Turk J Gastroenterol *2014; 25: 628-33*

The role of *Bifidobacterium lactis* B94 plus inulin in the treatment of acute infectious diarrhea in children

BOWEL

Ali İşlek[1], Ersin Sayar[1], Aygen Yılmaz[1], Betil Özhak Baysan[2], Derya Mutlu[2], Reha Artan[1]
[1]Department of Pediatric Gastroenterology, Akdeniz University Faculty of Medicine, Antalya, Turkey
[2]Department of Clinical Microbiology, Akdeniz University Faculty of Medicine, Antalya, Turkey

Original Article

ABSTRACT

Background/Aims: In contrast to many other studies of probiotic species, the number of publications evaluating *Bifidobacterium lactis* and its combinations with prebiotics as treatments for acute infectious diarrhea is limited. We investigated the synbiotic effects of *B. lactis* B94 plus inulin on acute infectious diarrhea.

Materials and Methods: The study was conducted on children with acute diarrhea between the ages of 2 and 60 months. The patients were administered 5×10^{10} colony-forming units (CFU) of *B. lactis* B94 plus 900 mg inulin or placebo, once a day for five days. Stools were examined for *Rotavirus, Adenovirus, Entamoeba histolytica, Salmonella, Shigella, Campylobacter, Clostridium difficile, Cryptosporidium*, and parasites.

Results: We examined 79 patients in the synbiotic group and 77 patients in the placebo group. The duration of diarrhea was significantly reduced in the synbiotic group in comparison with the placebo group (3.9±1.2 days vs. 5.2±1.3 days, respectively; $p<0.001$). Moreover, the number of diarrheal stools on the third day was significantly lower in the synbiotic group than in the placebo group (5.5±2.9 vs. 8.3±3.01, respectively; $p<0.001$). Diarrhea in the synbiotic-group patients with rotavirus infection was of a significantly shorter duration (3.2±1.3 days vs. 5.2±1.3 days, respectively; $p=0.001$). Duration of diarrhea in patients who started the synbiotic treatment within the first 24 h was shorter than that in the patients who started the treatment later (3.9±1.1 days vs. 4.8±1.8 days, respectively; $p=0.002$).

Conclusion: Treatment with 5×10^{10} CFU of *B. lactis* B94 plus 900 mg inulin shortened the duration of acute watery diarrhea by an average of 31 h. This decrease was most pronounced in cases of *Rotavirus* diarrhea.

Keywords: *Bifidobacterium lactis* B94, inulin, acute gastroenteritis, childhood

INTRODUCTION

Acute infectious diarrhea or acute gastroenteritis (AGE) is one of the most common medical condition occurring during childhood. Worldwide, 1-1.5 million children are affected by AGE every year. Eighty percent of those affected are under two years of age and most are 6-11 month-old infants. Children under the age of five are affected by AGE 3-4 times per year, and 1%-3% of these cases are acute or severe. The mortality rate because of diarrhea is 1%-4%. AGE is more frequent during winter and autumn. Patients present with frequent and watery stools, abdominal pain, and/or vomiting. Despite widespread education regarding dehydration and oral rehydration therapy, AGE continues to be a significant cause of morbidity and mortality in children (1,2).

Probiotics are a new source for the treatment of AGE, which is a major health problem with socioeconomic consequences. Probiotics are defined as "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host" (3). Probiotic microorganisms are mainly members of the *Lactobacillus*, *Bifidobacterium*, and *Streptococcus* families. Yeasts, such as *Saccharomyces boulardii*, have also been studied and are widely used for probiotic purposes (4-6). The mechanisms responsible for the positive effects of probiotics on the gastrointestinal system are adhesion and colonization to intestinal mucosa, production of antibacterial factors, competition with bacterial substrates, and stimulation of mucosal and systemic immunities (7-13).

Address for Correspondence: Ali İşlek, Department of Pediatric Gastroenterology, Akdeniz University Faculty of Medicine, Antalya, Turkey
E-mail: islekali@hotmail.com
Received: 19 August, 2014 **Accepted:** 27 October, 2014
 • Available online at www.turkjgastroenterol.org • DOI: 10.5152/tjg.2014.14022

Probiotics have been used in many indications, but they are most extensively studied in connection with acute infectious diarrhea. Some meta-analyses have concluded that further research in different age groups and various doses of different probiotics is required to evaluate the role of probiotics in the management of infectious diarrhea (6,14,15).

