HK J Paediatr (new series) 2022;**27**:182-187

Original Article

Investigation of the Effectiveness of *Lactobacillus reuteri* DSM 17938 in the Treatment of Infantile Colic: A Double-blind Placebo-controlled Randomised Study

A **Basturk**

Abstract

Purpose: This study aims to investigate the effectiveness of *Lactobacillus reuteri* DSM 17938 in the treatment of infantile colic in infants fed with breast milk or formula. ***Methods:*** The study sample consisted of 38 infants aged from 1 to 4 months. The infants were randomly divided into two groups. The first group (20 infants) was given 10^8 colony-forming units of *Lactobacillus reuteri* DSM 17938 once daily for 28 days regardless of the diet (breast milk or baby formula), while the second group (18 infants) was given placebo, again once daily. The mothers or caregivers of the infants were given charts to indicate the infants' daily crying episodes, restlessness times. ***Findings:*** At the end of the 28-day treatment, mean total crying episode times were 945±420 minutes in the probiotics group and 1205±450 minutes in the placebo group, with statistically significant difference (p=0.041). Similarly, after treatment, mean total restlessness times were 1005±390 minutes in the probiotics group and 1155±345 minutes in the placebo group, with statistically significant difference (p=0.037). In addition, after treatment, daily crying episodes and restlessness times were reduced down to 23 and 25 minutes in the probiotics group, respectively, and the difference was statistically significant (p=0.017 and p=0.026, respectively). In the placebo group, daily crying episodes and restlessness times were reduced down to 42 and 41 minutes after treatment, respectively, but the difference was not statistically significant (p=0.319 and p=0.273, respectively). ***Conclusion:*** The use of *Lactobacillus reuteri* DSM 17938 in infants with infantile colic significantly reduces crying episodes and restlessness.

Key words *Crying episode; Lactobacillus reuteri DSM 17938; Infantile colic*

Introduction

Infantile colic was first described by Wessel et al[1] in 1954 as crying or restlessness lasting for three or more hours a day, three or more days a week, for three or more weeks in an otherwise healthy infant. These episodes are also called "first three months colic". This is seen in 5-25% of infants.[2,3] Excessive crying is a very common complaint in the first three months after birth. Infantile colic can be observed in 2-week-old infants at the earliest, and the restlessness and crying episodes usually peak at 6-8 weeks after birth, often regressing around the 3rd-4th month.[1,4-6] These episodes of crying, restlessness and excessive gas may begin and regress in the absence of any clear trigger or cause, making it difficult to prevent or treat infantile colic.[1,4-7] The aetiology of infantile colic is still uncertain, although some authors have proposed a number of theories. These include excessive gas production and intestinal contraction, hypersensitivity to cow's milk protein, temporary lactase deficiency, maternal

Department of Pediatric Gastroenterology, Faculty of Medicine, Gaziantep University, Universite Bulvari-Campus 27310, Gaziantep, Turkey

A **Basturk** *MD*

Correspondence to: Dr A **Basturk**
Email: drahmetbasturk@hotmail.com

Received March 27, 2021

depression, insufficient bond between mother-infant or excessive stimulation of parents, difficult temperament of the infant and insecure attachment of the parents.[5-9] Most importantly, research has shown gut microbiota to have a crucial role in the aetiology of infantile colic.[10-13] Infants with and without infantile colic are reported to have considerably varying gut flora.[11,13,14] Studies supporting this have shown infants with infantile colic to have insufficient *lactobacilli* and excessive *Clostridium difficile, Escherichia spp.*, and *Klebsiella spp.* in their gut flora.[11,15] Probiotics are defined as "living microorganisms that provide health benefits to their host when in sufficient amounts".[16]

Our study was carried out with the aims of investigating the effectiveness of *Lactobacillus reuteri* DSM 17938 *(L. reuteri)* on complaints of crying episodes and restlessness commonly seen in infants with infantile colic.

