Lactobacillus GG in the prevention of antibiotic-associated diarrhea in children

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Objective: The objective of this study was to determine the efficacy of *Lactobacillus casei sps. rhamnosus (Lactobacillus GG)* (LGG) in reducing the incidence of antibiotic-associated diarrhea when coadministered with an oral antibiotic in children with acute infectious disorders.

Study design: Two hundred two children between 6 months and 10 years of age were enrolled; 188 completed all phases of the protocol. LGG, $1 \times 10^{10} - 2 \times 10^{10}$ colony forming units per day, or comparable placebo was administered in a double-blind randomized trial to children receiving oral antibiotic therapy in an outpatient setting. The primary caregiver was questioned every 3 days regarding the incidence of gastrointestinal symptoms, predominantly stool frequency and consistency, through telephone contact by blinded investigators.

Results: Twenty-five placebo-treated but only 7 LGG-treated patients had diarrhea as defined by liquid stools numbering 2 or greater per day. *Lactobacillus GG* overall significantly reduced stool frequency and increased stool consistency during antibiotic therapy by the tenth day compared with the placebo group.

Conclusion: *Lactobacillus GG* reduces the incidence of antibiotic-associated diarrhea in children treated with oral antibiotics for common childhood infections. (J Pediatr 1999;135:564-8)

Outpatient use of antibiotics is common in pediatrics, most often for a variety of minor infections of the respiratory tract, integument, and urinary tract. Certain antibiotics, especially those with a relatively broad spectrum, frequently result in diarrhea.[1] The incidence of antibiotic-associated diarrhea in children ranges from 20% to 40% of those receiving broad-spectrum antibiotics.[2]

The mechanism by which antibiotic-associated diarrhea occurs most likely relates to disturbances of microbial flora in the gastrointestinal tract. More than 500 species of bacteria inhabit the gut, and a balance of these organisms is crucial to normal gastrointestinal function.[3,4] Disruption of the microbial flora may result in the overgrowth of pathogenic organisms such as *Clostridium difficile* or may disturb the metabolism of carbohydrates, resulting in malabsorption of osmotically active particles.

See editorial, p. 535.

Recently, probiotic organisms have been advocated for use in stabilizing gut microflora in conditions where disturbances of normal bacterial flora result in gastrointestinal symptoms.[5,6] Because antibiotic-associated diarrhea is such a condition, use of a probiotic would seem appropriate in this situation. Unfortunately, many purported probiotics are not effective because of their inability to survive in gastric and bile secretions, inability to colonize the gastrointestinal tract, and ineffective binding to intestinal epithelial cells.[7-9] *Lactobacillus casei sps. rhamnosus (Lactobacillus GG)* has been shown to do all 3 and consequently would seem to be a suitable probiotic in this situation.[10-12] Consequently, we elected to initiate a randomized, double-blind, placebo-controlled trial of coadministration of *Lactobacillus GG* with antibiotics in children being treated for a variety of minor infections to assess the efficacy of this bacterium as a probiotic in the prevention of antibiotic-associated diarrhea.

LGG *Lactobacillus GG*

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METHODS

Based on power analysis ($\alpha = .05$ and .80 power), 202 children between the ages of 6 months and 10 years were recruited from a busy private primary care pediatric practice for participation in this study. The population base was largely representative of a middle class, Midwestern city (population 50,000). However, this practice receives many rural and minority (Native-American) referrals. All were being evaluated during the month of September for symptoms of acute infection of the upper or lower respiratory tract, the urinary tract, soft tissues, or skin. Only children who were prescribed a 10-day course of antibiotics were included. Children with any chronic disease, serious acute infection, or diarrhea at the time of antibiotic initiation were excluded. Once the decision was made to treat with oral antibiotics, parents were offered the opportunity for their child to participate in the study with the use of a consent form and materials approved by the Human Subject Protection Committee of Children's Hospital in Omaha, Nebraska.

