Keywords Bifidobacterium animalis subsp. lactis, probiotics, nosocomial infection, prevention, children

ABSTRACT

Background: The incidence of nosocomial infections in children in developed countries is still high, ranging from 8% to 30%, and standard preventive measures, such as increased hygiene, are not sufficiently efficacious. One of the potential strategies for their prevention is the use of probiotics.

Objective: The aim of the study was to investigate the role of Bifidobacterium animalis subsp. lactis in preventing nosocomial infections in the acute hospital setting.

Design: We conducted a randomized, double-blind, placebo-controlled trial in 727 hospitalized children (aged 1–18 y). The children were randomly allocated to receive placebo (placebo group, n = 365) or B. animalis subsp. lactis at a dose of 10⁹ colony-forming units/d (intervention group, n = 362) once daily for the entire duration of the hospital stay. Nosocomial infections were defined as infections that occurred >48 h after hospital admission and that were not present or incubating at the time of admission.

Results: Analysis was performed on an intention-to-treat basis. There was no difference in the study primary outcome or incidence of common nosocomial gastrointestinal and respiratory tract infections between groups (22 vs. 29 infections, respectively; incidence rate ratio = 0.76; 95% CI: 0.41, 1.36; P = 0.32). No difference was found for the duration of common nosocomial infections [mean (range): 3.58 (1–7) vs. 3.79 (1–8) d, in placebo vs. intervention group; P = 0.74]. There was also no difference between the intervention and placebo groups for any of the other secondary outcomes (incidence of gastrointestinal and respiratory tract infections separately, duration of gastrointestinal and respiratory infections, and duration of hospitalization) and exploratory outcomes (gastrointestinal and respiratory symptoms, severity of gastrointestinal and respiratory tract infections, and the use of antibiotics).

Conclusions: The results of this study show that the use of B. animalis subsp. lactis failed to prevent nosocomial infections in an acute-setting pediatric hospital in children who were >1 y of age. However, it should be taken into account that the overall incidence of nosocomial infections was lower than expected. This trial was registered at clinicaltrials.gov as NCT01702766. Am J Clin Nutr 2015;101:680–4.

INTRODUCTION

The incidence of nosocomial infections in children in developed countries is still high, ranging from 8% to 30% depending on the time of the year and type of hospital ward (1–3). Nosocomial infections or hospital-acquired infections, by definition, develop during a hospital stay, meaning that they are not present or incubating at the time of admission (4); infections that occur >48 h after admission are considered nosocomial. Overall, gastrointestinal and respiratory tract infections account for the majority of hospital-acquired infections (1–3). The incidence of nosocomial infections in Croatian hospitals is similar to that in Western Europe and the United States (5). Nosocomial infections have several negative effects: they prolong the hospital stay, worsen the treatment outcome, and significantly increase health care expenses (6). Moreover, standard preventive measures, such as increased hygiene, are not sufficiently efficacious (7).

The efficacy of probiotics in the prevention of nosocomial diarrhea in pediatric patients was investigated in several studies (6, 8–12). The results of the studies were generally positive for nosocomial diarrhea, but with regard to respiratory tract infections at a regular pediatric ward, data are more limited (6). Similar to other indications for probiotic use, the effects seem to be strain dependent. Bifidobacterium animalis subsp. lactis is a probiotic strain with long-term use and proven safety but until now was not investigated in the prevention of nosocomial infections in a large cohort. Therefore, the aim of this study was to investigate the potential of B. animalis subsp. lactis in preventing nosocomial infections in the same hospital setting by using the same protocol as in our previous study with a different probiotic strain (6).