Prebiotics are non-digestible food ingredients that positively affect the health of the host by selectively activating the growth and/or activity of a limited number of microorganism species found in the intestinal flora. In addition, prebiotics can increase the effects of probiotics because of their synbiotic relationships. Only a small number of studies have assessed the effects of prebiotics on acute diarrhea. Synbiotics are combinations of probiotics and prebiotics that can synergistically promote the growth of beneficial bacteria or newly added species in the colon (16).

In this study, we investigated the effects of *B. lactis* B94 plus a prebiotic inulin on the duration of acute infectious diarrhea, taking into account the etiology of infectious agents.

MATERIALS AND METHODS

The study was conducted on patients between the ages of 2 and 60 months and who presented with acute diarrhea to the Pediatric Emergency and Pediatric Gastroenterology Departments of the Akdeniz University Hospital between October 2012 and May 2013. Approval from the Akdeniz University Clinical Research Ethics Committee was obtained. Patients who had experienced watery, mucous, or bloody diarrhea with stool frequency of more than four times a day and in whom diarrhea lasted for less than seven days were included in the study. Immune deficiency, chronic diseases, usage of antibiotics or immunosuppressive drugs within the last two months, and usage of drugs that may affect motility and malabsorption were the criteria for exclusion from the study. A pediatrician examined all the patients. The degree of dehydration, stool appearance, stool consistency, and stool frequency was recorded. Parents who wanted their children to participate in the study gave their written and oral informed consent.

All the patients received routine treatment such as oral and/or intravenous fluid therapy, and nutritional support, and breastfeeding was promoted. The patients who required intravenous fluids were treated in the emergency rooms or outpatient treatment rooms and were then discharged. The patients were randomized and assigned to the synbiotic and placebo groups in a double-blind manner using a preformed randomization list. Each patient was given a code.

Stool samples were evaluated for *Salmonella*, *Shigella*, *Campylobacter*, *Cryptosporidium*, *Adenovirus*, *Rotavirus*, *Entamoeba histolytica*, and *Clostridium difficile*. Microscopy was used to determine parasitic infestations.

The patients in the synbiotic group were administered *B. lactis* B94 plus 900 mg inulincontaining preparation (Maflor® sachet, Mamsel, Turkey) once a day for five days. The patients in the placebo group received a maltodextrin-containing placebo with the same appearance as the synbiotic once a day for five days. The patients were advised to take the preparations with water. The parents were phoned every day for 10 days and were asked if their child took the preparations. They answered questions about stool frequency, vomiting frequency (if any), stool consistency, fever, and any dietary problems. The parents were also asked whether they needed any medical support. Statistical analyses were performed by a gastroenterologist using patient codes only and who was unaware of the treatment administered. In this study, our primary endpoint was the duration of diarrhea. The secondary endpoints were the number of stools on the third day, percentage of patients with diarrhea on the 5th day of the intervention, and duration of diarrhea for each etiological agent.

Evaluation of stool samples

Stool cultures

Aliquots of >2 g of fresh stool samples were placed in stool-sample containers with lids. The samples were cultivated in Conkey and XLD agar media. For Campylobacter isolation, Campy BAP media were used and *Yersinia* spp. samples were cultivated in cefsulodin-irgasan-novobiocin (CIN) agar media. When enterohemorrhagic *Escherichia coli* infection was suspected, the samples were cultivated in MacConkey agar and BCIG (5-bromo-4-chloro-3-indolyl-b -D-glucuronide sorbitol MacConkey agar) selective media. CIN agar cultures were incubated at 25°C in normal aerobic atmosphere for two days. Campy BAP agars cultures were incubated at 42°C for five days a microaerophilic environment for five days. Other cultures were incubated at 35°C-37°C in normal aerobic atmosphere for 24 h.

Antigen tests

Rotavirus and Adenovirus: The stool samples were analyzed for rotavirus and adenovirus antigens using an immunochromatographic EIA kit (RIDA®QUICK Rotavirus/Adenovirus Combi test, R-Biopharm AG, Germany) according to the manufacturer's instructions.