Material and Method

Patients

This study was conducted between October 2017 and April 2018 on 38 infants aged from 1 to 4 months who were fed breast milk or baby formula. The Rome IV criteria[4] were used for infantile colic diagnosis. Ethics committee approval was obtained from the local ethics committee before the study, along with written and oral consent forms from the families of the infants. Infants aged between 1-4 months diagnosed as infantile colic according to the criteria of Rome IV[17] were included the study. Infants were excluded if they were diagnosed with otitis media, urinary tract infection, stomatitis, meningitis, peristalsis problems, gastroesophageal reflux, invagination, hernia, corneal abrasion, foreign body in the eye, hair tourniquet, child abuse, drugs that pass through breast milk, cow's milk allergy, vaccine reaction, drug withdrawal in newborns, west syndrome, delayed maturation in neurological maturation, arrhythmia (supraventricular tachycardia) and congestive heart failure. Patients and infants who had used any probiotics in the last month were also excluded.

Double-blinded Study Design, Randomisation and Treatment

Patients diagnosed with infantile colic in the pediatric gastroenterology outpatient clinic of our hospital were directed to a Pediatric Gastroenterology Nurse and given boxes with unknown contents and only a code number. Two separate treatments were administered as probiotic and placebo. The two treatments were given by drugs completely identical in terms of colour, odor, taste and packaging properties, but with differing code numbers on the package, which were unknown to the doctor, the nurse and the patient and only known to the manufacturer.

This study was carried out prospectively, with the first group receiving probiotics treatment and the second group receiving placebo treatment. The first group was given 10^8 colony-forming units (CFU) of *L. reuteri* once a daily for 28 days regardless of the diet (breast milk or baby formula), while the second group was given a placebo completely identical in terms of colour, odor, taste and packaging properties. While the probiotic product is 5 ml and packaged to contain 5 drops of 10^8 CFU *L.reuteri*, sunflower oil, medium chain triglyceride oil and silicon dioxide (Biogaia® $1x10^8$ CFU/5 drops), the placebo product has the same content without *L.reuteri*. Receiving treatment was terminated if symptoms like abdominal pain, distention or vomiting were developed which were not seen before the treatment.

Evaluation of Outcome Measures

The primary outcome measures are complete regression of complaints and improvement in daily crying times by more than 50% after the 28-day probiotic treatment. The secondary outcome measures are improvement in mean total daily crying episodes and restlessness times and improvement in frequency of probiotic side effects such as nausea and vomiting after the 28-day treatment.

Mean total crying and restlessness times after the 28-day treatment were compared between both groups, along with any improvement in daily crying episodes and restlessness times. Those with more than 50% regression in daily crying times were evaluated separately. In data collection, those containing the same code number were collected under the same group. The results were compared according to the code numbers. The data were analysed by a statistician blinded to the contents of each code number, and the statistical significance between the groups was examined. The contents of the codes were ultimately revealed, and the probiotic and placebo names corresponding to the codes were written in tables and figures. A chart was given to the relatives of the patients and they were recommended to mark the treatment they used daily. Patients with deficiencies in the schedule were considered as non-compliant with treatment.

Statistical Analysis

The SPSS 15.0 (SPSS Inc. Chicago, IL, USA) version package software and the Microsoft Office Excel 2010 version were used in statistical analyses. Data comparison was done using the Mann-Whitney U Test and the Chi-Squared Test. In descriptive statistical analysis, numerical variables are given as mean ± standard deviation, and categorical data are given as number and percentage. The level of significance was taken as $p<0.05$.

