ABSTRACT. Objectives. Antimicrobial treatment may disturb the colonization resistance of gastrointestinal microflora, which may induce clinical symptoms, most commonly diarrhea. The severity of antibiotic-associated diarrhea may range from a brief, self-limiting disease to devastating diarrhea with electrolyte disturbances, dehydration, crampy abdominal pain, pseudo-membranous colitis, toxic megacolon, or even death. The incidence of diarrhea in children receiving a single antimicrobial treatment is unclear. In addition to more critical use of antimicrobials, adjunctive preventive measures to antibiotic-associated diarrhea are needed. The objective of this study was to evaluate the incidence of diarrhea in children with no history of antimicrobial use during the previous 3 months. Another aim of this study was to assess the preventive potential of Lactobacillus rhamnosus GG (Lactobacillus GG; American Type Culture Collection 53103), a probiotic strain with a documented safety record and a therapeutic effect in viral gastroenteritis on antibiotic-associated diarrhea.

Methods. Oral antimicrobial agents were prescribed for the treatment of acute respiratory infections at the clinics of the Health Care Center of the City of Tampere or Tampere University Hospital, Finland, to 167 patients who were invited to participate in the study. Of the patients, 48 were lost to follow-up; therefore, the final study population consisted of 119 children from 2 weeks to 12.8 years of age (mean: 4.5 years). All study subjects met the inclusion criteria: they had not received any antimicrobial medication during the previous 3 months, they did not suffer from gastrointestinal disorders, and they did not need intravenous antimicrobial treatment.

The patients were randomized to receive placebo or $2 \times 10^{10}$ colony-forming units of Lactobacillus GG in capsules given twice daily during the antimicrobial treatment. Lactobacillus GG and placebo capsules were indistinguishable in appearance and taste. The parents kept a daily symptom diary and recorded stool frequency and consistency at home for 3 months. Diarrhea was defined as at least three watery or loose stools per day for a minimum of 2 consecutive days. In the case of diarrhea, viral (adenovirus, rotavirus, calicivirus and astrovirus) and bacterial (Salmonella, Shigella, Yersinia, Campylobacter, Clostridium difficile, Staphylococcus aureus, and yeasts) analyses were studied in fecal samples. The metabolic activity of the gut microflora was assessed by analysis of fecal urease, b-glucosidase, and b-glucuronidase activities. The primary outcome measure was diarrhea during the first 2 weeks after the beginning of the antimicrobial treatment, because this period most likely reflects the effects of antimicrobial use. Secondary outcome measures were the activities of fecal urease, b-glucuronidase, and b-glucosidase.

Results. On the entire follow-up, 80% of any gastrointestinal symptoms were reported during the first 2 weeks after the beginning of the antimicrobial treatment. The incidence of diarrhea was 5% in the Lactobacillus GG group and 16% in the placebo group within 2 weeks of antimicrobial therapy ($\chi^2 = 3.82$. The treatment effect (95% confidence interval) of Lactobacillus GG was $-11\%$ ($-21\%$–$0\%$). In diarrheal episodes, the viral and bacterial analyses were positive for Clostridium difficile in 2 cases and for Norwalk-like calicivirus in 3 cases. The age of the patients with diarrhea was between 3 months and 5 years in 75% of cases in both groups. The severity of diarrhea was comparable in the study groups, as evidenced by similar stool frequency (mean: 5 per day; range: 3–6) and the duration of diarrhea (mean: 4 days; range: 2–8).

The activities of fecal urease and b-glucuronidase, but not b-glucosidase, changed significantly after the beginning of the antimicrobial treatment in the Lactobacillus GG group and in the placebo group alike. The decrease in urease and b-glucuronidase activities was reversible in patients with no diarrhea, but in patients with diarrhea, the modifications in gut microflora were more profound and prolonged. The activities of the three enzymes were normalized within 3 weeks, evidenced by stable enzyme activities in samples collected 3 weeks, 1 month, and 3 months after the beginning of the antimicrobial treatment, compared with those obtained before treatment.

Discussion. In the present study, after a single antimicrobial treatment, the incidence of diarrhea was 16%. The higher incidence of antibiotic-associated diarrhea in previous reports may be attributable to a recent antimicrobial therapy that disturbs intestinal flora and exposes to complications. Also, in the present study, changes in the metabolic activity of the intestinal flora were observed, evidenced by a transient decline in fecal enzyme activities.

Different probiotic preparations, including lactobacilli, are recommended frequently to prevent antibiotic-associated diarrhea. Although probiotics have been shown to be efficient in the prevention and the treatment of viral gastroenteritis, their usefulness during antimicrobial therapy in children has not been elucidated be-
Antimicrobial agents are the most frequently prescribed medicines in children, because acute infectious diseases are prevalent in this age group. The demonstration that acute infections are primarily of viral origin has not reduced the use of antibiotics, nor has the fact that antibiotics afford only marginal alleviation of the clinical symptoms.