Cryptosporidium spp. and E. histolytica: After native-lugol staining, each specimen was examined under a microscope for the presence of protozoan trophozoites or cysts. Modified Ziehl-Neelsen acid-fast staining method was used to detect *Cryptosporidium* spp. oocysts. An immunochromatographic assay (CerTest Crypto, CerTest Biotec S.L., Spain) was used to test the specimens for the presence of *Cryptosporidium* spp. antigens. Samples positive for cysts and/or trophozoites of the *E. histolytica/E. dispar* complex were examined for *E. histolytica*-specific galactose adhesin antigen using an ELISA-based kit (TechLab *E. histolytica* II test, TechLab Inc., Blacksburg, VA, USA) according to the manufacturer's instructions.

C. difficile: Stool samples were analyzed for *C. difficile* glutamate dehydrogenase (GDH) antigen and toxin A/B using a mem-

Original Article

Table 1. Demographic characteristics of patients

Characteristics	Synbiotic group	Placebo group	p
Age (mean, months)	38.4±23.6 (2-60)	37.6 ± 21.5 (2-60)	0.84
Gender (F/M)	37/42	35/42	0.86
Onset of diarrhea (day)	1.4±0.9	1.6±0.8	0.64
Stool frequency	10.6±3.2	9.3±3.4	0.82
Vomiting	26	30	0.45
Fever (>38°C)	29	27	0.97
Dehydration			
Mild	44	47	0.33
Moderate	25	22	0.32
Severe	10	8	0.99

Table 2. Microorganisms identified in the synbiotic and placebo groups

Microorganism	Synbiotic group	Placebo group	p
Rotavirus	26 (32.9%)	27 (35%)	0.77
Adenovirus	10 (12.6%)	8 (10.3%)	0.59
Salmonella spp.	2 (2.5%)	2 (2.5%)	0.99
E. histolytica	2 (2.5%)	2 (2.5%)	0.99

Table 3. Patient characteristics during the study

Status	Synbiotic group	Placebo group	p
Duration of diarrhea (day)	3.9±1.2	5.2±1.3	<0.001
Stool frequency in 3rd day	5.5±2.9	8.3±3.01	<0.001
Number of patients with diarrhea in 5th day	14 (17.7%)	30 (38.9%)	0.002
Duration of vomiting (day)	1.22±1.12	1.31±1.21	0.62
Duration of fever (day)	2.12±1.56	2.23±1.38	0.51

brane-enzyme immunoassay kit (C. DIFF QUIK CHEK COMPLETE, TechLab Inc., Blacksburg, VA, USA).

Statistical analysis

Descriptive statistics are presented as frequency and percentage, mean and standard deviation, or median, minimum, and maximum values. Fisher's exact test and Pearson's chi-square test were used to analyze categorical data. The Mann-Whitney U test was used to analyze the differences between the two groups. P values <0.05 were considered statistically significant. The analyses were performed using the SPSS 18.0 software package.

RESULTS

Out of the 198 patients admitted to the pediatric emergency and gastroenterology departments, 19 did not wish to participate; thus, 179 patients were included in the study. The patients were divided into two groups in a double-blind manner; 90 patients were assigned to the synbiotic group and 89 to the placebo group. During the study period, 11 patients in the synbiotic group and 12 patients in the placebo group were excluded from the study as they used antibiotics, did not take the required preparations, or did not communicate (Figure 1). Before the treatment, there was no difference between the groups in terms of age, gender, degree of dehydration, number of vomiting episodes, fever, frequency of stools, or initial period of diarrhea. Demographic characteristics of the patients are shown in Table 1.

