of incontinence per week after the acquisition of toileting skills, a history of excessive stool retention or of painful and hard bowel movements, or presence of a large fecal mass in the rectum with large diameter stools which may obstruct the toilet. Accompanying symptoms may include irritability, decreased appetite, and/or early satiety. The accompanying symptoms should disappear immediately following passage of a large stool.[1,2] Several already mentioned mechanisms of action of *Lactobacillus reuteri*, such as the production of short-chain fatty acids, interference with pathogenic bacteria growth and a direct action upon enteric nerves, render this probiotic a good candidate for the treatment of constipation.

The efficacy of *Lactobacillus reuteri* DSM 17938 for the treatment of chronic functional constipation in infants was evaluated in a double-blind, randomized, placebo-controlled trial by Coccorullo et al.[58] Forty-four infants at least 6 months old were randomly assigned to *L. reuteri* DSM 17938 10^8 CFU daily or a placebo treatment for 8 weeks. The use of laxatives was not allowed. Primary outcome measures were the frequency of bowel movements per week, stool consistency, and presence of inconsolable crying episodes, recorded by parents. The median frequency of defecation increased in *L. reuteri* group from 2.82 per week at the beginning to 4.77 per week at week 8 ($P = 0.0001$), and was significantly higher from defecation frequency of placebo group at week 2 ($P = 0.042$), 4 ($P = 0.008$) and 8 ($P = 0.027$). In addition, from 86.5 % of patients in *L. reuteri* group, who had hard consistency of stools at the beginning of the trial, there were only 50 % such infants after 2, and 18 % after 4 and 8 weeks of supplementation. However, stool consistency also improved in the placebo group, therefore there was no statistically significant difference between the probiotic and placebo groups in the stool consistency and the number of crying episodes per week. No adverse effects were observed during study period. This study confirmed *Lactobacillus reuteri* DSM 17938 as an efficient and safe therapeutic option, which can be used alone or in combination with other treatment modalities in infants and children with functional constipation.

Conclusion

Probiotics acting upon different mechanisms in FGIDs represent an interesting therapeutic option for these disorders. Modes of action are specific for each probiotic strain, so the results from clinical studies with one strain must not be automatically implicated to other strains. *Lactobacillus reuteri* is endogenous *Lactobacillus* species in human gastrointestinal tract. It possesses many specific properties that could be usable in the therapy of different FGIDs, among them the capacity to produce short-chain fatty acids and an antimicrobial substance reuterin as well as to affect intestinal sensitivity and motility acting directly on the enteric nerves. The effectiveness of two specific *Lactobacillus reuteri* strains, ATCC 55730 and DSM 17938, for the therapy of different

FGIDs in children was studied in several clinical trials. As strain ATCC 55730 contains potentially transmissible plasmid DNA carrying genes for antibiotic resistance, its daughter strain DSM 17938, possessing all positive characteristics of the mother strain, is recommended for safe application.

The results of high-quality clinical studies revealed the efficacy of *Lactobacillus reuteri* for the treatment of infantile colic and regurgitation, especially when it is associated with delayed gastric emptying. In addition, individual studies pointed out its potential usefulness in functional constipation and functional abdominal pain. However, more clinical studies should be performed including different age groups as well as bigger number of patients, in order to make firm conclusions about *L. reuteri* efficacy in the last two FGIDs. Despite this, considering an extremely low risk for any serious adverse effect and the available evidence of effectiveness, *Lactobacillus reuteri* can be recommended for use in constipation and especially in functional abdominal pain, as no therapeutic option for the latter condition has been proved to be really effective.

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