Antimicrobial treatment may disturb the colonization resistance of gastrointestinal microflora, which may induce clinical symptoms, most commonly diarrhea. The incidence of antibiotic-associated diarrhea has been estimated to vary between 5% and 25% in adults and between 8% and 30% in children. The discrepancies in the incidence may have been attributable to differences in the definition of diarrhea, the antimicrobial agent used, the number of daily doses, the duration of the treatment, and the time from previous antimicrobial treatments. The severity of antibiotic-associated diarrhea may range from a brief, self-limiting disease to devastating diarrhea with electrolyte disturbances, dehydration, crampy abdominal pain, pseudomembranous colitis, toxic megacolon, or even death.

In addition to more critical use of antimicrobials, adjunctive preventive measures to antibiotic-associated diarrhea are needed. The objective of this randomized, double-blind, placebo-controlled follow-up study was to evaluate the incidence of diarrhea after antimicrobial treatment in children with no history of antimicrobial use during the previous 3 months, because recent antimicrobial treatments may cause confusion. Another aim of this study was to assess the preventive potential of probiotics on antibiotic-associated diarrhea. For this purpose, we applied *Lactobacillus rhamnosus* GG (Lactobacillus GG; American Type Culture Collection 53103), a probiotic strain with a documented safety record and a therapeutic effect in viral gastroenteritis, as the probiotic for the treatment of children receiving antibiotics.

### METHODS

#### Patients

Oral antimicrobial agents were prescribed for the treatment of acute respiratory infections at the clinics of the Health Care Center of the City of Tampere or Tampere University Hospital to 167 patients, who were invited to participate in the study. Of the patients, 20 receiving placebo and 28 receiving *Lactobacillus GG* during antimicrobial treatment were lost to follow-up or discontinued because of difficulties in the transportation of the study samples. Therefore, the final study population consisted of 119 children (Table 1) from 2 weeks to 12.8 years of age (mean: 4.5 years, with 72% <6 years of age). All study subjects met the inclusion criteria: they had not received any antimicrobial medication during the previous 3 months, they did not suffer from gastrointestinal disorders, and they did not need intravenous antimicrobial treatment. Five patients were hospitalized; all others were treated as outpatients.

**TABLE 1. Clinical Characteristics of the Patients**

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Lactobacillus GG (n = 61)</th>
<th>Placebo (n = 58)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Otitis</td>
<td>45</td>
<td>43</td>
</tr>
<tr>
<td>Tonsillitis</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Pneumonia</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>Bronchitis</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Sinusitis</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Antimicrobial agent</td>
<td>Penicillin</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>Amoxicillin</td>
<td>41</td>
</tr>
<tr>
<td></td>
<td>Kephalosporins</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Erythromycin</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Trimetoprim-sulpha</td>
<td>5</td>
</tr>
<tr>
<td>Previous antibiotics: None</td>
<td>9</td>
<td>10</td>
</tr>
<tr>
<td>0–1 prescription/y</td>
<td>40</td>
<td>36</td>
</tr>
<tr>
<td>2 or more prescriptions/y</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>Child care</td>
<td>Home</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>Kindergarten</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>Private family</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>School</td>
<td>13</td>
</tr>
</tbody>
</table>

**Conclusion.** A probiotic strain, *Lactobacillus GG*, is effective in the prevention of diarrhea in children receiving antimicrobial treatment to respiratory infections. *Pediatrics* 1999;104(5). URL: http://www.pediatrics.org/cgi/content/full/104/5/664; antibiotic-associated diarrhea, *lactobacillus, glucuronidase, glucosidase, urease.*
tors. Intestinal bacterial enzyme activities reflect both the metabolic bility and the quantitative composition of intestinal bacteria.18 Fecal samples were prepared and the enzyme activities were measured as previously described.13 Enzyme activities are expressed as nmol of substrate metabolized per minute per milligram of protein in the fecal supernatant.

Detection of Lactobacillus GG in Feces
To study colonization, the recovery of Lactobacillus GG in feces in 23 randomly selected patients from samples collected 7 days after the start of antimicrobial treatment was studied as previously described.12 Analysis showed that the patients in the Lactobacillus GG group were colonized by Lactobacillus GG. Two patients were not colonized with the organism with a detection limit of $10^3$ colony-forming units per gram of feces. These were not excluded, because biopsy studies have indicated mucosal colonization even in cases in which fecal counts were below detection limit.13

Viral and Bacterial Analyses
Adenovirus-antigen and rotavirus-antigen were assessed using the enzyme-linked immunoassay. Fecal samples were cultured for Salmonella, Shigella, Yersinia, Campylobacter, Clostridium difficile, Staphylococcus aureus, and yeasts. Clostridium difficile toxin A was analyzed by enzyme immunoassay. These analyses were studied from fresh fecal samples. Frozen fecal samples negative under electron microscopy were analyzed further by reverse transcription polymerase chain reaction for Norwalk-like (genogroup I and II) caliciviruses14 (with confirmation by hybridization with specific probes) and for astroviruses.15

Statistical Methods
The results are presented as means with range. The $\chi^2$ test, Wilcoxon signed-rank test, and analysis of variance for repeated measurements were used in statistical comparisons.