Table 4. Duration of diarrhea for different etiological agents

Microorganisms	Synbiotic group	Placebo group	p
Rotavirus (day)	3.2±1.3	5.6±1.4	0.001
Adenovirus (day)	5.6±3.6	5.9±4.0	0.11
Others (day)	4.2±1.1	5.3±1.7	0.002

Table 5. Duration of diarrhea in the synbiotic group depending on the treatment starting time

Status	Synbiotic group	p
First 24 h	3.9±1.1	0.002
>24 h	4.8±1.8	

There was no difference between the groups in terms of identified etiological factors (Table 2). In both groups, stool examinations could not be performed for two of the patients. These four cases were not included in the calculation of diarrhea duration based on etiological factors. In 49.3% of the synbiotic-group patients and 48% of the placebo-group patients, no specific etiological agents were found. In the synbiotic group, the detection rates for Rotavirus, Adenovirus, Salmonella, and *E. histolytica* were 33.7%, 12.9%, 2.5%, and 2.5%, respectively, in comparison with 36%, 10%, 2.6%, and 2.6%, respectively, in the placebo group. The duration of diarrhea was significantly shorter in the synbiotic group than in the placebo group (3.9±1.2 days and 5.2±1.3 days, respectively; p<0.001). The number of diarrheal stools on the third day was significantly smaller in the synbiotic group than in the placebo group (5.5±2.9 and 8.3±3.01, respectively; p<0.001). The number of cases with diarrhea continuing on the fifth day was significantly larger in the placebo group than in the synbiotic group (38.9% and 17.7%, respectively; p=0.002). There was no significant difference between the groups in terms of duration of vomiting and fever (Table 3).

We also analyzed the effects of etiological factors. The duration of diarrhea was 3.2±1.3 days in the rotavirus-infected synbiotic-group patients and 5.6±1.4 days in the placebo-group patients with the same infection (p=0.001). There was no significant difference between the durations of diarrhea in the synbiotic- and placebo-group patients with Adenovirus gastroenteritis (5.6±3.6 and 5.9±4.0, respectively; p=0.11). The duration of diarrhea caused by *E. histolytica* was similar in both groups (data not provided) (Table 4). The duration of diarrhea was shorter for patients who started the synbiotic therapy within the first 24 h than for those who started their treatment later [3.9±1.1 and 4.8±1.8, respectively, p=0.002, Table 5]. There

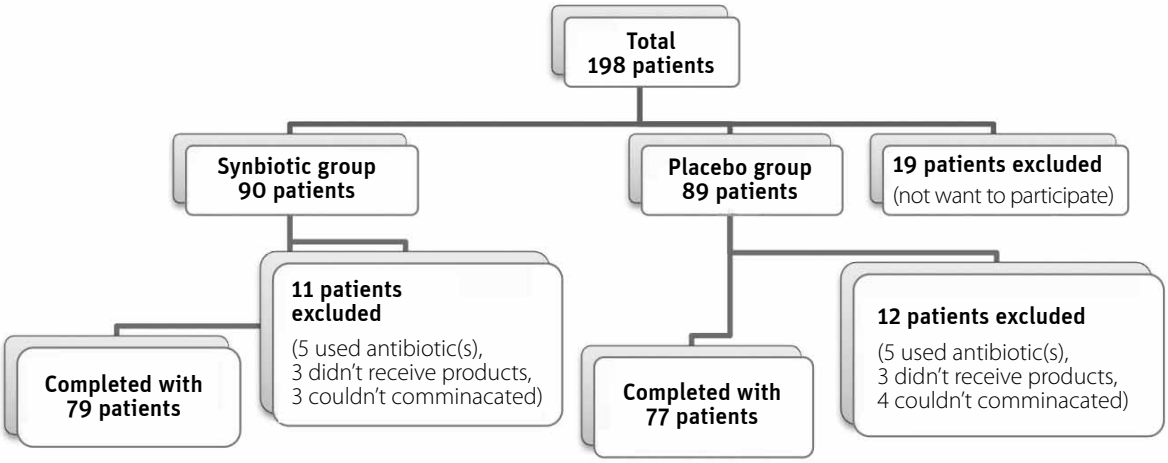

Figure 1. Patients recruitment flow chart.

were no side effects associated with *B. lactis* B94 and inulin treatment.

DISCUSSION

Treatment of acute infectious diarrhea is mainly designed to compensate for the loss of water and electrolytes (1) and to maintain the correct gastrointestinal microenvironment (17,18). Probiotics are intended for this secondary objective, i.e., the restoration of deteriorated intestinal microflora. The most investigated probiotics in this field are *Lactobacilli* and *Saccharomyces boulardii* (6).