Results

In this study, 56 patients were diagnosed with infantile colic. Seven patients were excluded as they refused to participate. The remaining 49 patients consisted of two randomly selected groups with double-blind treatment (26 in the probiotic group and 23 in the placebo group). However, 6 patients in the probiotic group and 5 in the placebo group were excluded during follow-up as they did not complete the treatment. Therefore, the probiotic group consisted of 20 patients, while the placebo group consisted of 18. The flow chart of the patients is shown in Figure 1. There was no difference between the groups in terms of findings at admission, age or gender distribution (Table 1). The patients had a mean age of 3.23±0.24 months and a female/male ratio of 0.92. At the end of the 28-day treatment, mean total crying episode times were 945±420 minutes in the probiotics group and 1205±450 minutes in

Table 1 Comparison of groups according to demographic characteristics and application symptoms

Parameters	Placebo	Probiotic	p
n	18	20	0.745*
Age (month)	3.05±0.12	3.49±0.36	0.307*
Gender	10 Male	11 Male	0.714
	8 Female	9 Female	
Breast milk	12	13	0.418
Baby formula	6	7	0.561
Daily crying crises (time-min)	49	47	0.826
Daily restlessness (time-min)	46	45	0.724

*Mann Whitney U test was applied and Chi Square test was applied to others.

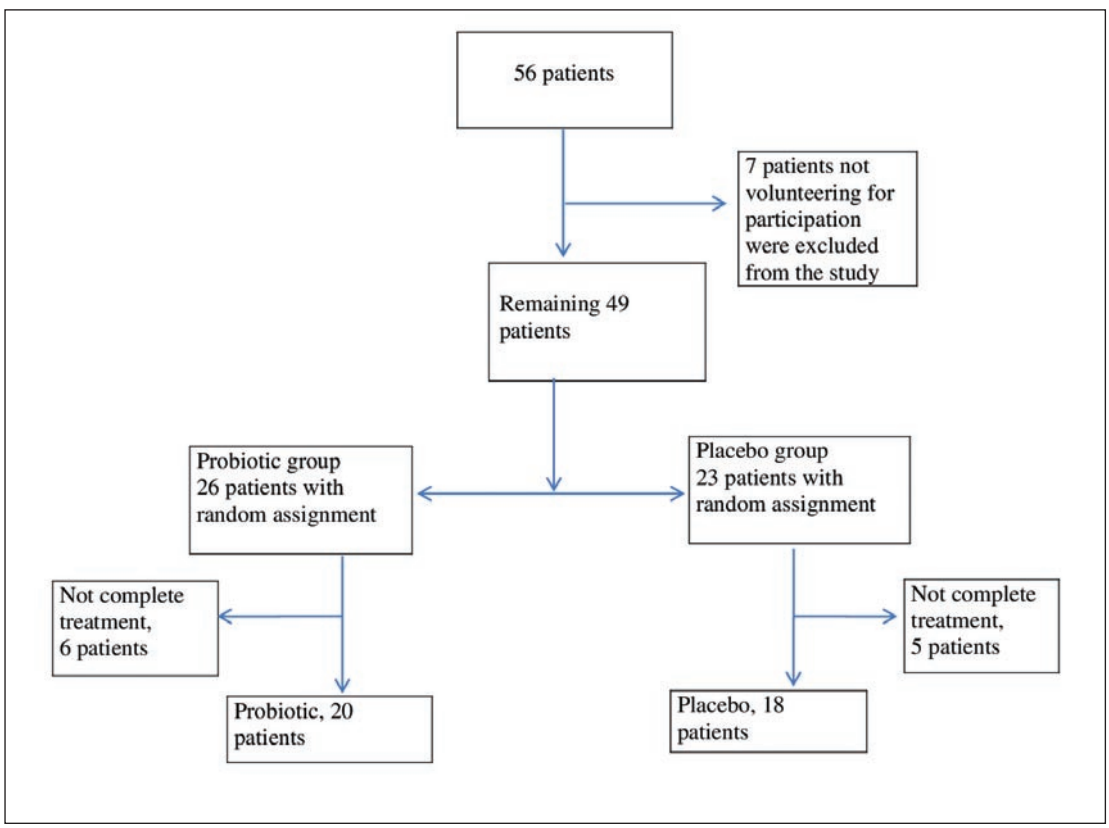

Figure 1 Patient flow chart.