METHODS

Study protocol

All patients who were older than 12 mo and hospitalized at the Pediatric Department of the Children’s Hospital Zagreb from November 2012 to July 2013 were eligible for the study. Recruitment lasted until the sample size was reached. We excluded children <12 mo of age; children with immunodeficiency, neoplasm,
or chronic severe illnesses; children who had received probiotic and/or prebiotic products before enrollment (2 wk before hospitalization); and those with an anticipated hospital stay of <3 d or who were rehospitalized. This study was a prospective, randomized, double-blind, placebo-controlled parallel study registered at clinicaltrials.gov (NCT01702766).

The test product was a sachet containing 1 g of powder. Both preparations, active and placebo, were supplied by the probiotic strain producer Chr. Hansen, Denmark. Products were packed in identical sachets, which differed only by color (green or orange). The sachet contained 1 g maltodextrin powder, with or without *B. animalis* subsp. *lactis* at a dose of 10^7 CFUs. The study probiotic was *B. animalis* subsp. *lactis* BB-12; BB-12 is a registered trademark of Chr. Hansen A/S, Hørsholm, Denmark. Both products, active and placebo, had the same taste (without flavor), color, and smell. Products were stored at room temperature and the shelf life was 1 y. Both the research staff and patients were unaware of the nature of the product. The unblinding procedure was performed after the study was completed and after the statistical analyses were finalized.

The study product was consumed daily in the morning together with breakfast. The powder was mixed in ~20 mL of water and consumed immediately thereafter under the supervision of a pediatric resident. During the entire intervention period the subjects were not allowed to consume any probiotic or prebiotic products other than the study products supplied to them by the study personnel.

The primary endpoint was the incidence of nosocomial infections (gastrointestinal and respiratory infections) and the number of children with nosocomial infections. Nosocomial infections were defined as infections that developed 48 h after hospital admission and were not present or incubating at the time infections were defined as infections that developed 48 h after hospital admission and were not present or incubating at the time of admission (4). Gastrointestinal tract infections were defined as diarrhea (≥3 loose or watery stools in 24 h or an increase of >50% in the number of stools in 24 h) or vomiting (defined by a physician and not considered as a result of other symptoms, including cough, or diseases including gastroesophageal reflux disease or neurological conditions) or both. Respiratory tract infections were defined as pharyngitis, otitis, common cold, pneumonia, bronchitis, and bronchiolitis. All infections were diagnosed by an attending pediatrician.

Secondary endpoints were as follows: number of children with gastrointestinal infections, number of children with respiratory infections, duration of symptoms of nosocomial infections (gastrointestinal and respiratory infections), and duration of hospitalization. Exploratory variables included the following: number of children with diarrheal episodes, number of children with vomiting episodes, number of gastrointestinal infections with determined infective cause [nature of infective etiology (when determined)], severity of gastrointestinal infections based on the Vesikari scale (13), number of children with upper respiratory infections (common cold, pharyngitis, or otitis), number of children with lower respiratory infections (pneumonia, bronchitis, and bronchiolitis), number of respiratory infections with determined infective cause [nature of infective etiology (when determined)], severity of respiratory infections according to the physician based on visual analog scale, and use of antibiotics.

Patients were checked every day for signs and symptoms of respiratory infections; all data with regard to nasal discharge, sore throat, erythema of pharynx, cough, fever, wheezing, and dyspnea were recorded. All diagnoses concerning upper respiratory tract infections were based on clinical signs and symptoms. In children with symptoms and laboratory tests (complete blood count and C-reactive protein concentration) suggestive of bacterial infection, nasopharyngeal or pharyngeal swabs were collected and tested for bacteria. In patients with symptoms of pneumonia, laboratory tests were performed (complete blood count and C-reactive protein concentration), as well as pharyngeal swab, blood culture, and chest radiograph.

All data for gastrointestinal infections on number of stools per day, number of vomiting episodes per day, fever, and dehydration risk were assessed; and data on the need for parenteral rehydration were collected on a daily basis. Each patient who developed gastrointestinal symptoms had his or her stool tested for bacteria, rotavirus, adenovirus, and norovirus. Antibiotic-associated diarrhea was excluded (diarrhea in patients who were treated with antibiotics and without positive stool test was not included as nosocomial infection). Seven days after hospital discharge, all patients were contacted to establish whether they developed an infection that was in the incubation stage at discharge; however, no infections were recorded after the patients were discharged.