Despite numerous studies, the management of infectious diarrhea with probiotics has some unsolved problems related to the diarrhea description, improvement criteria, probiotic type, probiotic dose, study quality, and probiotic effectiveness evaluation (6).

A number of meta-analyses have evaluated the efficacy of probiotics in the treatment of AGE. In the most recent Cochrane meta-analysis, data collected from 63 randomized controlled trials (RCTs) and 8014 subjects of all ages were evaluated for the efficacy of probiotics in the treatment of AGE. Among those RCTs, 56 were carried out in infants and young children. Forty-six RCTs tested a single probiotic, and 17 RCTs tested a combination of probiotics. The most commonly studied probiotics were *Lactobacillus* GG (LGG), *S. boulardii*, and *Enterococcus* lactic acid bacteria strain SF68. The Cochrane review reported that LGG reduces the duration of diarrhea (by 27 h on average), mean stool frequency on day 2 [mean difference (MD): 0.8, 95% CI: 1.3 to 0.2], and the risk of diarrhea lasting 4 days [risk ratio (RR): 0.6, 95% CI: 0.4-0.9]. The meta-analysis found that the treatment with *S. boulardii* or *Enterococcus faecium* (strain SF68) reduces the risk of diarrhea lasting 4 days or longer (RR: 0.37, 95% CI: 0.2-0.65 and RR: 0.21, 95% CI: 0.08-0.52; respectively). The authors have pointed out that more research is needed to assist clinicians in the use of particular probiotic regimens in specific patient groups (19).

A recent systematic review focused on studies of LGG treatment in AGE-affected children. Fifteen RCTs involving 2963 children were examined in this review. LGG significantly reduces the duration of diarrhea in comparison with placebo or no treatment; it is most effective when used at a daily dose of $\geq 10^{10}$ CFU. However, in comparison with controls, this treatment had no effect on the total stool volume (20).

An updated meta-analysis by Szajewska, which included 9 RCTs (five placebo-controlled) involving 1117 participants (age, 2 months-12 years) has shown that the treatment of AGE with 250-750 mg/day of *S. boulardii* reduces the duration of diarrhea by approximately 1 day (21).

In another recent meta-analysis of 13 RCTs, the authors reported that *S. boulardii* (250-750 mg/day) significantly reduces the duration of diarrhea (by approximately 24 h) and hospitalization (by approximately 20 h). They emphasized that *S. boulardii* shortens the initial phase of watery stools; mean number of stools starts to decrease on day 2, and a significant reduction is observed on days 3 and 4 (22).

A recent systematic review evaluated the effectiveness of *L. reuteri* DSM 17938 (2 RCTs) and *L. reuteri* ATCC 55730 (3 RCTs) in the treatment of AGE in children. In comparison with placebo or no treatment, *L. reuteri* DSM 17938 significantly reduces the duration of diarrhea (MD: 32 h, 95% CI 41 to 24) and increases the chance of recovery on day 3 (RR: 3.5, 95% CI: 1.2-10.8). Similar results have been obtained *with L. reuteri* ATCC 55730 (23). *L. reuteri* ATCC 55730 and *L. reuteri* DSM 17938 have been compared because *L. reuteri* ATCC 55730 strain carries transferable tetracycline and lincomycin resistance. It has been replaced by a new strain, *L. reuteri* DSM 17938, with no unwanted plasmid-borne resistance factors (6,23).

A systematic review of four RCTs evaluated the efficacy of heat-inactivated *L. acidophilus* strain LB. The intake of *L. acidophilus*

LB ranged from 2 to 4.5 days at a dose of 10^{10} CFU. In three RCTs with hospitalized children, *L. acidophilus* LB significantly reduces the duration of diarrhea in comparison with placebo (mean difference: 21.6 h, 95% CI: 26.5 to 16.6). In one RCT carried out in outpatients, the duration of diarrhea was unaffected. The use of *L. acidophilus* LB increases the chance of cure on day 3 in 2 RCTs and on day 4 in 2 RCTs. The authors concluded that there is insufficient evidence to recommend *L. acidophilus strain* LB for treating diarrhea in children (24).