the placebo group, with a statistically significant difference (p=0.041). Similarly, after treatment, mean total restlessness times were 1005±390 minutes in the probiotics group and 1155±345 minutes in the placebo group, with a statistically significant difference (p=0.037). In addition, after treatment, daily crying episodes and restlessness times were decreased by 23 and 25 minutes in the probiotics group, respectively (p=0.017 and p=0.026, respectively). This difference was also statistically significant. In the placebo group, daily crying episodes and restlessness times were reduced down to 42 and 41 minutes after treatment, respectively, but the difference was not statistically significant (p=0.319 and p=0.273, respectively) (Table 2). The number of infants with a 50% decrease in mean crying time after the 28-day treatment was 12 in the probiotics group and 5 in the placebo group, with a significant difference (p=0.046) (Figure 2). No side effect has been observed in any of the patients during the study.

Table 2 Symptoms of infantile colic before and after 28 days of treatment, by study group. Values are numbers of patients with each complaint

Symptoms	**Placebo**			**Probiotic**		
	BT	AT	p^{1}*	BT	AT	p^{1}*
Daily crying crises (time-min)	49	42	0.319	47	23	0.017
Daily restlessness (time-min)	46	41	0.273	45	25	0.026
		AT			**AT**	**p^{2}***
Total crying crises (time-min)		1205±450			945±420	0.041
Total restlessness (time-min)		1155±345			1005±390	0.037

BT: Before treatment, AT: After treatment, *Chi-square test

p^{1} Comparison of daily crying and restless periods of placebo and probiotic groups before and after treatment

p^{2} Comparison of total crying and restlessness times of both groups after treatment