**Ethics**

The study was conducted following the principles of the Helsinki Declaration and good clinical practice guidelines. The protocol was approved by the Children’s Hospital Ethical Committee. Written informed consent was obtained from the parent or guardian of each child included in the study.

**Randomization and blinding**

The trial was a prospective, randomized, double-blind, placebo-controlled study. Randomization was performed by using Random Allocation Software in which every patient received a number and obtained the preparation successively. To avoid disproportionate numbers of patients in each group, randomization was performed in blocks of 6 subjects (3 receiving the probiotic product and 3 receiving the placebo). To ensure allocation concealment, sequentially numbered, opaque sealed envelopes were used and an independent person prepared the randomization schedule. All study personnel, parents, and guardians were unaware of the group assignments. Randomization codes were secured until all of the data were analyzed.

**Sample size**

With 2 primary endpoints and a difference in incidence of 7% (the incidence in the placebo group was estimated as 12% and the incidence in the active group was 5%), based on our previous study (6) the estimated sample size was a total of 658 (or 329 children/arm) with a power of 80% (α of 5%). With an estimated dropout rate of 10%, the total sample size needed was 724 or 362 children per arm.

**Statistical analysis**

Student’s *t* test was used to compare mean values of continuous variables approximating a normal distribution. For non-normally distributed variables, the Mann-Whitney test was used.
The chi-square test was used, as appropriate, to compare categorical variables. The difference between study groups was considered significant when \( P < 0.05 \). All statistical tests were 2-tailed and performed at the 5% level of significance. All analyses were conducted on an intention-to-treat basis, including all patients in the groups to which they were randomly assigned. All patients, including the patients who discontinued the intervention, were followed up until the end of intervention. Incidence rate ratios were calculated by Poisson regression models. Statistical analysis was performed by using the computer software SPSS 19.0 (IBM SPSS) and MedCalc statistical software (version 12.7.8) (MedCalc Software). The statistical plan and complete statistical analysis were performed before unblinding, and all analyses were performed according to the written statistical analysis plan.

RESULTS

Demographic and other baseline characteristics

Overall, data were analyzed for 365 children in the placebo group and 362 in the intervention group (Figure 1). Demographic characteristics are presented in Table 1. A total of 338 (92.6%) children in the placebo group and 341 (94.2%) in the intervention group were compliant to the study product (\( P = 0.39 \)).

Primary efficacy endpoints

There were 22 infections in the placebo group and 29 in the intervention group, with an incidence rate in the placebo group of 0.33 (95% CI: 0.21, 0.50) and 0.44 (95% CI: 0.29, 0.63) in the intervention group (incidence rate ratio = 0.76; 95% CI: 0.41, 1.36; \( P = 0.32 \), Poisson regression model). The number of children with common nosocomial infections is presented in Table 2.

Secondary efficacy endpoints

The incidence rate of respiratory tract infection was 0.20 in the placebo group (95% CI: 0.10, 0.33) and 0.18 (95% CI: 0.09, 0.32) in the intervention group (incidence rate ratio = 1.08; 95% CI: 0.45, 2.59; \( P = 0.85 \), Poisson regression model). The differences in the number of children with respiratory tract infections are presented in Table 2. None of the children had recurrent respiratory tract infection.

The incidence rate of gastrointestinal infection was 0.14 (95% CI: 0.06, 0.26) in the placebo group and 0.26 (95% CI: 0.15, 0.41) in the intervention group (incidence rate ratio = 0.53; 95% CI: 0.21, 1.25; \( P = 0.11 \), Poisson regression model). The differences in the number of children with gastrointestinal infections are presented in Table 2. None of the children had recurrent gastrointestinal infection. Differences between duration of common nosocomial infections and duration of hospitalization are presented in Table 3.


