

# Efficacy of *Lactobacillus* GG in prevention of nosocomial diarrhea in infants

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**Objective:** Nosocomial diarrhea is a major problem in pediatric hospitals worldwide. We evaluated the efficacy of orally administered *Lactobacillus* GG (LGG) in the prevention of this disease in young children.

**Study design:** Eighty-one children aged 1 to 36 months who were hospitalized for reasons other than diarrhea were enrolled in a double-blind trial and randomly assigned at admission to receive LGG (n = 45) at a dose of  $6 \times 10^9$  colony-forming units or a comparable placebo (n = 36) twice daily orally for the duration of their hospital stay.

**Results:** LGG reduced the risk of nosocomial diarrhea ( $\geq 3$  loose or watery stools/24 h) in comparison with placebo (6.7% vs 33.3%; relative risk: 0.2; [95% CI: 0.06–0.6]; number needed to treat: 4 [95% CI: 2–10]). The prevalence of rotavirus infection was similar in LGG and placebo groups (20% vs 27.8%, respectively; relative risk: 0.72; 95% CI: 0.33–1.56). However, the use of LGG compared with placebo significantly reduced the risk of rotavirus gastroenteritis (1/45 [2.2%] vs 6/36 [16.7%], respectively; relative risk: 0.13; 95% CI: 0.02–0.79; number needed to treat: 7; 95% CI: 3–40).

**Conclusions:** Prophylactic use of LGG significantly reduced the risk of nosocomial diarrhea in infants, particularly nosocomial rotavirus gastroenteritis. (J Pediatr 2001;138:361-5)

In children, nosocomial infectious diarrhea is commonly caused by enteric viral pathogens, especially rotavirus.<sup>1,2</sup> Depending on population, type of hospital, and standard of care, the reported incidence rate ranges from 4.5<sup>3</sup> to 22.6<sup>4</sup> episodes per 100 admissions. Infants

and toddlers are at the highest risk of acquiring nosocomial viral gastroenteritis.<sup>3</sup> On the other hand, enteric bacteria are rarely responsible for sporadic episodes of nosocomial diarrhea in children (<1% of cultured cases),<sup>5</sup> with *Clostridium difficile* being the most preva-

lent bacterial pathogen.<sup>6,7</sup> A common noninfectious cause of nosocomial diarrhea is antibacterial therapy; the mechanism is presumably related to alterations of normal bowel microflora and colonization by resistant flora.

|     |                         |
|-----|-------------------------|
| CFU | Colony-forming units    |
| LGG | <i>Lactobacillus</i> GG |
| RR  | Relative risk           |

Nosocomially acquired diarrhea can prolong hospital stay and increase medical costs.<sup>4</sup> We have shown that 48.8% of episodes of rotavirus nosocomial gastroenteritis prolonged hospital stay by 5.9 days per episode on average, resulting in a significant increase in total cost of hospital treatment.<sup>1</sup> Thus there is a strong need for cost-effective measures to prevent hospital-acquired diarrhea.

In 1994, Saavedra et al<sup>8</sup> reported that probiotics may be effective in prevention of diarrhea in hospitalized children. In a double-blind, placebo-controlled trial, infants aged 5 to 24 months who were admitted to a long-term medical care hospital for treatment of non-gastrointestinal conditions were randomized to receive a standard infant formula or the same formula with *Bifidobacterium bifidum* and *Streptococcus thermophilus*. Over a 17-month period, 31% of the patients receiving the control formula, but only 7% of those receiving the supplemented formula, developed diarrhea; and 39% of the subjects who received the control formula and only 10% of those who received the supplemented formula shed rotavirus at some time during their hospitalization.

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Supported in part by a grant from the Medical University of Warsaw (H.S.).

Submitted for publication Mar 20, 2000; revision received June 29, 2000; accepted Aug 30, 2000.