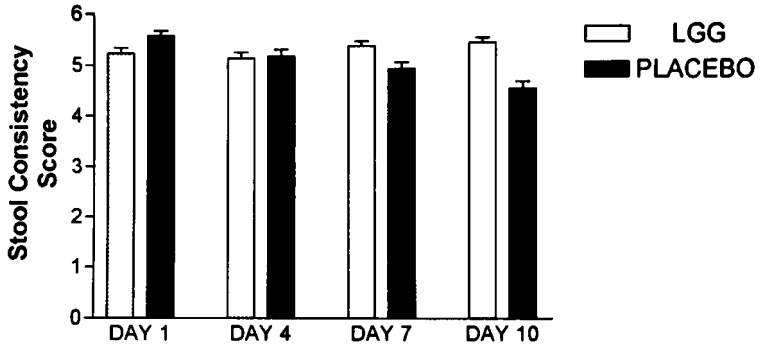
Patients receiving any oral antibiotic were included in the study and were randomized with a computer-generated randomization table to receive either LGG or a placebo in capsule form. Product randomization by blinded numeric codes was performed by the supplier before the product was shipped to the investigation site. Codes were kept by the supplier until all data were collected. The LGG and placebo were packed in identical bottles with identical capsule covers.

Children weighing <12 kg were given 1 capsule (10 billion colony-forming units), and those >12 kg were given 2 capsules (20 billion colony-forming units of live LGG) once daily with a meal. The dosing regimen was based on previous pediatric studies that demonstrated effective colonization at these dosage levels.[13-15] The LGG capsules were obtained from CAG Functional Foods in Omaha, Nebraska, and contained LGG and inulin as a prebiotic filler. Placebo was composed of inulin. Those unable to swallow capsules were instructed to open the capsules and mix the contents with food not above room temperature. Parents were instructed not to alter the child's diet in any way during the course of treatment. No other obvious probiotic-containing supplements were allowed during the course of the study. After capsules were dispensed, parents were instructed to refrigerate them to ensure viability.

After oral and written consent was obtained, parents were instructed on the administration of the probiotic and given a copy of the assessment parameters for reference when contacted by the investigators. Parents were contacted within 24 hours of initial enrollment for baseline data collection by one of the investigators and subsequently were contacted every 3 days until antibiotic courses were completed or diarrhea ceased. At each contact, stool frequency and consistency were assessed and graded numerically. In addition, the presence or absence of visible blood content in the stool, abdominal pain, nausea, vomiting, bloating, and appetite suppression was assessed. If any child was unable to consume the assigned product for whatever reason, or if the primary caregiver was unable to provide complete data, the child was to be removed from the study.

The Stool Consistency Continuum is composed of a group of 8 line drawings depicting stools varying from watery to hard and dry (Fig 1).[16] Parents were asked to compare each stool passed to a drawing and assign it a numeric consistency score. Stool frequency was determined by counting the number of stools passed during a 24-hour period. If present, abdominal pain was also given an intensity score based on a visual analog scale. Investigation of diarrhea causes was to be pursued if clinical presentation suggested an infectious cause (vomiting, abdominal cramping, and loose, bloody frequent stools) or if dehydration appeared likely. All other parameters were assessed solely on the basis of their presence or absence. At the conclusion of the study, all children were given a $25.00 gift certificate for a toy store.

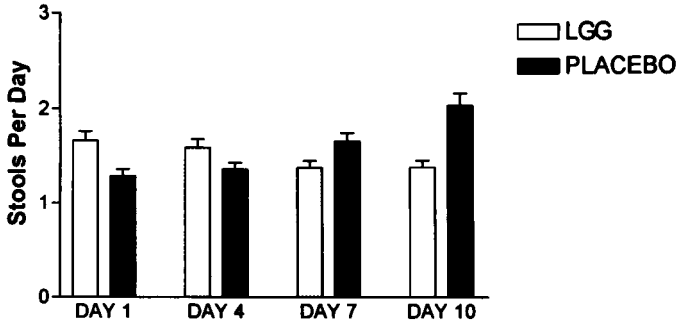
Fig 1. Line drawings depicting stool consistency and given numeric value. From Young RJ, Beerman LE, Vanderhoof JE. Increasing oral fluids in chronic constipation in children. Gastroenterol Nurs 1998;21:156-61. Reprinted with permission.



Stool Consistency Score

DAY OF STUDY	LGG	SEM	PLACEBO	SEM
DAY 1	5.230	0.110	5.570	0.100
DAY 4	5.130	0.120	5.170	0.130
DAY 7	5.370	0.090	4.920	0.130
DAY 10	5.430	0.090	4.530	0.140

Fig 2. Mean stool consistency per day ± SEM for each observation period for both active and placebo groups is shown. Active is displayed in *solid bars* and placebo in *open bars*. By day 10, significant difference in stool consistency was observed ($P < .02$).



