

# *Lactobacillus reuteri* (DSM 17938) in Infants with Functional Chronic Constipation: A Double-Blind, Randomized, Placebo-Controlled Study

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**Objectives** To evaluate the beneficial effects of *Lactobacillus reuteri* (DSM 17938) in infants with functional chronic constipation.

**Study design** A double-blind, placebo-controlled, randomized study was conducted from January 2008 to December 2008 in 44 consecutive infants at least 6 months old (mean age  $\pm$  SD,  $8.2 \pm 2.4$  SD; male/female, 24/20) admitted to the Gastrointestinal Endoscopy and Motility Unit of the Department of Pediatrics, University "Federico II" of Naples, with a diagnosis of functional chronic constipation. The 44 infants with chronic constipation were randomly assigned to 2 groups: group A (n = 22) received supplementation with the probiotic *L reuteri* (DSM 17938) and group B (n = 22) received an identical placebo. Primary outcome measures were frequency of bowel movements per week, stool consistency, and presence of inconsolable crying episodes, recorded in a daily diary by parents.

**Results** Infants receiving *L reuteri* (DSM 17938) had a significantly higher frequency of bowel movements than infants receiving a placebo at week 2 ( $P = .042$ ), week 4 ( $P = .008$ ), and week 8 ( $P = .027$ ) of supplementation. In the *L reuteri* group, the stool consistency was reported as hard in 19 infants (86.4%) at baseline, in 11 infants (50%) at week 2, and in 4 infants (18.2%) at weeks 4 and 8. However, there was no significant difference between *L reuteri* and placebo groups in the stool consistency at all weeks ( $P = .63$ , week 2;  $P = .38$ , week 4;  $P = .48$ , week 8). Similarly, there was no statistically difference in the 2 groups in the presence of inconsolable crying episodes. No adverse effects were reported.

**Conclusions** The administration of *L reuteri* (DSM 17938) in infants with chronic constipation had a positive effect on bowel frequency, even when there was no improvement in stool consistency and episodes of inconsolable crying episodes. Because of their safety profile, probiotics may be an attractive option in the treatment of functional constipation. (*J Pediatr* 2010; ■: ■-■).

Constipation is a common and distressing pediatric problem, with a prevalence ranging from 7% to 30% in both western and non-western countries.<sup>1</sup> No organic cause is found in 90% to 95% of children with constipation.<sup>2</sup> Only 60% of children with constipation have successful treatment with laxatives,<sup>3</sup> and a sizable proportion of children needs long-term therapy. After 1 year of treatment, constipation persisted in 53% of children, and 52% of them were still constipated 5 years later.<sup>4</sup> Moreover, approximately 30% of children beyond puberty continue to struggle with constipation symptoms, such as infrequent, painful evacuation of stools and fecal incontinence.<sup>3</sup> Development of new therapeutic strategies is necessary to treat these challenging patients more effectively.

There is growing interest in the use of probiotics in organic and functional gastrointestinal disorders. Probiotics are reported to be effective in the treatment of inflammatory bowel disease, traveller diarrhea, and constipation.<sup>5,6</sup> One rationale for using probiotics to treat constipation is a report of dysbiosis in the intestinal flora of patients with chronic constipation.<sup>7</sup> Functional constipation in children therefore would seem, on the whole, to induce no bacterial overgrowth, but only changes in selected bacterial species. The stools of the children with constipation showed a disturbed microbiota characterized by a high clostridia count in comparison with other genera (*Bacteroides* and *Escherichia coli*) and a substantial frequency of clostridia and enterobacteriaceae species, which are rarely isolated in healthy children. In addition, probiotics might improve intestinal motility; bifidobacteria and lactobacilli produce lactic acid, acetic acid, and other acids, resulting in a lowering of pH in the colon. A lower pH enhances peristalsis of the colon and subsequently decreases colonic transit time, which is beneficial in the treatment of constipation.<sup>8</sup>

A limited number of studies have been published about the effects of probiotics on constipation in children, but so far there is no hard evidence to recommend the use of probiotics in children affected by chronic constipation.

The aim of our study was to evaluate the beneficial effects of *Lactobacillus reuteri* (DSM 17938), one of the few endogenous *Lactobacillus* species in the human gastrointestinal tract, in infants with functional chronic constipation.

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Noos (Italy) provided probiotic and placebo for the study and it paid for color reproduction charges. The authors declare no conflicts of interest.