RESULTS
Clinical Characteristics of the Patients
The mean age of the patients in the Lactobacillus GG group was 4.7 years (range: 2 weeks to 11.8 years of age), and in the placebo group, 4.4 years (range: 2 weeks to 12.8 years of age). The groups were also comparable in clinical diagnosis, antimicrobial agents used, history of antibiotic use, and mode of day care (Table 1). The parents reported no adverse effects of Lactobacillus GG or placebo.

The Frequency of Diarrhea After the Beginning of the Antimicrobial Treatment
On the entire follow-up, 80% of any gastrointestinal symptoms were reported during the first 2 weeks after the beginning of the antimicrobial treatment. In 3 (5%) patients in the Lactobacillus GG group and in 9 (16%) patients in the placebo group, the change in stool consistency and frequency during the first 2 weeks fulfilled the criteria of diarrhea ($\chi^2 = 3.82; P = .05$). The treatment effect (95% confidence interval) of Lactobacillus GG was $-11\%$ ($-21\%$–$0\%$). In diarrheal episodes, the viral and bacterial analyses were positive for Clostridium difficile in 2 cases (1 in both groups with toxin A-positive in the patient in the Lactobacillus GG group) and Norwalk-like calicivirus in 3 cases (1 in the Lactobacillus GG group and 2 in the placebo group), whereas, the viral and bacterial analyses were negative for rotavirus, astrovirus, Salmonella, Shigella, Yersinia, Campylobacter coli, Campylobacter jejuni, Staphylococcus aureus, and yeasts. The age of the patients with diarrhea was between 3 months and 5 years in 75% of cases, in both groups alike. The severity of diarrhea was comparable in the study groups, evidenced by similar stool frequency (mean: 5 per day; range: 3–6) and the duration of diarrhea (mean: 4 days; range: 2–8). In all cases, the diarrhea was self-limiting.

Fecal Urease, $\beta$-Glucosidase, and $\beta$-Glucuronidase Activity
The activities of fecal urease and $\beta$-glucuronidase, but not $\beta$-glucosidase, changed significantly after the beginning of the antimicrobial treatment ($P = .0001$ and $P < .0001$, respectively) in the Lactobacillus GG group and in the placebo group alike. The decrease in urease and $\beta$-glucuronidase activities was reversible in patients with no diarrhea, but in patients with diarrhea, the modifications in gut microflora were more profound and prolonged (Fig 1). The activities of the three enzymes were normalized within 3 weeks, evidenced by stable enzyme activities in samples collected 3 weeks, 1 month, and 3 months after the beginning of the antimicrobial treatment, compared with those obtained before treatment (data not shown).

DISCUSSION
The most common complication of antimicrobial therapy is antibiotic-associated diarrhea. In the present study, the incidence of diarrhea after a single antimicrobial treatment was 16%. We chose to analyze only the diarrhea episodes that occurred during the first 2 weeks after the beginning of the antimicrobial treatment, because the later that diarrhea occurs, the more unlikely it is caused by an antimicrobial agent. The higher incidence of antibiotic-associated diarrhea in previous reports may be attributable to a recent antimicrobial therapy that disturbs intestinal flora and exposes to complications. Also, in the present study, changes in the metabolic activity of the intestinal flora were observed, evidenced by a transient decline in fecal enzyme activities.

Different probiotic preparations, including lactobacilli, are recommended frequently to treat and prevent disturbances in intestinal microflora and antibiotic-associated diarrhea. Although probiotics have been shown to be efficient in the prevention16 and the treatment17 of viral gastroenteritis, their usefulness during antimicrobial therapy in children has not been elucidated before. We observed that the administration of Lactobacillus GG to children receiving antimicrobial therapy for respiratory infection reduced the incidence of antibiotic-associated diarrhea to one third. This is in agreement with a previous study demonstrating beneficial effect of Lactobacillus GG in the treatment of relapsing Clostridium difficile colitis.17 The profitable effect may be mediated by a number of functions of probiotics,18 ie, production of antimicrobial substances, local competition of adhesion receptors and nutrients, and stimulation of intestinal antigen specific and nonspecific immune responses.19

The most effective way to prevent antibiotic-associated diarrhea is still critical use of antimicrobials, as recommended recently for the treatment of acute otitis media in children.20 However, when antimicro-

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of Tampere University Hospital, the Emil Aaltonen Foundation, and the Academy of Finland.

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Fig 1. The mean activities of fecal urease, β-glucuronidase, and β-glucosidase in patients with diarrhea (closed circle) and in those with no diarrhea (open circle) before antimicrobial treatment and 1 and 2 weeks after the beginning of the antimicrobial treatment. Asterisks indicate significantly different (P ≤ .05) from patients with no diarrhea.
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Taina Arvola, Kirsi Laiho, Sari Torkkeli, Hannu Mykkänen, Seppo Salminen, Leena Maunula and Erika Isolauri

*Pediatrics* 1999;104;e64
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