Stools Per Day

DAY OF STUDY	LGG	SEM	PLACEBO	SEM
DAY 1	1.670	0.100	1.290	0.070
DAY 4	1.590	0.090	1.360	0.070
DAY 7	1.380	0.070	1.660	0.090
DAY 10	1.380	0.070	2.030	0.130

Fig 3. Mean number of stools per day ± SEM for each observation period for both active and placebo groups is shown. Active is displayed in *solid bars* and placebo in *open bars*. By day 10, significant difference in mean stool frequency was observed ($P < .02$).

Stool consistency scores and stool frequency were analyzed by a mixed design, groups observation point, analysis of variance. Newman-Keuls pairwise comparisons were performed. Chi-squared analysis was also performed to evaluate the occurrence of the stool consistency score of <4 on either day 7 or day 10 of the observation period.

RESULTS

The study was completed by 188 children (median age 4 years); 14 failed to complete the study, primarily because of antibiotic noncompliance or inability of the investigators to contact the primary caregiver at the assigned follow-up time. None of the participants failed to complete the 10-day course of antibiotics because of a change in stool consistency or frequency. There were no failures resulting from untoward effects of either LGG or placebo. Both active and placebo groups were similar for age distribution, sex, and type of antibiotics, and all who completed the study had no difficulty consuming the prescribed amount. The most common antibiotics prescribed, the reason for their use, and other patient characteristics are listed in Table I. Compliance was measured by capsule counting at the conclusion of the study.

For the purposes of data evaluation, diarrhea was defined as the presence of at least 2 liquid stools per day on at least 2 observation periods during the course of this study. With this definition 25 (26%) patients who received placebo but only 7 (8%) who received LGG had diarrhea during antibiotic administration. The mean duration of diarrhea was 4.70 days in the LGG group and 5.88 days in the placebo group ($P = .05$). Mean stool consistency scores at each observation point for the active and placebo groups are shown in Fig 2. There was a gradual trend toward decreasing stool consistency over time, with the active group differing significantly from the placebo group by the tenth day ($P < .02$). A stool consistency score of <4 on either day 7 or day 10 was significantly more common in the placebo-treated group than in the LGG-treated group; 48% of the children treated with placebo had a stool consistency score of <4 during the course of therapy, whereas only 17% of the children in the LGG group had a score of <4 ($P < .001$). Mean stool frequencies for the active and placebo groups at each observation point are shown in Fig 3. Again, by day 10 the active group differed significantly from the placebo group ($P < .02$).

Clostridium difficile colonization was not evaluated in the study, because no child with significant diarrhea at the beginning of the study was enrolled, and none had diarrhea that failed to resolve shortly after the end of antibiotic therapy. Routine monitoring of LGG colony counts in the capsules was performed throughout the study and showed no significant variance in total colony count. No differences were seen between the active and placebo groups in any of the other parameters assessed.

Table I. Study demographics

	Active (n = 93)	Placebo (n = 95)
Sex		
Male	43	42
Female	50	53
Age	3 y 11 mo	4 y
Diagnosis		
Otitis	55	54
Pharyngitis	17	20
Bronchitis	8	11
Dermatologic	7	4
Sinusitis	5	5
Other	1	1
Antibiotic used		
Amoxicillin	33	32
Amoxicillin/clavulanate potassium	20	13
Cefprozil	4	9
Clarithomycin	9	9
Other	27	32

DISCUSSION

Probiotics are defined as live organisms that, when ingested, result in health benefits including amelioration or prevention of a specific disease state.[6] These organisms generally enhance the intestinal microflora by replenishing suppressed bacteria and inhibiting the growth of more pathogenic flora.[17] Some probiotics including LGG also actively secrete antimicrobial substances, which inhibit the growth of certain other organisms.[12] Many strains of bacteria claimed to be useful as probiotics are destroyed in the acidic environment of the stomach or die when exposed to bile acids in the duodenum and proximal jejunum.[7] It has been demonstrated that LGG can be cultured in the stool for up to 2 weeks after oral administration is ceased.[12,18]

