MAJOR ARTICLE

Absence of Efficacy Of Nonviable *Lactobacillus acidophilus* for the Prevention of Traveler's Diarrhea: A Randomized, Double-Blind, Controlled Study

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Background. Diarrhea is the most common illness associated with international tourism. We evaluated the efficacy of a probiotic preparation of nonviable *Lactobacillus acidophilus* (hereafter referred to as LA) for the prevention of traveler's diarrhea.

Methods. We conducted a randomized, double-blind, controlled trial. Travelers were randomized to receive either LA or placebo twice daily from 1 day before their departure to 3 days after their return. On each day of the trip and the week following the return, travelers had to record the number and consistency of stools and their adherence to the treatment. Diarrhea was defined as $\geqslant$3 unformed stools in a 24-h period.

Results. From January 2001 to September 2004, a total of 174 subjects were randomized to each treatment group. Half of the travelers went to West Africa, and organized tours or backpacking were the most common modes of traveling. The incidence of diarrhea did not differ between the 2 groups; it was 61.4 cases per 100 person-months in the LA group (95% confidence interval [CI], 44.1–85.5) and 43.4 cases per 100 person-months in the placebo group (95% CI, 30.0–62.9) ($P = .14$). Adjustment for travel duration and other variables did not reveal any difference between the 2 groups (adjusted hazard ratios comparing the LA and placebo groups were 1.43 [95% CI, 0.87–2.36] in an intent-to-treat analysis and 1.38 [95% CI, 0.79–2.39] in an efficacy analysis).

Conclusions. There was no beneficial effect of treatment with LA for the prevention of travelers' diarrhea. More studies are required to assess the efficacy of other specific probiotics (e.g., a *Lactobacillus rhamnosus* GG preparation) for preventing traveler's diarrhea.

Diarrheal illness is the most common health impairment associated with international tourism in terms of frequency—it affects 20%–50% of travelers—and economic impact [1, 2]. Bacterial infection accounts for ~80% of cases, among which, enterotoxinogenic *Escherichia coli, Shigella* species, and *Salmonella* species are the most common pathogens. Although most cases of traveler's diarrhea (TD) are self-limited and benign, the illness causes discomfort for the traveler and represents a significant socioeconomic burden for both the traveler and his or her country of origin [2]. Precautions regarding dietary habits are the main measures of prevention, but efficacy is low, and measures are insufficiently followed. Therefore, intensive efforts have been undertaken to find an effective prophylactic medication, particularly for children, pregnant women, and persons with preexisting illness, who are at an increased risk of developing complicated and long-lasting disease. Antibacterials, such as quinolones, provide excellent protection, but their use is limited because of potential—sometimes severe—adverse reactions and the risk of increasing drug resistance [3]. Together with nonabsorbable antibiotics, such as rifaximin [4], another recent approach to avoid adverse effects of antibacterial

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medication is the use of probiotics, which consist of preparations of either bacteria—especially lactobacilli and bifidobacteria—or yeast (e.g., *Saccharomyces* species) [5]. Several clinical studies have shown the therapeutic and/or prophylactic efficacy of specific probiotics against acute gastroenteritis and antibiotic-associated diarrhea (including *Clostridium difficile* infection) [6–11]. However, evidence of efficacy against TD has remained inconclusive, because there have been a limited number of studies, including some with methodological drawbacks; although some studies have reported a reduction in the risk of TD following treatment with probiotics [12–14], others have not found any beneficial effect [15, 16]. In a double-blind, randomized, controlled trial, we evaluated the efficacy of a probiotic preparation of nonviable *Lactobacillus acidophilus* (hereafter referred to as LA) for the prevention of TD.

PATIENTS AND METHODS

Study sites. The study was conducted in France from January 2001 to September 2004. The hospitals involved were the travel clinic of Avicenne University Hospital (Bobigny), Bichat-Claude Bernard University Hospital (Paris), Institut Pasteur (Paris), Saint-Antoine University Hospital (Paris), Raymond Poincarré University Hospital (Garches), Delafontaine Hospital (Saint-Denis), and Gonesse Hospital (Gonesse).