Some studies also examined the effect of AGE treatment using other *Lactobacillus* species, some *Bifidobacterium* species, *Streptococcus thermophilus,* and other probiotics (25-28). However, there is not enough evidence to recommend these probiotics for routine use in AGE (6). According to the latest ESPGHAN systematic review, the use of the *L. rhamnosus* GG (low quality of evidence, strong recommendation) and *S. boulardii* (low quality of evidence, strong recommendation) may be considered in the management of children with AGE in addition to rehydration therapy. Less compelling evidence is available for *L. reuteri* DSM 17938 (very low quality of evidence, weak recommendation) and heat-inactivated *L. acidophilus* LB (very low quality of evidence, weak recommendation). Other strains or combinations of strains have been tested, but the evidence of their efficacy is weak or preliminary (6).

To the best of our knowledge, there is no published data on effects of administration of *B. lactis* B94 in AGE. In the latest ESPGHAN position paper and in another meta-analyses, no RCT has tried to evaluate the effect of singly administered *Bifidobacterium* species because of the lack of data (6,19). The effects of *Bifidobacterium* have often been assessed in conjunction with other probiotic species. In our study, we investigated the effect of *B. lactis* B94 and inulin on AGE. We demonstrated that *B. lactis* B94 and inulin shortened the duration of diarrhea by 31 h on average. This effect was more prominent when the synbiotic was used within the first 24 h of diarrhea onset. The number of diarrheal stools on the third day was significantly smaller in the synbiotic group than in the placebo group. We also examined stool frequency on the second day. Although the reduction in stool frequency on the third day was more pronounced, the number of diarrheal stools on the second day was also significantly smaller in the synbiotic group than in the placebo group (6.6±3.4 and 9.3±3.9, respectively; $p<0.001$). Our findings add some new insight to the field of probiotics. A particularly important feature of this study was the assessment of the effect of specific etiological factors on the efficacy of *B. lactis* B94 in the treatment of AGE. The treatment reduced the duration of diarrhea in the synbiotic group infected with *Rotavirus*; however, cases of *Adenovirus* and *E. histolytica* diarrhea were unaffected. The patients infected with *Salmonella* had no fever and did not undergo antibacterial treatment. The diarrhea improved on the second and third day in *Salmonella*-infected patients in the synbiotic group and on the third day in two *Salmonella*-infected cases in the placebo group. The diarrhea persisted through the tenth day in four patients in the synbiotic group and seven patients in the placebo group (p=0.32). One limitation of our study was that the patients were enrolled between October 2012 and May 2013. The patients who had diarrhea in the summer were not included, which might have affected the results. The absence of any reported major side effects associated with the applied probiotic-prebiotic combination indicated good tolerance for *B. lactis* B94; the treatment appears to be safe. However, it should be noted that there were no immunocompromised patients in this study.

Inulin is a long-chain fructooligosaccharide obtained from chicory extract (29). Some studies have reported that the addition of prebiotics to the diet results in increased levels of bifidobacteria and lactobacilli in the intestinal flora (30,31). The effects of prebiotics, including inulin, have been extensively investigated in infant nutrition studies; stool properties of infants receiving prebiotics are similar to those of breastfed infants (32). However, the effects of prebiotics on acute diarrhea have not been investigated in detail. The prebiotic used in this study has synbiotic characteristics; therefore, our results might be due to synbiotic effects. However, there is only sparse data on the positive effects and effective doses of *B. lactis* and prebiotics in acute diarrhea. Further studies in this field are still needed. Although prebiotics are generally well tolerated, they can cause bloating, abdominal pain, and diarrhea when taken in excessive amounts. In our study, the patients were given 900 mg inulin, and no symptoms of discomfort were observed. The daily effective doses of inulin-type fructans range from 4 g to 15 g. However, some studies have observed an increase in the number of stool bifidobacteria at a dose of 1 g/day (30,31). Further studies are needed to determine an effective dosage of inulin.

Treatment with $5\times10_{10}$ *CFU of B. lactis* B94 and 900 mg inulin reduced the duration of acute watery diarrhea by 31 h on average. This reduction was the most pronounced in cases of *Rotavirus* diarrhea.

Ethics Committee Approval: Ethics committee approval was received for this study.

