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

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**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 conditions 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).
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 synergistic relationships. Only a small number of studies have assessed the effects of prebiotics on acute diarrhea. Symbiotics 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 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 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 who were randomized and assigned to the symbiotic 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 symbiotic 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 symbiotic 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 Comey 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 \( \text{Escherichia coli} \) infection was suspected, the samples were cultivated in MacConkey agar and BCIG (5-bromo-4-chloro-3-indolyl-\( \text{D} \)-glucuronide sorbitol MacConkey agar) selective media. CIN agar cultures were incubated at 25°C in normal aerobic atmosphere for two days. Campy BAP agar 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-
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 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.

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).
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 ≥10¹⁰ 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
Lactobacillus

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. bouardii (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 symbiotic was used within the first 24 h of diarrhea onset. The number of diarrheal stools on the third day was significantly smaller in the symbiotic 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 symbiotic 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 symbiotic 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 symbiotic 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 symbiotic 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 symbiotic characteristics; therefore, our results might be due to symbiotic 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 5x10^10 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.


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