FIGURE 1 Flowchart of study recruitment.

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>Demographic characteristics and reason for hospitalization</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Placebo group ( n = 365 )</td>
</tr>
<tr>
<td>Age, (^2) y</td>
<td>9.78 (1.03–18.44)</td>
</tr>
<tr>
<td>Female sex, ( n ) (%)</td>
<td>198 (54.2)</td>
</tr>
<tr>
<td>Reason for hospitalization, ( n ) (%)</td>
<td></td>
</tr>
<tr>
<td>Intoxication</td>
<td>3 (0.8)</td>
</tr>
<tr>
<td>Urinary tract disorder</td>
<td>59 (16.2)</td>
</tr>
<tr>
<td>Neurological disorder</td>
<td>132 (36.2)</td>
</tr>
<tr>
<td>Noninfectious gastrointestinal disorder</td>
<td>90 (24.7)</td>
</tr>
<tr>
<td>Cardiologic disorder</td>
<td>26 (7.1)</td>
</tr>
<tr>
<td>Genetic disorder</td>
<td>9 (2.5)</td>
</tr>
<tr>
<td>Noninfectious pulmonary disorder</td>
<td>46 (12.6)</td>
</tr>
<tr>
<td>Immunologic disorder</td>
<td>0</td>
</tr>
<tr>
<td>Hematologic disorder</td>
<td>0</td>
</tr>
</tbody>
</table>

\(^{1}\)Derived by using nonparametric Mann-Whitney and chi-square tests.

\(^{2}\)Values are medians; ranges in parentheses.
**Exploratory endpoints**

The types of respiratory tract infection and visual analog scale are presented in Table 2. The infective cause of respiratory tract infections was not determined. One child in the placebo group and 4 in the intervention group received antibiotics ($P = 0.23$).

Presenting gastrointestinal symptoms and Vesikari scores are shown in Table 2. Of 727 hospitalized children, only 3 developed rotavirus infections: 2 in the placebo group and 1 in the intervention group. An adenovirus infection was identified in one child from the intervention group ($P = 0.57$).

Binary logistic regression for overall infections is presented in Table 4. There was a significant association between prolonged hospital stay and the risk of all assessed infections. Children who were of older age had a higher chance of acquiring an infection.

**Adverse events**

There were no adverse events recorded.

**DISCUSSION**

This study found no effect of the probiotic strain *B. animalis* subsp. *lactis* at a dose of $10^9$ CFUs/d in the prevention of nosocomial respiratory and gastrointestinal infections. Emerging evidence shows that different probiotic strains have different effects in the prevention or in the treatment of various diseases (14). With respect to the prevention of nosocomial diarrhea in children, several studies assessed the efficacy of probiotics (6, 8–11) and yielded different results. The strongest effect was found for *Lactobacillus rhamnosus* GG. This probiotic strain was investigated in 3 randomized controlled trials (RCTs) (6, 8, 9), which are summarized in a meta-analysis that found an overall positive effect of *L. rhamnosus* GG on the prevention of nosocomial diarrhea (15). Two studies evaluated other probiotics; however, they were performed in a chronic hospital setting. Saavedra et al. (10) investigated the efficacy of *Bifidobacterium bifidum* and *Streptococcus thermophilus* in infants and children aged 5–24 mo and found a positive preventive effect of *B. bifidum* (6.9% vs. 31%; RR: 0.2; 95% CI: 0.06, 0.8). The other study compared the probiotic strain *B. animalis* subsp. *lactis* with placebo in 90 infants with a prolonged stay in residential child care centers, and similarly to our results, failed to confirm a reduction in the cumulative incidence of diarrhea in the probiotic-treated group (28.3% vs. 38.6%; RR: 0.7; 95% CI: 0.4, 1.3) (11). However, that study found fewer mean numbers of days with diarrhea and a lower risk of developing diarrhea in infants receiving a supplemented formula (11).
The largest RCT on the role of probiotics in the prevention of nosocomial infections was performed in our center. A total of 742 hospitalized children were recruited, and results showed a significant risk reduction for nosocomial gastrointestinal (5.1% vs. 12.0%; RR: 0.4; 95% CI: 0.25–0.7) and respiratory tract infections (2.1% vs. 5.5%; RR: 0.38; 95% CI: 0.18, 0.85) in children who received *L. rhamnosus* GG (6).