Study subjects. To be eligible, a person had to be ⩾18 years old, plan on traveling to an area associated with an intermediate or high risk of diarrheal illness [17] for <21 days, and leave France for their travel within 2 months after enrolling in the study. Exclusion criteria were pregnancy, any immunodeficiency or chronic digestive disease, a previous suspected reaction to lactose, being born in a developing country, and current treatment with an antacid, a proton pump inhibitor, or any drugs associated with a potential digestive adverse event. Written, informed consent was obtained from all participants. The study protocol was approved by the ethical committee of St. Germain-en-Laye Hospital (St. Germain-en-Laye, France).

Procedures. At enrollment, information on sociodemographic factors and travel characteristics (including the country visited, duration of visit, and type of accommodation or mode of travel) were collected. Subjects were individually randomized to receive LA or placebo. Randomization was performed at Axcan Pharma Laboratory (Houdan, France), in accordance with the following criteria: a 1:1 ratio of patients receiving LA versus placebo, stratification according to hospital, and a randomization block size of 4 subjects. Both LA and the placebo had the same appearance and taste. No participant, investigator, or hospital pharmacist was aware of the treatment assignments. A key revealing individual assignments was released to the investigators once the database had been monitored, cleaned, and locked after completion of follow-up.

The study treatment consisted of 1 sachet dose of nonviable *Lactobacillus* (each dose contained 10^{10} bacteria mixed with a fermented culture medium) or placebo twice daily. Bacteria were made nonviable by heating at 110°C for 1 h (the antimicrobial activity of the mixture was maintained) [18]. Participants were instructed to begin treatment on the day before departure and continue until 3 days after their return or until their first episode of diarrhea. Moreover, they were given a single dose (800 mg) of ciprofloxacin to take optionally for treatment of diarrhea.

Follow-up. On the day before departure, each day of the trip, and each day of the week following their return, participants had to complete a log recording the number of sachets taken, the number and consistency of stools (normal, soft, or unformed), and details on any other events or medications taken. In the instance of diarrheal illness, participants indicated the related symptoms, the type of medications used, and how diarrhea affected their quality of life (e.g., the need to seek medical advice or the cancellation of scheduled activities). At the end of follow-up, participants sent the log to the coordinating center. They were contacted by phone if they had not sent it within 15 days after the presumed date of return.

Study definitions. Diarrhea was defined as the passage of ⩾3 unformed stools in a 24-h period. The duration of diarrhea was defined as the time between the first 3 unformed stools and the first normal stool. Subjects were considered to be nonadherent to the study treatment if they either did not take >2 consecutive sachet doses or >4 nonconsecutive sachet doses. Subjects whose logs were incomplete were considered to be lost to follow-up as of the date on which the log was last filled out properly.

Statistical analysis. Baseline characteristics were compared between treatment groups, according to follow-up status. Differences between means of normally distributed continuous variables were compared using Student's *t* test, distribution of non-Gaussian continuous variables were compared using the Mann-Whitney *U* test, and differences between proportions were compared using the χ^2 test or Fisher's exact test.

The primary efficacy end point was the occurrence of diarrhea. The incidence of diarrhea was estimated as the number of cases per person-months at risk; 95% CIs were determined assuming a Poisson distribution of cases. The at-risk period started with the intake of the first sachet dose and lasted until the first day of diarrheal illness, loss to follow-up, or 1 week after the return, whichever came first. Periods during which subjects did not fill in the log were removed from the period at risk (16 and 12 subjects in the LA and placebo groups, respectively, had gaps in their logs). The hazard ratios comparing the risk of diarrhea between the LA and placebo groups were estimated using Cox proportional hazard models. Multivariate analysis was performed using the same models, adjusting for the duration of travel (<10 days, 10–15 days, and

Table 1. Baseline characteristics of the randomized study subjects, by treatment group.

Characteristic	LA group (n = 174)	Placebo group (n = 174)	P[a]
Study enrollment, by year			
2001	6 (3)	6 (3)	
2002	55 (32)	52 (30)	.98
2003	89 (51)	90 (52)	
2004	24 (14)	26 (15)	
Sex, M:F ratio	1.04	0.82	.33
Age, mean years (95% CI)	38 (36–40)	38 (36–40)	.71
Travel duration, mean days (95% CI)	12 (11–13)	14 (13–14)	.004
Type of travel or accommodation			
Backpacking	38 (22)	51 (29)	
Staying with locals	22 (13)	14 (8)	
Organized tour	74 (42)	72 (42)	. 36
Business trip	14 (8)	16 (9)	
Other	26 (15)	21 (12)	
Destination			
West Africa	95 (55)	79 (45)	
East Africa	12 (7)	14 (8)	
Central Africa	14 (8)	14 (8)	
North Africa	0 (0)	2 (1)	
Oceania	7 (4)	8 (5)	.52
South America	20 (11)	25 (14)	
Asia	21 (12)	30 (17)	
Central America/the Caribbean	2 (1)	1 (1)	
Middle East	3 (2)	1 (1)	

NOTE. Data are no. (%) of subjects, unless otherwise indicated. Recruitment of subjects at each health care center for each treatment group was similar (P = .86). LA, probiotic preparation of nonviable *Lactobacillus acidophilus*.