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BM Bowel movement

## Methods

A double-blind, placebo-controlled, randomized study was conducted from January 2008 to December 2008 in 44 consecutive formula-fed infants >6 months of age (mean age  $\pm$  SD,  $8.2 \pm 2.4$  SD; male/female: 24/20) referred for functional chronic constipation to the Gastrointestinal Endoscopy and Motility Unit of the Department of Pediatrics, University “Federico II” of Naples, Italy.

Functional constipation is defined by the Rome III Criteria as having at least two of these symptoms:  $\leq 2$  defecations per week; history of excessive stool retention and painful or hard bowel movements; presence of a large fecal mass in the rectum; history of large-diameter stools.<sup>9</sup>

Infants with organic causes of constipation such as Hirschsprung’s disease, spinal bifida (occulta), hypothyroidism or other metabolic or renal abnormalities, and mental retardation, infants taking oral laxatives or antibiotics, and infants who were fed breast milk and formula with the addition of probiotics, prebiotics, or both were excluded. Probiotic or prebiotic food supplements were not allowed through the study period.

Weaning started in a standard fashion at 5 months of age with fruit followed by weaning purees with a dose of 0.5 g/Kg/day of fiber, according to guideline proposed by the American Academy of Pediatrics Committee on Nutrition.<sup>10</sup> At enrollment, a medical history was collected and all patients underwent clinical examination, including rectal digital examination.

The 44 infants were randomly assigned to 2 groups according to an automatically generated randomization list: group A (n = 22) received supplementation with the probiotic *L reuteri* (DSM 17938), and group B (n = 22) was given an identical placebo. *L reuteri* (DSM 17938) was administered at a dose of  $10^8$  colony-forming units in 5 drops of a commercially available oil suspension (Reuterin, Noos S.r.l.; BioGaia AB, Stockholm, Sweden), 30 minutes after feeding, once per day for 8 weeks. This oil suspension is stable for 24 months at 2°C to 8°C (as documented by the manufacturer). During the study period, parents were instructed to keep the product refrigerated. Excellent compliance has been defined as no violation of the protocol for the study product intake.

The use of laxatives was not allowed during the treatment period, whereas glycerin suppository was used only when there was no defecation for >5 days.

Parents received a stool diary to record the frequency of daily bowel movements, stool consistency, inconsolable crying episodes, and the use of enema of their children. Evaluation was conducted during the visits at the outpatient clinic 2, 4, and 8 weeks after the study start. During each visit, the physician assessed the patient’s diary and examined the child. Treatment success was defined as  $\geq 3$  defecation per week, and stool consistency was defined as hard, normal, and watery. The interpretation of inconsolable infant crying was left to the mothers. Mothers were asked to report the number of crying episodes that were difficult to soothe in their babies.

Primary outcome measures were frequency of bowel movements per week, stool consistency, and presence of inconsolable crying episodes. Secondary outcome was comparison of the frequency of defecation, stool consistency, and presence of inconsolable crying episodes in the two groups.

Informed consent was obtained from parents of all the enrolled patients, and the study was approved by the independent ethics committee of the University of Naples, Federico II.

## Statistical Analysis

All data were stored in a common database and statistically analyzed with SPSS software version 8.0 (SPSS, Chicago, Illinois). Comparisons in proportions were made with the  $\chi^2$  test. The Fischer exact test was dependent on the number of observations. A *P* value <.05 was considered to be statistically significant.

The power evaluation for both univariate and multivariate tests has been computed with the SPSS Multivariate Anova: population rate, 2.9%; smallest difference, 25%; first type error, 0.05; second type error, 0.05; *P* < .05; power, 95%; case/control, 1/1.

## Results

Forty-four infants >6 months old with chronic constipation were enrolled, and all patients completed the study. Of the 44 infants with constipation, 22 were assigned randomly to treatment with *L reuteri* (DSM 17938), and 22 were assigned to receive a placebo. The study groups were well matched in age, sex, and constipation characteristics. Age, sex, and baseline characteristics are shown in the **Table**.

No differences have been identified in the diet of infants treated with *L reuteri* and that of the placebo group.