Our current RCT was performed in the same setting but with a different probiotic strain, *B. animalis* subsp. *lactis*, which was used for the first time in the acute pediatric hospital. The different findings in this compared with our previous study (6) could have several explanations. First, it is well established that the probiotic effects are strain dependent and that the positive effect of one strain should not be extrapolated for probiotics in general (16, 17). This was clearly shown by Szażewska’s group (8) whereby, in the same hospital setting, the use of *L. rhamnosus* GG reduced nosocomial diarrhea, but the use of *Lactobacillus reuteri* did not (12). The other explanation for a lack of effect is the overall low rate of nosocomial infections; in our previous study (6) there were 91 nosocomial infections in 742 children compared with only 51 in 727 children in the present study. However, although the number of nosocomial infections was lower, the number of included children was very high, and none of the investigated outcomes had a tendency to show the difference.

This study has several limitations. The number of nosocomial infections was low and most of the infections were of short duration and of unproven etiology in both groups. Moreover, on the basis of the study protocol, infants, who are generally more prone to infections, were not included in the study, which meant that our results could not be extrapolated to this specific age group. However, the strengths of the study overcome the weaknesses. This study had adequate randomization, a large sample size, a double-blind design, comprehensive follow-up, strict surveillance, and was an intention-to-treat analysis.

In conclusion, the use of *B. animalis* subsp. *lactis* cannot be recommended for the prevention of nosocomial infections in acute setting pediatric hospitals in children >1 y old. However, it should be taken into account that the overall incidence of nosocomial infections was lower than expected. Furthermore, the results of our study add further evidence that every probiotic strain should be tested for every specific clinical indication.

We thank the following pediatric residents at Children’s Hospital Zagreb who contributed to the study by administering the products to the patients and managing patient data log sheets: Elizabeta Ozimec; Iva Kolacek Vacka, Marija Jančić, Ljubica Bohan, and Katja Dumić Kabat. The authors’ responsibilities were as follows—IH and SK: designed the research, wrote the manuscript, and had primary responsibility for final content; IH, VTP, A Mocić Pavić, and AM Pasini: conducted the research and provided essential reagents or essential materials; and IH: analyzed data and performed statistical analysis. All of the authors read and approved the final manuscript. None of the authors had a conflict of interest. Chr. Hansen, Denmark, had no role in the design, implementation, analysis, or interpretation of the data.

### REFERENCES

10. Saavedra JM, Bauman NA, Oung I, Perman JA, Yolken RH. Feeding of *Bifidobacterium* *bifidum* and *Streptococcus* thermophilus to infants in hospital for prevention of diarrhea and shedding of rotavirus. Lancet 1994;344:1046–9.

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**TABLE 4**

Binary logistic regression for overall nosocomial infections

<table>
<thead>
<tr>
<th></th>
<th>RR (95% CI)</th>
<th>P†</th>
</tr>
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<tbody>
<tr>
<td>Placebo group</td>
<td>0.72 (0.38, 1.36)</td>
<td>0.31</td>
</tr>
<tr>
<td>Duration of hospitalization</td>
<td>1.08 (1.01, 1.15)</td>
<td>0.03</td>
</tr>
<tr>
<td>Age in years</td>
<td>0.92 (0.87, 0.98)</td>
<td>0.01</td>
</tr>
</tbody>
</table>

†Derived by using binary logistic regression.