[a] Proportions and means were compared using the χ^2 test and Student's t test, respectively.

⩾16 days), which was significantly different between the 2 groups at baseline. Other baseline variables were tested in the model, because differential rates of loss to follow-up between the 2 treatment groups could introduce some imbalance. The proportional hazards assumption was investigated graphically using a test based on the Schoenfeld residuals. The primary analysis was an intent-to-treat (ITT) analysis with all randomized subjects, except those excluded a posteriori and those who did not fill in or return their logs, as detailed in Results. An efficacy analysis was also performed with only subjects who completed follow-up and had no deviations from the treatment or study protocol. Kaplan-Meier survival curves were estimated by treatment group. Finally, the tolerability of the medications was assessed by comparing the prevalence and type of declared medical events in the 2 groups.

Sample size. The sample size was calculated to document a 50% reduction in the cumulative incidence of diarrhea in the LA group, compared with the placebo group (15% vs. 30%; $\alpha = 0.05$; $\beta = 0.20$, by 2-sided tests). We aimed to recruit 175 subjects per group, estimating that the rate of subjects lost to follow-up could be 30%.

Data entry was performed with MS Excel, version 2002 (Microsoft). Cleaning and statistical analyses were performed with Stata statistical software, version 8.0 (Stata).

RESULTS

Study population. In all, 348 subjects were enrolled in the study, with 174 in each group (table 1). The baseline characteristics of the 2 treatment groups were similar, except for the travel duration, which was longer in the placebo group than in the LA group (14 days vs. 12 days; P = .004).

A total of 22 subjects were excluded a posteriori from the trial (13 in the LA group and 9 in placebo group), either because they did not receive the study treatment (because their travel plans were cancelled or they did not obtain their medication from the pharmacy) or because they did not fulfill eligibility criteria and, therefore, were not followed up (figure 1). A total of 81 subjects either did not fill in or return their logs and were considered to be lost to follow-up. They did not differ

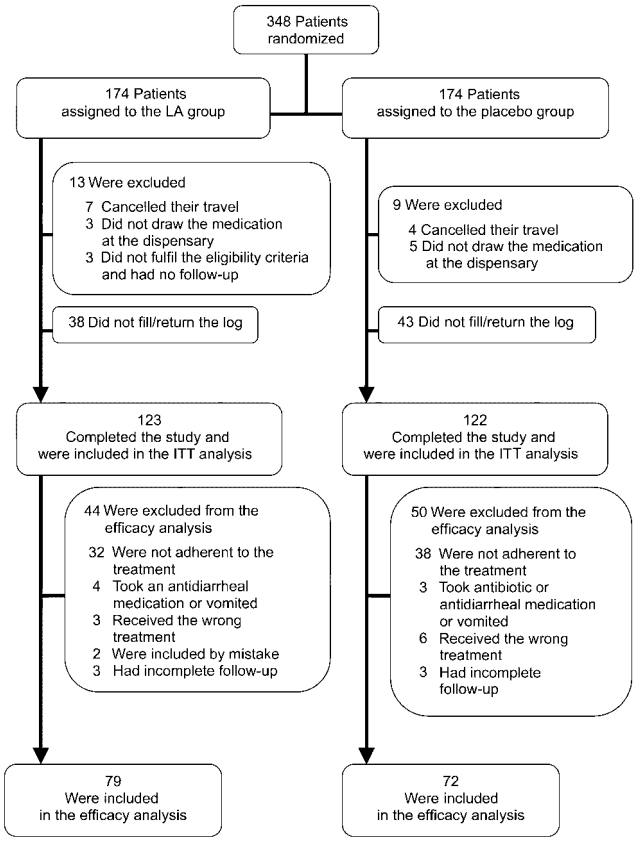

Figure 1. Flow diagram of subjects included in the randomized, controlled, double-blind trial on the efficacy of a probiotic preparation of nonviable *Lactobacillus acidophilus* for the prevention of travelers' diarrhea. ITT, intent to treat.