Informed Consent: Written informed consent was obtained from patients who participated in this study.

Peer-review: Externally peer-reviewed.

Author contributions: Concept - A.I., R.A.; Design - A.I., R.A.; Supervision - A.Y.; Resource - A.I., E.S.; Materials - A.I., D.M., B.O.B.; Data Collection&/or Processing - A.I., E.S.; Analysis&/or Interpretation - A.I., R.A.; Literature Search - A.I., R.A.; Writing - A.I.; Critical Reviews - A.I., R.A.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study has received financial support from the Scientific Research Fund of Akdeniz University.

REFERENCES

1. Koletzko S, Osterrieder S. Acute infectious diarrhea in children. Dtsch Arzetbl Int 2009; 106: 539-47.
2. Walker CL, Rudan I, Liu L, et al. Global burden of childhood pneumonia and diarrhoea. Lancet 2013; 20; 381: 1405-16. **[CrossRef]**
3. Hill C, Guarner F, Reid G, et al. Expert consensus document. The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. Nat Rev Gastroenterol Hepatol 2014; 11: 506-14. **[CrossRef]**
4. Kullen MJ, Bettler J. The delivery of probiotics and prebiotics to infants. Curr Pharm Des 2005; 11: 55-74. **[CrossRef]**
5. Michail S, Sylvester F, Fuchs G, Issenman R. Clinical efficacy of probiotics: review of the evidence with focus on children. J Pediatr Gastroenterol Nutr 2006; 43: 550-7. **[CrossRef]**
6. Szajewska H, Guarino A, Hojsak I, Indrio F, Kolacek S, Shamir R, Vandenplas Y, Weizman Z. Use of probiotics for management of acute gastroenteritis: a position paper by the ESPGHAN Working Group for Probiotics and Prebiotics. J Pediatr Gastroenterol Nutr 2014; 58: 531-9. **[CrossRef]**
7. De Preter V, Vanhoutte T, Huys G, Swings J, Rutgeerts P, Verbeke K. Effect of lactulose and *Saccharomyces boulardii* administration on the colonic urea-nitrogen metabolism and the bifidobacteria concentration in healthy human subjects. Aliment Pharmacol Ther 2006; 23: 963-74. **[CrossRef]**
8. Cabana MD, Shane AL, Chao C, Oliva-Henker M. Probiotics in primary care pediatrics. Clin Pediatr 2006; 45: 405-10. **[CrossRef]**
9. Fedorak RN, Madsen KI. Probiotics and prebiotics in gastrointestinal disorders. Curr Opin Gastroenterol 2004; 20: 146-55. **[CrossRef]**
10. Michail S, Abernathy *F. Lactobacillus* plantarum reduces thein vitro secretory response of intestinal epithelial cells in enteropathogenic Escherichia coli infection. J Pediatr Gastroenterol Nutr 2002; 35: 350-5. **[CrossRef]**
11. Link-Amster H, Rochat F, Saudan KY, Mignot O, Aeschlimann JM. Modulation of a specific humoral immune response and changes in intestinal flora mediated through fermented milk intake. FEMS Immunol Med Microbiol 1994; 10: 55-64. **[CrossRef]**
12. Arunachalam K, Gill HS, Gill HS, Chandra RK. Enhancement of natural immune function by dietary consumption of *Bifidobacteria lactis* (HN019). Eur J Clin Nutr 2000; 54: 263-7. **[CrossRef]**
13. Kalia M, Isolauri E, Soppi E, Virtanen E, Laine S, Arvilommi H. Enhancement of the circulating antibody secreting cell response in human diarrhoea by a human *Lactobacillus* strain. Pediatr Res 1992; 32: 141-4. **[CrossRef]**
14. Szajewska H, Mrukowicz JZ. Probiotics in the treatment and prevention of acute infectious diarrhea in infants and children: a systematic review of published randomized, double-blind, placebo-controlled trials. J Pediatr Gastroenterol Nutr 2001; 33: 17-25. **[CrossRef]**
15. Allen SJ, Okoko B, Martinez E, Gregorio G, Dans LF. Probiotics for treating infectious diarrhea. Cochrane Database Syst Rev 2003;(4): CD003048.