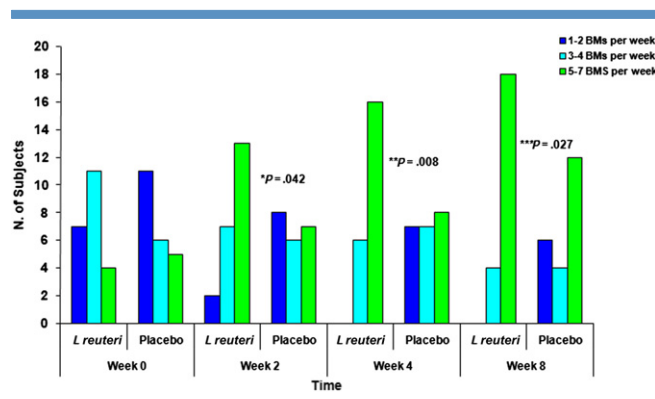
The frequency of bowel movements (BMs) per week increased in infants who received *L reuteri* (DSM 17938) (**Figure 1**). The frequency of BMs was 2.82 per week at week 0, compared with 4.77 at week 8 (*P* = .0001; 95% CI,  $-2.75$  to  $-1.16$ ).

In addition, infants treated with *L reuteri* had a significantly higher defecation frequency than placebo at week 2 of treatment (*P* = .042), at week 4 of treatment (*P* = .008), and at week 8 of treatment (*P* = .027).

In the *L reuteri* group, the stool consistency was reported to be hard in 19 infants (86.4%) at baseline, in 11 infants (50%)

**Table.** Age, sex, and baseline characteristics

	<i>L reuteri</i> group (n = 22)	Placebo group (n = 22)
Age, months (mean $\pm$ SD)	8.2 $\pm$ 2.4	8.8 $\pm$ 2.1
Sex (female/male)	14/8	6/16
<b>BMs/week</b>		
1-2/week	31.8%	50%
3-4/week	50%	27.3%
5-7/week	18.2%	22.7%
Hard BMs	66.4%	59.1%
Rectal impaction	72.7%	52.4%



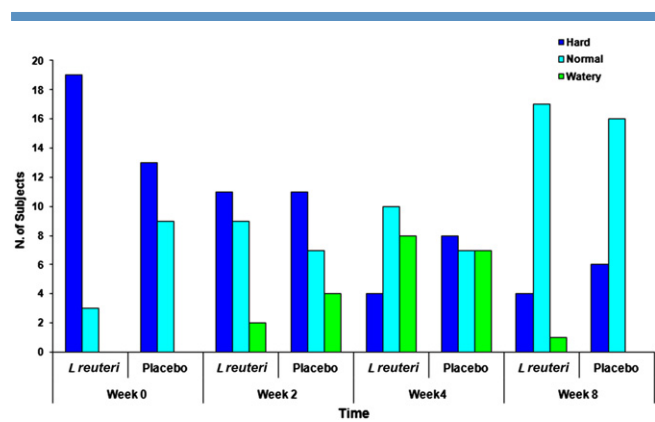
**Figure 1.** Frequency of BMs in the *L reuteri* and placebo groups. Infants treated with *L reuteri* had a significantly higher defecation frequency than infants in the placebo group at week 2 of treatment ( $P = .042$ ), at week 4 ( $P = .008$ ), and at week 8 ( $P = .027$ ).

at week 2, and in 4 infants (18.2%) at weeks 4 and 8 ( $P = .34$ , week 2;  $P = .01$ , week 4;  $P = .01$ , week 8).

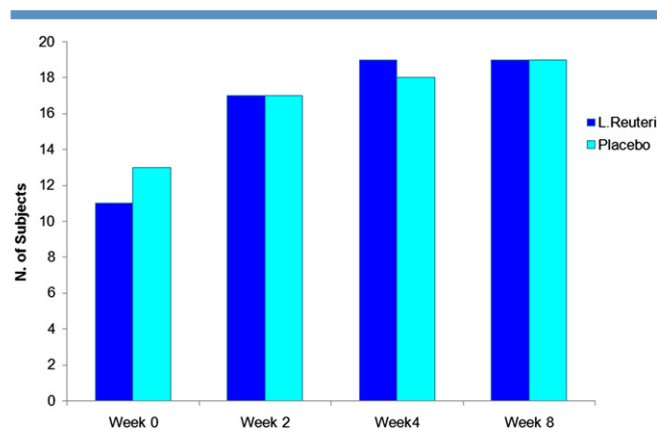
However, there was no statistically significant difference between the *L reuteri* and placebo groups in the stool consistency at all weeks ( $P = .63$ , week 2;  $P = .38$ , week 4;  $P = 0.48$ , week 8; **Figure 2**).