This organism was initially described by Gorbach et al[12] from Tufts University, who isolated it from human fecal samples and subsequently demonstrated its probiotic capabilities. It was initially classified as a *Lactobacillus rhamnosus*, subsequently as a *Lactobacillus casei*, and ultimately assigned the subspecies GG. It is one of the most extensively studied and best characterized probiotic organisms. It has been shown to be effective in a number of clinical situations including reducing the incidence of traveler's diarrhea, shortening the duration and severity of rotavirus diarrhea, and ameliorating erythromycin-induced diarrhea in adults.[13-15,19-21] Preliminary evidence suggests that the organism may even be beneficial in the prevention or amelioration of intestinal allergy in infants.[22] It has also been shown to be useful in small uncontrolled studies of patients with relapsing *Clostridium difficile* diarrhea, having successfully broken the cycle of recurrence in a number of patients in whom *Clostridium difficile*-associated diarrhea repetitively recurred after vancomycin or metronidazole therapy was withdrawn.[23-25]

Table II. Antibiotic sensitivity of *Lactobacillus GG*

Antibiotics	MIC (μg/mL)
Ciprofloxacin	0.20
Ampicillin	0.50
Cefotaxime	4.00
Penicillin	1.00
Cephalothin	16.00
Erythromycin	0.25
Tetracycline	2.00
Trimethoprim	> 4.00
Sulfamethoxazole	76.00
Amoxicillin/Clavulanate	0.50

MIC, Minimum inhibitory concentration.

Because these organisms vary significantly from one another in their probiotic properties, careful documentation of each organism's efficacy is required before it is considered for use as a probiotic in any specific condition. Unfortunately, lack of awareness of this concept has resulted in the widespread use of a number of poorly studied "probiotic" organisms with minimal therapeutic value. Recently, studies examining the viability of a number of commercially available probiotics have shown that these preparations frequently do not contain the number of live organisms stated and occasionally contain no live organisms at all.[9] Ideally, final verification of the organism's viability is based on colonization studies, as has previously been done with LGG.[12,18] Careful handling and quality control is necessary to ensure overall viability of a probiotic product.

Antibiotic-associated diarrhea is a classic example of the deleterious effect of disturbing normal gastrointestinal flora both quantitatively and qualitatively. Twenty percent to 40% of all children receiving broad-spectrum antibiotics

have diarrhea.[2] In our study 26% of the placebo-treated children had diarrhea when it was defined as at least 2 liquid stools on at least 2 observation periods during antimicrobial therapy. Antibiotic-associated diarrhea was reduced to 8% in our study with the coadministration of LGG. This diarrhea is often merely a nuisance and rarely causes dehydration. However, it can occasionally result in hospitalization, markedly increasing the cost of antimicrobial therapy. More commonly, antibiotic-associated diarrhea may be a cause of compliance failure, because parents will discontinue administration of the antibiotic as a result of frustration over loose stools. Children with diarrheal stools are often not admitted to day-care, and subsequently absenteeism from work may be an additional economic factor to be considered when the cost of the untoward effects of antimicrobial therapy is calculated.

The antimicrobial resistance pattern of LGG was published in 1982.[26] The subject of antibiotic resistance to lactobacilli in general has been reviewed more recently.[27] Current antimicrobial resistance (shown in Table II), however, does not necessarily correlate with colonization resistance. A recent study has also demonstrated that *L rhamnosus* GG does not contain plasmids; therefore, the transfer of chromosomal vancomycin resistance from LGG to enterococcal species was not detected.[28] It produces L-lactate almost exclusively and therefore is unlikely to cause D-lactic acidosis even in highly susceptible patients with intestinal stasis and bacterial overgrowth.

Possible limitations of our study included the use of parental interview as a data collection method. Provision of the simplistic data monitoring tool for the parent to refer to when interviewed was to avoid problems with pure recall as a collection method. The specific type of acute disease or antibiotic type was also not controlled, but statistical analysis of these groups revealed no difference between them for any of the variables.