Table 2. Risk of diarrhea among travelers, by Cox regression model.

Analysis	Incidence[a] (95% CI)		Adjusted HR[b] (95% CI)	P[c]
	LA group	Placebo group		
ITT (n = 245)	61.4 (44.1–85.5)	43.4 (30.0–62.9)	1.43 (0.87–2.36)	.16
Efficacy[d] (n = 151)	86.6 (60.5–123.8)	63.9 (42.1–97.1)	1.38 (0.79–2.39)	.26

NOTE. Diarrhea was defined as the occurrence of ⩾3 unformed stools in a 24-h period. HR, hazard ratio; ITT, intent to treat; LA, probiotic preparation of nonviable *Lactobacillus acidophilus*.

[a] Incidence is defined as no. of cases per 100 person-months.

[b] HRs were adjusted for travel duration.

[c] P values were calculated using the Cox proportional hazards model, adjusted for travel duration.

[d] Subjects who deviated from the study protocol were excluded.

from subjects who completed the study, except that these patients were generally younger (data not shown). Therefore, the ITT analysis was restricted to the remaining 245 subjects (123 and 122 in the LA and placebo groups, respectively).

Diarrhea and efficacy of LA. Diarrhea occurred in 63 (25.7%) of 245 subjects. Most (87%) of the diarrhea episodes took place during the subjects' trips, beginning a mean of 9 days after the subject arrived and lasting for a mean of 3.6 days (95% CI, 2.8–4.4 days). Related symptoms were recorded in 83% of cases and consisted of abdominal pain, fever, headache, nausea, or vomiting. Eight patients (4 in each group) reported fever. Seventy percent of the patients (40 of the 57 subjects for whom the information was available) took medication when diarrhea occurred; of these, 35% took ciprofloxacin, and 83% took medication to manage their symptoms, such as loperamide, nifuroxazide, racecadotril, or diosmectite. Nine patients (16%) were confined to their room. One patient in the placebo group sought medical advice.

Diarrhea occurred in 35 (28.5%) of 123 travelers in the LA group and in 28 (23.0%) of 122 travelers in the placebo group; incidence rates were 61.4 cases (95% CI, 44.1–85.5) and 43.4 cases (95% CI, 30.0–62.9) per 100 person-months, respectively (P = .14). In univariate analysis, no associations were found between risk of diarrhea and age, sex, the country visited, type of accommodation or mode of travel, or travel duration. Travel duration was forced into the multivariate model, because durations were significantly different between the 2 groups at baseline. In multivariate analysis, there was no difference in the risk of diarrhea between treatment groups (hazard ratio, 1.43; 95% CI, 0.87–2.36) (table 2). Survival curves estimated using the Kaplan-Meier method are presented in figure 2.

A total of 94 subjects were excluded from the efficacy analysis. For 9 persons, the investigator did not provide treatment according to randomization protocol. Seven subjects reported having taken an antidiarrheal medication or antibiotic or reported vomiting before diarrhea occurred, 2 subjects were recruited by mistake (their travel duration exceeded 60 days), and 6 had incomplete follow-up. A total of 70 subjects (32 [26% of 123 subjects] in the LA group, and 38 [31% of 122 subjects] in the placebo group) were considered to have not adhered to the treatment regimen. Only 4 subjects (1 in the LA group and 3 in the placebo group) did not take any medication.

The efficacy analysis was restricted to subjects who received the correct treatment and followed study protocol and for whom full follow-up data were available (151 subjects; 79 and 72 in the LA and placebo groups, respectively). Diarrhea occurred in a total of 52 subjects; 30 (38%) of 79 subjects in the LA group and 22 (31%) of 72 in the placebo group had diarrhea, resulting in incidence rates 86.6 cases (95% CI, 60.5–123.8) and 63.9 cases (95% CI, 42.1–97.1) per 100 person-months, respectively (P = .29). After adjusting for travel duration, there was no statistically significant difference in the risk of diarrhea between treatment groups (hazard ratio, 1.38; 95% CI, 0.79–2.39) (table 2).

Among the 245 subjects included in the ITT analysis, 87 (36%) reported at least 1 medical event during follow-up (47 [38%] of 123 in the LA group and 40 [33%] of 122 in the placebo group; P = .38). A total of 125 events were declared (64 in the LA group vs. 61 in the placebo group). Most events (34%) were gastrointestinal symptoms; headache (14%); ear, nose, and throat symptoms (15%); or cutaneous eruption (13%). The number and type of events did not differ between the 2 groups.