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0022-3476/2001/\$35.00 + 0 9/21/111321

doi:10.1067/mpd.2001.111321

**Table I.** Baseline characteristics of study groups

|                               | LGG                         | Placebo                     | P value |
|-------------------------------|-----------------------------|-----------------------------|---------|
| No. of subjects               | 45                          | 36                          |         |
| Age (mo)                      | 11.6 ± 8.7                  | 9.9 ± 8.1                   | .32*    |
| Sex (M/F)                     | 27/18                       | 20/16                       | .69†    |
| Weight (g)                    | 8736.2 ± 2839.8             | 8125.6 ± 2745.9             | .33*    |
| Hospitalization (d)           | 9.5 ± 4.2<br>(min 2–max 17) | 8.3 ± 3.6<br>(min 3–max 23) | .24*    |
| Reasons for hospitalization   |                             |                             |         |
| Ear, nose, & throat disorders | 14                          | 6                           |         |
| Respiratory                   | 24                          | 22                          |         |
| Urinary tract infections      | 4                           | 4                           |         |
| Neurologic disorders          | —                           | 4                           |         |
| Orthopedics                   | 1                           | —                           |         |
| Hematologic disorders         | 1                           | —                           |         |
| Endocrine                     | 1                           | —                           |         |

Results are presented as mean ± SD.  
\*Wilcoxon test.  
†Chi-square test.

A number of studies have shown the efficacy of other probiotic agents, including *Lactobacillus GG*, in the prevention of diarrhea.<sup>9-13</sup> However, there are no data on the efficacy of LGG in the prevention of nosocomial diarrhea. Because several pediatric clinical trials showed the efficacy of LGG in the treatment of rotavirus gastroenteritis<sup>14-19</sup> and preliminary data suggest it may also be effective in treatment of *C difficile* diarrhea,<sup>20,21</sup> we evaluated the efficacy of orally administered LGG in the prevention of nosocomial diarrhea in young children.

## METHODS

The recruitment of the children was conducted in two pediatric hospitals in Poland (Warsaw and Kielce) from September 1998 to December 1999. All children aged 1 to 36 months who were admitted to the hospital for reasons other than diarrhea were eligible for entry into the study. Children with a history of probiotics use within 7 days before admission, acute gastroenteritis within 3 days before admission, symptoms other than diarrhea suggest-

ing gastroenteritis (eg, vomiting), underlying intestinal disease, or the presence of visible blood in the stool, as well as those who were breast fed, were excluded.

The investigation was carried out as a double-blind, placebo-controlled study. Infants were randomly assigned at admission to receive LGG in a dose of  $6 \times 10^9$  colony-forming units per sachet (2.46 g) or a comparable placebo twice daily for the entire duration of their hospital stay. Both LGG and placebo were manufactured and supplied by Dicofarm SpA (Rome, Italy) as a powder in identical sachets and kept refrigerated until use. Both LGG and placebo were reconstituted in a small amount of water and administered with feedings.

Patients were evaluated daily for stool number and consistency. In case of loose or watery stools occurring within 3 days after discharge, patients were advised to contact hospital physicians. Stool samples, obtained weekly and during an episode of diarrhea, were analyzed for bacteria with standard stool cultures and rotavirus antigen. The latter was detected in stool samples by a commercial latex aggluti-

nation test with a rotavirus-specific monoclonal antibody (Slidex Rota-Kit 2; BioMerieux, Lyon, France).

## Definitions

For the purposes of this study, diarrhea was defined as the passage of 3 or more loose or watery stools in a 24-hour period. Rotavirus infection was diagnosed when rotavirus antigen was detected in a stool specimen. Rotavirus gastroenteritis was diagnosed when rotavirus antigen was detected in stool of a child who presented with acute gastroenteritis.

## Statistical Analysis

Chi-square test or the Fisher exact test, as appropriate, was used for comparisons of proportions, and Wilcoxon test was used for comparison of the mean values of diarrhea duration and patients' weight and age. Data were analyzed with S-plus (Mathsoft, Inc) and Epi-Info software (Epi-Info, Epidemiology Program Office, Centre for Disease Control and Programme on AIDS, WHO). Relative risk, 95% CI, and number needed to treat were calculated by using Arcus statistical software (Medical Computing, Aughton, UK). The differences between study groups were considered significant when the *P* value was <.05 or when 95% CI for RR did not exceed 1.0 (equivalent to *P* < .05).

## Ethical Considerations

Parents were fully informed about the aims of the study, and written consent was obtained from at least one parent. The study protocol was reviewed and approved by the ethical review committee.

## RESULTS

The characteristics of the 81 patients at study entry are presented in Table I. All children randomly assigned to placebo and intervention groups received the study product throughout

the duration of hospitalization. There were no withdrawals from the study or protocol violations, so the final analysis was performed as intention-to-treat.

Main and secondary outcome measures are summarized in Table II. Of the 81 recruited children, 15 (18.5%) experienced diarrhea in the course of hospitalization but none returned with diarrhea up to 3 days after discharge. The use of LGG as compared with placebo was associated with a significantly reduced risk of diarrhea (RR: 0.2; 95% CI: 0.06–0.6). Four (95% CI: 2–10) patients would need to be treated with LGG to prevent a single episode of nosocomial diarrhea. In both groups, rotavirus was the most common infectious agent associated with nosocomial diarrhea (Table III).