16. Macfarlane GT, Steed H, Macfarlane S. Bacterial metabolism and health-related effects of galacto-oligosaccharides and other prebiotics. J Appl Microbiol 2008; 104: 305-44.
17. Wallace B. Clinical Use of Probiotics in the Pediatric Population. Nutr Clin Pract 2009; 24: 50-9. **[CrossRef]**
18. Senok AC, Ismaeel AY, Botta GA. Probiotics: facts and myths. Clin Microbiol Infect 2005; 11: 958-66. **[CrossRef]**
19. Allen SJ, Martinez EG, Gregorio GV, Dans LF. Probiotics for treating acute infectious diarrhoea. Cochrane Database Syst Rev 2010; CD003048.
20. Szajewska H, Skorka A, Ruszczynski M, Gieruszczak-Białek D. Meta-analysis: Lactobacillus GG for treating acute gastroenteritis in children updated analysis of randomised controlled trials. Aliment Pharmacol Ther 2013; 38: 467-76. **[CrossRef]**
21. Szajewska H, Skorka A. Saccharomyces boulardii for treating acute gastroenteritis in children: updated meta-analysis of randomized controlled trials. Aliment Pharmacol Ther 2009; 30: 955-63. **[CrossRef]**
22. Dinleyici EC, Eren M, Ozen M, Yargic ZA, Vandenplas Y. Effectiveness and safety of Saccharomyces boulardii for acute infectious diarrhea. Expert Opin Biol Ther 2012; 12: 395-410. **[CrossRef]**
23. Szajewska H, Urbanska M, Chmielewska A, Weizman Z, Shamir R. Meta-analysis: Lactobacillus reuteri strain DSM 17938 (and the original strain ATCC 55730) for treating acute gastroenteritis in children. Benef Microbes 2014; 24: 1-9. **[CrossRef]**
24. Szajewska H, Ruszczynski M, Kolacek S. Meta-analysis shows limited for using Lactobacillus acidophilus LB to treat acute gastroenteritis in children. Acta Paediatr 2014; 103: 249-55. **[CrossRef]**
25. Huang YF, Liu PY, Chen YY Nong BR, et al. Three-combination probiotics therapy in children with salmonella and rotavirus gastroenteritis. J Clin Gastroenterol 2014; 48: 37-42. **[CrossRef]**
26. Grandy G, Medina M, Soria R, Teran CG, Araya M. Probiotics in the treatment of acute rotavirus diarrhoea. A randomized, double-blind, controlled trial using two different probiotic preparations in Bolivian children. BMC Infect Dis 2010; 10: 253. **[CrossRef]**
27. Foster LM, Tompkins TA, Dahl WJ. A comprehensive post-market review of studies on a probiotic product containing Lactobacillus helveticus R0052 and Lactobacillus rhamnosus R0011. Benef Microbes 2011; 2: 319-34. **[CrossRef]**
28. Rerksuppaphol S, Rerksuppaphol. L. Lactobacillus acidophilus and Bifidobacterium bifidum stored at ambient temperature are effective in the treatment of acute diarrhoea. Ann Trop Paediatr 2010; 30: 299-304. **[CrossRef]**
29. Vandenplas Y. Oligosaccharides in infant formula. Br J Nutr 2002; 87: 293-6. **[CrossRef]**
30. Knol J, Boehm G, Lidestri M, et al. Increase of faecal bifidobacteria due to dietary oligosaccharides induces a reduction of clinically relevant pathogen germs in the faeces of formula-fed preterm infants. Acta Paediatr 2005; 94: 31-3. **[CrossRef]**
31. Bakker ZAM, Alles MS, Knol J, Kok FJ, Tolboom JJ, Bindel JG. Effects of infant formula containing a mixture of galacto- and fructo-oligosaccharides or viable Bifidobacterium animalis on the intestinal microflora during the first 4 months of life. Br J Nutr 2005; 94: 783-90. **[CrossRef]**
32. Braegger C, Chmielewska A, Decsi T, et al. Supplementation of infant formula with probiotics and/or prebiotics: A systematic review and comment by the ESPGHAN committee on nutrition. J Pediatr Gastroenterol Nutr 2011; 52: 238-50. **[CrossRef]**