DISCUSSION

We conducted a randomized, double-blind, controlled clinical trial to evaluate the efficacy of LA for the prevention of TD. We did not find any beneficial effect of treatment with LA on incidence of diarrhea.

One limitation of our study was the proportion of subjects lost to follow-up: 25% of the study group. This was not a surprising finding, because the population was healthy, with no

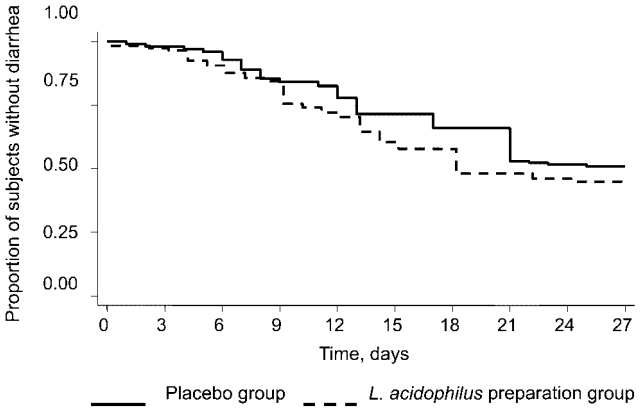

Figure 2. Kaplan-Meier estimated survival curves, showing the probability of not having diarrhea, by treatment group, after adjustment for travel duration.

strong incentive to participate in the study. It is unlikely that the loss of subjects to follow-up introduced a substantial bias in the ITT analysis, because the loss was balanced and nondifferential across the 2 study groups. Thus, we are quite confident in the validity of our analysis, which shows no statistically significant difference between the 2 groups in the incidence of diarrhea. These results were confirmed in a Cox model adjusting for potential confounders (e.g., travel duration). Finally, the level of adherence among participants was good (>70%), and an efficacy analysis restricted to adherent participants confirmed the results of the ITT analysis.

Our study provides a useful addition to the existing literature on the topic of the efficacy of probiotics in the prevention of TD; whereas previous studies gave conflicting findings, our findings are clear. Although protective effects have been shown with probiotic preparations of *L. rhamnosus* GG [12, 13] and *Saccharomyces boulardii* [14], a mixture of LA and *Lactobacillus bulgaricus* [15], LA or *Lactobacillus fermentum* preparations [16] have not been shown to be effective. Differences in the populations involved in the studies, the probiotic strains used (and their viability), and methodological and statistical problems (such as subgroup analyses or lack of ITT analyses) could explain these discrepancies.

We did not find any beneficial effect of LA, but we emphasize that our results do not prejudge the efficacy of other probiotics to prevent TD. Additional trials may still be worth considering with probiotics (e.g., *Lactobacillus rhamnosus* GG) that have demonstrated a protective effect for the prevention and the treatment of acute infectious diarrhea in children [7, 11]. We do not exclude the possibility that the use of nonviable microorganisms might have contributed to the lack of efficacy of LA. We chose nonviable bacteria to avoid rare—but reported—cases of systemic infection due to *Lactobacillus* [19, 20], especially because TD is a milder infection. *Lactobacillus* infections generally occur in severely ill or immunocompromised hosts, but cases have been described in individuals who are relatively mildly immunocompromised, as well [21]. Today, elderly people who may be immunocompromised because of illnesses, such as diabetes or nonprogressive cancer, are more and more likely to be travelers. Measures for the prevention of TD have to be applicable to these populations, too.

In the present study, >25% of the travelers (increasing to 64% when diarrhea is defined as $\geqslant$1 unformed stool per 24 h, as proposed elsewhere [22]) had a diarrheal episode. The mean duration of diarrhea was 3.5 days, and 16% of the patients were confined to their room. These findings once again underline the high incidence of diarrhea in travelers and reassert the need for safe and effective chemoprophylaxis. Antibacterials, such as quinolone-based prophylaxis, are not appropriate for small children and pregnant women—the populations most seriously affected by TD. To date, no probiotic medication has been found to provide clinically relevant protection against TD, and a general recommendation for the use of such preparations cannot be made. Additional trials with other probiotics are required. Because such medications are low priced and have excellent safety profiles and acceptance rates, they are ideal for tourists to use as self-medication [19].

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