The prevalence of rotavirus infection was similar in LGG and placebo groups (Table IV). However, LGG compared with placebo significantly reduced the risk of rotavirus gastroenteritis (RR: 0.13; 95% CI: 0.02–0.79). Seven patients (95% CI: 3–40) would need to be treated with LGG to prevent a single episode of nosocomial rotavirus gastroenteritis (Table IV).

LGG was well tolerated, and no adverse effects of the treatment were noted.

## DISCUSSION

Prophylactic administration of LGG significantly reduced the risk of nosocomial diarrhea in infants, particularly with respect to nosocomial rotavirus gastroenteritis. An LGG strain of a human origin characterized by its ability to survive a passage through the gastrointestinal tract was chosen for this study. In a number of clinical trials, only LGG showed consistent efficacy in the treatment of rotavirus gastroenteritis.<sup>14–18</sup> The efficacy of LGG in the prevention of rotavirus diarrhea is of great practical value until more cost-effective and convenient preventive methods are available. In spite of the high priority given to the develop-

**Table II.** Main and secondary outcome measures

|  | LGG<br>(n = 45)                 | Placebo<br>(n = 36)           | RR<br>(95% CI) | P value |
|--|---------------------------------|-------------------------------|----------------|---------|
| Main outcome measure   |                                 |                               |                |         |
| Incidence of diarrhea  | 3 (6.67%)                       | 12 (33.3%)                    | 0.2 (0.06–0.6) | .002*   |
| Secondary outcome measures   |                                 |                               |                |         |
| Age of children with diarrhea (mo) <sup>†</sup>                          | 6.1 ± 5.3<br>(min 2-<br>max 12) | 7.8 ± 8<br>(min 1-<br>max 30) |                | .94‡    |
| Onset time of diarrhea after admission (d) <sup>†</sup>                  | 3.3 ± 1.5                       | 3.4 ± 1.7                     |                | 1.00‡   |
| Duration of diarrhea (d) <sup>†</sup>                                    | 6.3 ± 1.3                       | 6.5 ± 2.6                     |                | 1.00‡   |
| No. of watery stools per 24 hours in children with diarrhea <sup>†</sup> | 4.2 ± 2.0                       | 4.5 ± 2.1                     |                | .94‡    |

RR, Relative risk.  
\*Chi-square test.  
<sup>†</sup>Results are presented as mean ± SD.  
<sup>‡</sup>Wilcoxon test.

**Table III.** Etiology of nosocomial diarrhea in study groups

| Etiologic factor               | LGG (n = 3) | Placebo (n = 12) | P value |
|--------------------------------|-------------|------------------|---------|
| Identified infectious etiology | 1 (33%)     | 7 (58.3%)        | .45*    |
| Rotavirus                      | 1 (33%)     | 6 (50%)          | .55*    |
| <i>Yersinia</i> species        | —           | 1 (8.3%)         | —       |
| Unknown                        | 2 (67%)     | 5 (41.7%)        | .45*    |

\*Fisher exact test.

**Table IV.** Efficacy of LGG against rotavirus

|   | LGG<br>(n = 45) | Placebo<br>(n = 36) | RR<br>(95% CI)    | P value           |
|---|-----------------|---------------------|-------------------|-------------------|
| Rotavirus infection*  | 9 (20%)         | 10 (27.8%)          | 0.72 (0.33–1.56)  | .41 <sup>†</sup>  |
| Rotavirus gastroenteritis <sup>‡</sup>                                  | 1 (2.2%)        | 6 (16.7%)           | 0.13 (0.02–0.79)  | .02 <sup>†</sup>  |
| Gastroenteritis in rotavirus-infected patients <sup>§</sup>             | 1/9 (11.1%)     | 6/10 (60%)          | 0.18 (0.032–0.86) | .04 <sup>  </sup> |
| Rotavirus gastroenteritis <sup>‡</sup> with onset ≥72 h after admission | —               | 5                   |                   | .01 <sup>†</sup>  |

RR, Relative risk.  
\*Asymptomatic and symptomatic patients with rotavirus antigen shedding in stool.  
<sup>†</sup>Chi-square test.  
<sup>‡</sup>Diarrhea with rotavirus antigen shedding in stool.  
<sup>§</sup>Diarrhea in patients with rotavirus antigen shedding in stool.  
<sup>||</sup>Fisher exact test.