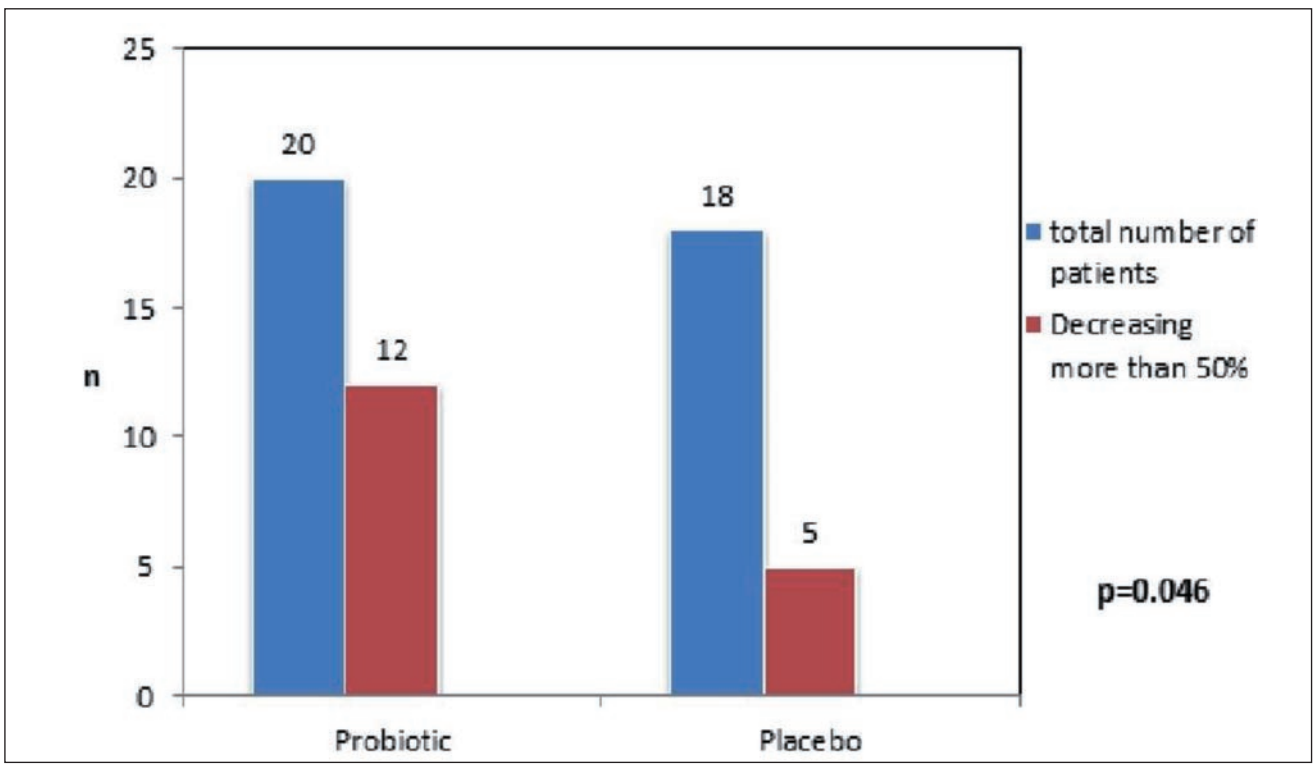

Figure 2 Comparison of the groups according to the number of babies whose crying time decreased by more than 50%.

Discussion

One comparative placebo study with *L. reuteri* on infants with infantile colic found that total crying and restlessness times and daily crying times were significantly decreased in the probiotics group.[18] Another study with *L. reuteri* on infants only fed with breast milk reported that the probiotics group showed significant improvement in daily crying episodes and maternal depression.[19] Szajewska et al[20] conducted a comparative placebo study with *L. reuteri* on infants only fed with breast milk and fed more than 50% of their diet with breast milk and found that daily crying times and crying episodes showed quicker improvement in the probiotics group. As a result of meta-analysis of 6 randomised controlled studies, Xu et al[21] *L. reuteri* possibly increased the effectiveness of treatment for infantile colic and decreased crying time at two to three weeks without causing adverse events.

Our results show similarity to those mentioned above. Our study showed both mean total daily crying times and restlessness times to be significantly improved in the probiotics group. The studies mentioned above[18-20] reported improvement rates of 5-38% in their placebo groups, consistent with our rate of 27%.

However, contrary to our study, there are also studies in the literature reporting insignificant findings. A comparative placebo study by Sung et al[22] with *L. reuteri* on infants fed with breast milk or baby formula showed no significant improvement in crying and restlessness times in the probiotics group. Another comparative placebo study by Savino et al[23] with *L. reuteri* reported no significant improvement in crying times in the probiotics group, but they observed a significant number of infants that yielded more than 50% improvement in crying times, similar to our results. Again, similarly, they observed no side effects in any patient.

Fatheree et al[24] with *L. reuteri* reported no significant improvement in crying, fussing times in the probiotics group but however, they have shown that it is safe to use as a single daily dose.

With clinical significance of 0.05, and a power of 80% to detect an absolute difference of 24% we needed a sample size of 30 patients for placebo and 30 patients for probiotic group. But our probiotic and placebo groups consisted of 20 and 18 patients respectively. This is the limitation of the study.

In conclusion, our study showed that *L. reuteri* helped significantly reduce crying episodes and restlessness times in infants with infantile colic. We observed no side effects in any of our patients due to *L. reuteri* use. However, we recommend that further studies with larger patient groups are needed for a definite judgement on the efficacy and safety of the use of *L. reuteri*.