REFERENCES

1. Bartlett JG. Antibiotic-associated diarrhea. Clin Inf Dis 1992;15:573-81.
2. Elstner CL, Lindsay AN, Book LS, Matsen JM. Lack of relationship of *Clostridium difficile* to antibiotic-associated diarrhea in children. Pediatr Inf Dis 1983;2:364-6.
3. Simon GL, Gorbach SL. Intestinal flora in health and disease. Gastroenterology 1984;86:174-93.
4. Rao SS, Edwards CA, Austen CJ, Bruce C, Read NW. Impaired colonic fermentation of carbohydrate after ampicillin. Gastroenterology 1988;94: 928-32.
5. Gibson GR, Roberfroid MD. Dietary modulation of the human colonic mocrobiota: including the concept of prebiotics. J Nutr 1994;125:1401-12.
6. Fuller R. Probiotics in human medicine. Ann Med 1990;22:37-41.
7. Charteris WP, Kelly PM, Morelli L, Collins JK. Selective detection, enumeration and identification of potentially probiotic Lactobacillus and Bifidobacterium species in mixed bacterial populations. Int J Food Microbiol 1997;35:1-27.
8. Gorbach SL. Lactic acid bacteria and human health. Ann Med 1990;22: 37-41.
9. Hamilton-Miller JMT, Shah S, Smith CT. "Probiotic" remedies are not what they seem. Br Med J 1996; 312:55-6.
10. Meurman JH, Antila H, Korhonen A, Salminen S. Effect of *Lactobacillus rhamnosus* strain GG (ATCC 51303) on the growth of *Streptococcus dobrinus in vitro.* Eur J Oral Sci 1995;103:253-8.
11. Alander M, Korpela R, Saxelin M, Vilponnen-Salmela T, Mattila-Sandholm T, vonWright A. Recovery of *Lactobacillus rhamnosus GG* from human colonic biopsies. Lett Appl Microbiol 1997;24:361-4.
12. Goldin BR, Gorbach SL, Saxelin M, Barakat S, Gualtieri L, Salminen S. Survival of Lactobacillus species (strain GG) in human gastrointestinal tract. Dig Dis Sci 1992;37:121-8.
13. Majamaa H, Isolauri E, Saxelin M, Vesikari T. Lactic acid bacteria in the treatment of acute rotavirus gastroenteritis. J Pediatr Gastroenterol Nutr 1995;20:333-9.
14. Raza S, Graham SM, Allen SJ, Sultana S, Cuevas L, Hart CA. *Lactobacillus GG* promotes recovery from acute nonbloody diarrhea in Pakistan. Pediatr Inf Dis J 1995;14:107-11.
15. Isolauri E, Juntunen M, Rautanen T, Sillanaukee P, Koivula T. A human Lactobacillus strain (Lactobacillus casei sp strain GG) promotes recovery from acute diarrhea in children. Pediatrics 1991;88:90-7.
16. Bergstrom N, Braden B, Laquezza A, Holmen A. The Braden Scale for predicting pressure sore risk. Nurs Res 1987;36:205-10.
17. Salminen S, Deighton M. Lactic acid bacteria in the gut in normal and disordered states. Dig Dis Sci 1992;10: 227-38.
18. Saxelin M, Pessi T, Salminen S. Fecal recovery following oral administration of Lactobacillus strain GG (ATCC 53103) in gelatine capsules to healthy volunteers. Internat J Food Microb 1995;25:199-203.
19. Siitonen S, Vapaatalo H, Salminen S, Gordin A, Saxelin M, Wikberg R, et al. Effect of *Lactobacillus GG* yoghurt in prevention of antibiotic-associated diarrhea. Ann Med 1990;22:57-9.
20. Pant AR, Graham SM, Allen SJ, Karikal S, Sabchareon A, Cuevas L, Hart CA. *Lactobacillus GG* and acute diarrhoea in young children in the tropics. J Trop Med 1996;42:162-5.
21. Hilton E, Kolakowski P, Smith M, Singer C. Efficacy of *Lactobacillus GG* as a diarrheal preventative in travelers. J Trav Med 1997;4:41-3.
22. Majamaa H, Isolauri E. Probiotics: a novel approach in the management of food allergy. J Allergy Clin Immunol 1997;99:179-85.
23. Gorbach, SL, Chang TW, Goldin BR. Successful treatment of relapsing *Clostridium difficile* colitis with *Lactobacillus GG*. Lancet 1987;2:1519.
24. Biller JA, Katz AJ, Flores AF, Buie TM, Gorbach SL. Treatment of recurrent *Clostridium difficile* colitis with *Lactobacillus GG*. J Pediatr Gastroenterol Nutr 1995;21:224-6.
25. Gorbach SL. Function of the normal human microflora. Scand J Infect Dis 1986;49:17-30.
26. Salminen S, Von Wright A. Lactic acid bacteria. New York: Marcel Dekker; 1982.
27. Charteris WP, Kelly PM, Morelli L, Collins JK. Antibiotic susceptibility of potentially probiotic Lactobacillus species. J Food Prot 1998;61:1636-43.
28. Tynkkynen S, Singh KV, Varmanen P. Vancomycin resistance factor of *Lactobacillus rhamnosus GG* in relationship to enterococcal vancomycin resistance (*van)* genes. Int J Food Microbiol 1998;41:195-204.