During the study period, we observed a significant increase of inconsolable crying episodes in the *L reuteri* group ( $P = .02$ ), whereas a trend toward significance was found in the placebo group ( $P = .08$ ). In addition, there was no statistically significant difference in the 2 groups in the presence of inconsolable crying episodes at all weeks ( $P = .64$ , week 2;  $P = .50$ , week 4;  $P = .66$ , week 8; **Figure 3**).

Compliance with the study preparation was excellent in 94.6% of the infants in the *L reuteri* group and in 86.9% of the infants in the placebo group. No adverse effects such as vomiting, bloating, and increased flatulence were reported.



**Figure 2.** Stool consistency in the *L reuteri* and placebo groups. There was no statistically significant difference in the 2 groups in the stool consistency at all weeks ( $P = .63$ , week 2;  $P = .38$ , week 4;  $P = .48$ , week 8).



**Figure 3.** Inconsolable crying episodes in the *L reuteri* and placebo groups. There was no statistically significant difference in the 2 groups in the presence of inconsolable crying episodes at week 0, 2, 4, 8.

## Discussion

This study demonstrates a significant efficacy of *L reuteri* (DSM 17938) on functional chronic constipation of infants. The frequency of BMs significantly increased from 2.82 per week at week 0 to 4.77 at week 8 ( $P = .0001$ ).

Subjects treated with *L reuteri* (DSM 17938) had a significantly higher defecation frequency than subjects receiving a placebo at week 2 of treatment ( $P = .042$ ), at week 4 ( $P = .008$ ), and at week 8 ( $P = .027$ ). We found a significant improvement of stool consistency in the *L reuteri* group during treatment, but no significant difference was shown when comparing the 2 groups. Similarly, there was no statistically significant difference in the 2 groups in the presence of inconsolable crying episodes. There were no clinical adverse events related to *L reuteri* (DSM 17938). However, we observed a significant increase of inconsolable crying episodes in the *L reuteri* group and a trend toward significance was shown in the placebo group. This seems to be in contrast to earlier studies, but we could hypothesize that the pathogenesis of the inconsolable crying episodes in this cohort is different from that of the infantile colics, which affect infants aged <6 months.<sup>9,11</sup> Because of the considerable variation in infant cry characteristics, it is impossible to define what is normal crying behavior and what is abnormal crying behavior. Bias cannot explain our findings. Because this study was based on parental reports, social, cultural, and environmental differences in maternal perceptions of crying might be an explanation. We do not believe that the increase of inconsolable crying episodes was related to the use of *L reuteri*, because we observed a higher rate of inconsolable crying episodes in both groups during the treatment. In addition, *L reuteri* was well tolerated, and no adverse events associated with this supplementation were reported in any of the existing trials in children.<sup>11-14</sup>

Loening-Baucke showed that the prevalence rate for constipation, defined by the NASPGHAN and the Rome II criteria, in the first year of life was 2.9%.<sup>15-17</sup> Symptoms develops

during the first year of life in approximately 40% of children with functional constipation; peak incidence occurs at the time of toilet training, between 2 and 4 years of age.<sup>18,19</sup> Constipation in infants often corresponds with the change from breast milk to commercial formula or with the introduction of solids in their diet. In some babies, an acute episode of constipation may occur in association with a change in the diet. Passage of dry and hard stools may cause anal fissures and pain. Genetic predisposition may play a role because constipation often dates back to the first months of life, and many patients have a positive family history of constipation.<sup>20</sup> A study by van den Berg et al evaluating the clinical course of severe functional constipation in early childhood demonstrated that, 6 months after initial evaluation, only 69% of the children had recovered, and a relapse in 15% of them was observed within 3 years.<sup>21</sup> In addition, it has been demonstrated that early age of onset and family history of constipation are predictive of persistence.<sup>22</sup> Despite therapy, constipation is still present in 30% of children after puberty.<sup>3</sup> It is clear that an early therapeutic intervention could beneficially contribute to the outcome of constipation in infant. Although there is evidence that gut flora is important in gut motility, there is little evidence that gut flora is abnormal in constipation.<sup>23,24</sup> Hyams et al had evaluated the stool characteristics of young infants and found that breastfed infants as group passed twice as many BMs than infants receiving cow's milk formulas and that infants receiving soy formula had hard/firm stools significantly more often than breastfed and other formula-fed infants.<sup>25</sup> This data may be related to differences in microflora. The number of bifidobacteria is higher in breastfed babies. Breast milk has an optimal whey protein composition, rich in  $\alpha$ -lactalbumin, lactose, and with low phosphorus content. Part of the lactose escapes digestion and is fermented, releasing water (that moves into lumen to soften stools) and short-chain fatty acids (that lower the pH and provide fuel for bifidobacteria). Lactose passes through normal digestion, resulting in the production of lactic acid, which has a low buffering capacity. Because of this, intestinal pH decreases and bifidobacteria proliferate.<sup>26</sup>