Declaration of Interest

None

References

1. Wessel MA, Cobb JC, Jackson EB, Harris GS, A C Detwiler AC. Paroxysmal fussing in infancy, sometimes called colic. Pediatrics 1954;14:421-35.
2. Clifford TJ, Campbell MK, Speechley KN, Gorodzinsky F. Sequelae of Infant Colic: Evidence of Transient Infant Distress and Absence of Lasting Effects on Maternal Mental Health. Arch Pediatr Adolesc Med 2002;156:1183-8.
3. Lucassen PL Assendelft WJ, van Eijk JT, Gubbels JW, Douwes AC, van Geldrop WJ. Systematic review of the occurrence of infantile colic in the community. Arch Dis Child 2001;84:398-403.
4. Brazelton TB. Crying in infancy. Pediatrics 1962;29:579-88.
5. Barr RG. The normal crying curve: what do we really know? Dev Med Child Neurol 1990;32:356-62.
6. Barr RG. Colic and crying syndromes in infants. Pediatrics 1998; 102:1282-6.
7. Barr RG, Kramer MS, Boisjoly C, McVey-White L, Pless IB. Parental diary of infant cry and fuss behaviour. Arch Dis Child 1988;63:380-7.
8. Illingworth RS. Crying in infants and children. Br Med J 1955;1: 75-8.
9. Poole SR. The infant with acute, unexplained, excessive crying. Pediatrics 1991;88:450-5.
10. de Weerth C, Fuentes S, Puylaert P, de Vos WM. Intestinal microbiota of infants with colic: development and specific signatures. Pediatrics 2013;131:550-8.
11. Lehtonen L, Korvenranta H, Eerola E. Intestinal microflora in colicky and noncolicky infants: bacterial cultures and gas-liquid chromatography. J Pediatr Gastroenterol Nutr 1994;19: 310-4.
12. Rhoads JM, Fatheree NY, Norori J, et al. Altered fecal microflora and increased fecal calprotectin in infants with colic. J Pediatr 2009;155:823-8.
13. Savino F, Cresi F, Pautasso S, et al. Intestinal microflora in breastfed colicky and non-colicky infants. Acta Paediatr 2004;93: 825-9.
14. Savino F, Bailo E, Oggero R, et al. Bacterial counts of intestinal Lactobacillus species in infants with colic. Pediatr Allergy Immunol 2005;16:72-5.
15. Savino F, Cordisco L, Tarasco V, Calabrese R, Palumeri E, Matteuzzi D. Molecular identification of coliform bacteria from colicky breastfed infants. Acta Paediatr 2009;98:1582-8.

16. World Health Organization 2001. Health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria. A joint FAO/WHO expert consultation, 2001. http://www.fao.org/3/a-a0512e.pdf.
17. Benninga MA, Faure C, Hyman PE, St James Roberts I, Schechter NL, Nurko S. Childhood Functional Gastrointestinal Disorders: Neonate/Toddler. Gastroenterology 2016;15;S0016-5085(16) 00182-7.
18. Chau K, Lau E, Greenberg S, et al. Probiotics for Infantile Colic: A Randomized, Double-Blind, Placebo-Controlled Trial Investigating Lactobacillus Reuteri DSM 17938. J Pediatr 2015; 166:74-8.
19. Mi GL, Zhao L, Qiao DD, Kang WQ, Tang MQ, Xu JK. Effectiveness of Lactobacillus Reuteri in Infantile Colic and Colicky Induced Maternal Depression: A Prospective Single Blind Randomized Trial. Antonie Van Leeuwenhoek 2015;107: 1547-53.
20. Szajewska H, Gyrczuk E, Horvath A. Lactobacillus Reuteri DSM 17938 for the Management of Infantile Colic in Breastfed Infants: A Randomized, Double-Blind, Placebo-Controlled Trial. J Pediatr 2013;162:257-62.
21. Xu M, Wang J, Wang N, et al. The Efficacy and Safety of the Probiotic Bacterium Lactobacillus reuteri DSM 17938 for Infantile Colic: A Meta-Analysis of Randomized Controlled Trials. PLOS ONE 10(10):e0141445.
22. Sung V, Hiscock H, Tang MLK, et al. Treating infant colic with the probiotic Lactobacillus reuteri: double blind, placebo controlled randomised trial. BMJ 2014;348:g2107.
23. Savino F, Cordisco L, Tarasco V, et al. Lactobacillus Reuteri DSM 17938 in Infantile Colic: A Randomized, Double-Blind, Placebo-Controlled Trial. Pediatrics 2010;126:526-33.
24. Fatheree NY, Liu Y, Taylor CM, et al. Lactobacillus reuteri for Infants with Colic: A Double-Blind, Placebo-Controlled, Randomized Clinical Trial. J Pediatr 2017;191:170-8.