ment of effective and safe vaccines for immunization against rotavirus, no satisfactory vaccine is currently available in practice. Passive administration of

bovine or human anti-rotavirus antibodies is a potential alternative to vaccination and probiotics for prevention of rotavirus illness.<sup>22</sup> Whether the for-

mer is more cost-effective than probiotics requires further investigation. Observing enteric precautions may reduce the risk of nosocomial infectious diarrhea, although several authors have reported moderate efficacy of these measures in prevention of viral gastroenteritis, suggesting the role of respiratory (droplet) transmission of infection in addition to the fecal-oral route, which was proposed for rotavirus and calicivirus.<sup>23</sup>

It is noteworthy that our study showed the potential benefit of LGG also in prevention of non-rotaviral nosocomial diarrhea. A recent therapeutic trial did not confirm the efficacy of LGG in culture-proven bacterial diarrhea (caused by *Salmonella*, *Shigella*, *Campylobacter*, *Yersinia*, or *Entamoeba* species).<sup>19</sup> On the other hand, in the same study,<sup>19</sup> it was demonstrated that in the subset of patients in whom stools yielded no identifiable pathogens, the administration of LGG significantly reduced the duration of diarrhea. It was speculated that the success of the probiotic therapy in patients with no identifiable enteric pathogen might be explained by its efficacy against undetected enteric viruses. The same may be true for the preventive effect of LGG in hospitalized children, because in addition to rotaviruses, several other viral pathogens were reported to cause nosocomial gastroenteritis in children including caliciviruses, astroviruses, adenoviruses,<sup>24,25</sup> and most recently, human torovirus.<sup>26</sup>

The preventive effect of LGG in non-rotaviral diarrhea demonstrated in the present study may be also due to the prevention of noninfectious diarrhea, that is, antibiotic-associated diarrhea. LGG reduces the incidence of antibiotic-associated diarrhea in children receiving antimicrobial treatment for common childhood infections.<sup>12,13</sup> In the present study the details of antibiotic therapy were not evaluated. Thus we may not conclude specifically on the preventive effect of LGG on antibiotic-associated diarrhea in children admitted to the hospital.

Several mechanisms have been proposed to explain the efficacy of probiotics in prevention and treatment of diarrheal diseases. The possible mechanisms include the synthesis of antimicrobial substances,<sup>27,28</sup> competition for nutrients required for growth of pathogens,<sup>29</sup> competitive inhibition of adhesion of pathogens,<sup>30-32</sup> modification of toxins or toxin receptors,<sup>33,34</sup> and stimulation of immune response to pathogens.<sup>35,36</sup> Recently, Mack et al<sup>37</sup> showed that *Lactobacillus* species (*L. rhamnosus* strain GG, as well as *L. plantarum* strain 299v) inhibit, in a dose-dependent manner, binding of *Escherichia coli* strains to intestine-derived epithelial cells grown in tissue culture by stimulation of synthesis and increased secretion of mucins. Interestingly, although *Bifidobacterium bifidum* and *Streptococcus thermophilus* were shown to prevent rotavirus infection in the study by Saavedra et al,<sup>8</sup> the prevalence of rotavirus infection in LGG- and placebo-treated infants in our study was similar, suggesting a different mechanism of action of these probiotic agents.

A number of issues must be addressed if administration of LGG is to be considered as a preventive measure to reduce the incidence of nosocomial diarrhea. First, the magnitude of an individual dose required for optimal protection is not known. Although in our study it was lower than in other preventive trials with LGG ( $6 \times 10^9$  CFU vs  $3.7 \times 10^{10}$  CFU in the study by Oberhelman et al<sup>9</sup> and  $1 \times 10^{10}$ – $2 \times 10^{10}$  CFU in the study by Vanderhoof et al<sup>12</sup>), we administered LGG twice daily (vs once daily in both the above cited preventive studies). Doses used in the therapeutic trials were also diverse. Thus comparative studies to determine the efficacy of various dosage regimens are needed. The second issue is the optimal schedule (high dose once daily vs lower doses more frequently) and the duration of treatment with LGG required to achieve the preventive effect. It has been speculated that

a beneficial prophylactic effect can only be expected with regular consumption of the probiotic agent.<sup>38,39</sup>

In conclusion, our findings confirm the efficacy of prophylactic LGG administration in the prevention of nosocomial diarrhea in infants, particularly nosocomial rotavirus gastroenteritis.

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