In adults, one double-blind, randomized trial showed that *L casei* Shirota improved gastrointestinal symptoms in patients with chronic constipation.<sup>27</sup> A small non-randomized open clinical trial in elderly subjects showed that supplementation with a combination of *L rhamnosus* and *Propionibacterium freudenreichii* increased defecation frequency compared with a group receiving *L reuteri* and an unsupplemented group. No reduction in laxatives use was observed.<sup>28</sup>

A randomized controlled trial addressed the use of probiotics in the treatment of children with constipation and concluded that *Lactobacillus GG* was not an effective adjunct to lactulose.<sup>29</sup> In addition, a recent double-blind, placebo-controlled, randomized study in children with chronic constipation showed an increase in defecation frequency, but there was no statistically significant difference in efficacy between magnesium oxide and *Lactobacillus casei rhamnosus* (Lcr35).<sup>30</sup> A pilot non-randomized, non-placebo controlled study showed that a mixture of probiotics, containing Bifido-

bacteria and *Lactobacilli*, has positive effects on frequency of bowel movements, stool frequency, number of fecal incontinence episodes, and presence of abdominal pain.<sup>31</sup>

In our study, we recruited infants >6 months old to avoid subjects with dyschezia. Dyschezia is seen in the first few months of life and can occur several times a day. The symptoms improve without intervention, and parents should abstain from rectal stimulation.<sup>15</sup>

In conclusion, this prospective, placebo-controlled, double-blind study demonstrates the efficiency and safety of *L reuteri* DMS 17938 in infants with functional constipation to increase stool frequency. On the basis of our results, probiotics as a natural, safe, and well-tolerated treatment may provide a simple and attractive way to treat infantile functional chronic constipation. A possible link between constipation and changes in intestinal flora needs to be evaluated in the future. ■

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## References

- van den Berg MM, Benninga MA, Di Lorenzo C. Epidemiology of childhood constipation: a systematic review. *Am J Gastroenterol* 2006;101:2401-9.
- Loening-Baucke V. Chronic constipation in children. *Gastroenterology* 1993;105:1557-64.
- van Ginkel R, Reitsma JB, Büller HA, van Wijk MP, Taminiu JA, Benninga MA. Childhood constipation: longitudinal follow-up beyond puberty. *Gastroenterology* 2003;125:357-63.
- Staiano A, Andreotti MR, Greco L, Basile P, Auricchio S. Long-term follow-up of children with chronic idiopathic constipation. *Dig Dis Sci* 1994;39:561-4.
- Doron S, Gorbach SL. Probiotics: their role in the treatment and prevention of disease. *Expert Rev Anti Infect Ther* 2006;4:261-75.
- Kruis W. Review article: antibiotics and probiotics in inflammatory bowel disease. *Aliment Pharmacol Ther* 2004;20(Suppl. 4):75-8.
- Zoppi G, Cinquetti M, Luciano A, Benini A, Muner A, Bertazzoni Minelli E. The intestinal ecosystem in chronic functional constipation. *Acta Paediatr* 1998;87:836-41.
- Picard C, Fioramonti J, Francois A, Robinson T, Neant F, Matuchansky C. Review article: bifidobacteria as probiotic agents—physiological effects and clinical benefits. *Aliment Pharmacol Ther* 2005;22:495-512.
- Hyman PE, Milla PJ, Benninga MA, Davidson GP, Fleisher DF, Taminiu J. Childhood functional gastrointestinal disorders: neonate/toddler. *Gastroenterology* 2006;130:1519-26.
- Williams CL, Bollella M, Wynder EL. A new recommendation for dietary fiber in childhood. *Pediatrics* 1995;96(5 Pt 2):985-8.
- Savino F, Pelle E, Palumeri E, Oggero R, Miniero R. *Lactobacillus reuteri* (American type culture collection strain 55730) versus simethicone in the treatment of infantile colic: a prospective randomized study. *Pediatrics* 2007;119:e124-30.
- Connolly E, Abrahamsson T, Bjorksten B. Safety of D(-)-lactic acid producing bacteria in the human infant. *J Pediatr Gastroenterol Nutr* 2005;41:489-92.



13. Ogden NS, Bielory L. Probiotics: a complementary approach in the treatment and prevention of pediatric atopic disease. *Curr Opin Allergy Clin Immunol* 2005;5:179-84.
14. Rosenfeldt V, Benfeldt E, Valerius NH, Paerregaard A, Michaelsen KF. Effect of probiotics on gastrointestinal symptoms and small intestinal permeability in children with atopic dermatitis. *J Pediatr* 2004;145:612-6.
15. Loening-Baucke V. Prevalence, symptoms and outcome of constipation in infants and toddlers. *J Pediatr* 2005;146:359-63.
16. Baker SS, Liptak GS, Colletti RB, Croffie JM, Lorenzo Di, Ector W, et al. Constipation in infants and children: evaluation and treatment. *J Pediatr Gastroenterol Nutr* 1999;29:612-26.
17. Rasquin-Weber A, Hyman PE, Cucchiara S, Fleisher DR, Hyams JS, Milla PJ, et al. Childhood functional gastrointestinal disorders. *Gut* 1999;45:5II60-8.
18. Caplan A, Walker L, Rasquin A. Validation of the Pediatric Rome II criteria for functional gastrointestinal disorders using the questionnaire on pediatric gastrointestinal symptoms. *J Pediatr Gastroenterol Nutr* 2005;41:305-16.
19. Voskuijl WP, Heijmans J, Heijmans HS, Taminiou JA, Benninga MA. Use of Rome II criteria in childhood defecation disorders: applicability in clinical and research practice. *J Pediatr* 2004;145:213-7.
20. Masi P, Miele E, Staiano A. Pediatric anorectal disorders. *Gastroenterol Clin North Am* 2008;37:709-30.
21. van den Berg MM, van Rossum CH, de Lorin F, Reitsma JB, Di Lorenzo C, Benninga MA. Functional constipation in infants: a follow up study. *J Pediatr* 2005;147:700-4.
22. Staiano A, Andreotti MR, Greco L, Basile P, Auricchio S. Long-term follow-up of children with chronic idiopathic constipation. *Dig Dis Sci* 1994;39:561-4.
23. Szajewska H, Setty M, Mrukowicz J, Guandalini S. Probiotics in gastrointestinal diseases in children: hard and not-so-hard evidence of efficacy. *J Pediatr Gastroenterol Nutr* 2006;42:454-75.
24. Husebye E, Hellstrom PM, Sundler F, Chen J, Midtvedt T. Influence of microbial species on small intestinal myoelectirc activity and transit in germ free rats. *Am J Physiol Gastrointest Liver Physiol* 2001;280:G368-80.
25. Hyams JS, Treem WR, Etienne NL, Weinerman H, MacGilpin D, Hine P, et al. Effect of infant formula on stool characteristics of young infants. *Pediatrics* 1995;95:50-4.
26. Vandenplas Y, Benninga M. Probiotics and functional gastrointestinal disorders in children. *J Pediatr Gastroenterol Nutr* 2009;48:S107-9.
27. Koebnick C, Wagner I, Leitzmann P, Stern U, Zunft HJ. Probiotic beverage containing *Lactobacillus casei* Shirota improves gastrointestinal symptoms in patients with chronic constipation. *Can J Gastroenterol* 2003;17:655-9.
28. Möllenbrink M, Bruckschen E. Treatment of chronic constipation with physiologic *Escherichia coli* bacteria. Results of a clinical study of the effectiveness and tolerance of microbiological therapy with the *E coli* Nissle 1917 strain (Mutaflor) *Med Klin (Munich)* 1994;89:587-589.
29. Banaszkievicz A, Szajewska H. Ineffectiveness of *Lactobacillus GG* as an adjunct to lactulose for the treatment of constipation in children: a double-blind, placebo-controlled randomized trial. *J Pediatr* 2005;146:364-9.
30. Bu LN, Chang MH, Ni YH, Chen HL, Cheng CC. *Lactobacillus casei rhamnosus Lcr35* in children with chronic constipation. *Pediatr Int* 2007;49:485-90.
31. Bekkali NL, Bongers ME, Van den Berg MM, Liem O, Benninga MA. The role of a probiotics mixture in the treatment of childhood constipation: a pilot study. *Nutr J* 2007;6:17.