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A Double-blind, Randomized, Placebo-controlled Trial of *Lactobacillus acidophilus* for the Treatment of Acute Watery Diarrhea in Vietnamese Children

Tran Thi Hong Chau, MD, Nguyen Ngoc Minh Chau, MSc,* Nhat Thanh Hoang Le, PhD,* Hao Chung The, MSc,* Phat Voong Vinh, MSc,* Nguyen Thi Nguyen To, MSc,* Nguyen Minh Ngoc, MD,† Ha Manh Tuan, MD,† Tang Le Chau Ngoc, MD,† Marion-Eliette Kolader, MD,‡ Jeremy J. Farrar, MD,*§ Marcel Wolbers, PhD,*§ Guy E. Thwaites, MD,*§ and Stephen Baker, PhD*§¶, on behalf of the Oxford-Vietnam Probiotics Study Group‖*

Background: Probiotics are the most frequently prescribed treatment for children hospitalized with diarrhea in Vietnam. We were uncertain of the benefits of probiotics for the treatment of acute watery diarrhea in Vietnamese children.
Methods: We conducted a double-blind, placebo-controlled, randomized trial of children hospitalized with acute watery diarrhea in Vietnam. Children meeting the inclusion criteria (acute watery diarrhea) were randomized to receive either 2 daily oral doses of 2×10^8 CFUs of a local probiotic containing *Lactobacillus acidophilus* or placebo for 5 days as an adjunct to standard of care. The primary end point was time from the first dose of study medication to the start of the first 24-hour period without diarrhea. Secondary outcomes included the total duration of diarrhea and hospitalization, daily stool frequency, treatment failure, daily fecal concentrations of rotavirus and norovirus, and *Lactobacillus* colonization.
Results: One hundred and fifty children were randomized into each study group. The median time from the first dose of study medication to the start of the first 24-hour diarrhea-free period was 43 hours (interquartile range, 15–66 hours) in the placebo group and 35 hours (interquartile range, 20–68 hours) in the probiotic group (acceleration factor 1.09 [95% confidence interval, 0.78–1.51]; $P = 0.62$). There was also no evidence that probiotic treatment was efficacious in any of the predefined subgroups nor significantly associated with any secondary end point.
Conclusions: This was a large double-blind, placebo-controlled trial in which the probiotic underwent longitudinal quality control. We found under these conditions that *L. acidophilus* was not beneficial in treating children with acute watery diarrhea.

Key Words: diarrhea, lactobacillus, norovirus, probiotic, randomized controlled trial, rotavirus, Vietnam

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Diarrheal diseases are a major global health issue, with the vast majority of the disease burden arising in young children residing in low-middle income countries.[1] It was estimated that >7 million children under the age of 5 years died in 2010; 15% of these deaths were attributable to diarrhea.[2,3] Typically, episodes of diarrhea are self-limiting, and patients often recover without ever obtaining a diagnosis identifying the etiologic agent. In those who are diagnosed, rotavirus is the most frequently identified pathogen in young children, followed by an array of other viral, parasitic and bacterial agents.[4] Vietnam is a rapidly developing low-middle income countries in Southeast Asia, with an estimated mortality rate of 23/1000 in children less than 5 years of age.[3] The total number of deaths in this age bracket in Vietnam in 2010 was 34,940, 11% of which were associated with diarrhea.[3] Oral rehydration solution, zinc, probiotics (in a multitude of formulations), and antimicrobials are the most commonly used treatments for children hospitalized with acute diarrhea in Vietnam, largely following WHO guidelines (with the exception of probiotics).[5] The use of probiotics in Vietnam is common in both hospitals and the community,[6] and we have previously estimated that >70% of 1500 children hospitalized with diarrhea in Ho Chi Minh City were prescribed a probiotic.[7]

In a Cochrane review of the effect of probiotics for the treatment of acute watery diarrhea, Allen et al[8] combined data from 8014 participants who were enrolled in 63 studies. The authors noted extensive heterogeneity in study design, definitions, infecting agents, probiotic organisms and dosages. Notwithstanding these caveats, a combined meta-analysis found probiotics to be effective in reducing the duration of diarrhea by a mean of 24.8 hours (95% confidence interval [95% CI], 15.9–33.6 hours), reduced the frequency of stools on the second day of treatment by a mean of 1.8 stools (95% CI, 0.45–1.14) and lowered the risk of developing persistent diarrhea by 59% (95% CI for risk, 0.32–0.53). The authors advocated larger, more robust trial designs specifically focusing on pathogen identification and the incorporation of standard definitions and end points to accurately inform clinical guidelines. There is currently no international regulatory agreement for the manufacture or clinical use of probiotics,[9,10] and additional scientific evidence is required to substantiate any potential health benefits of probiotics.[11]

We were uncertain as to the benefits of probiotics for the treatment of children with acute watery diarrhea in Vietnam. Therefore, we sought to address many of the limitations raised in the Cochrane review by conducting a double-blind, randomized, placebo-controlled trial. Specifically, we aimed to test the hypothesis that 5 days of 2 oral daily doses of 2×10^8 CFUs of *Lactobacillus acidophilus* (*L. acidophilus*),

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*The Hospital for Tropical Diseases, Wellcome Trust Major Overseas Programme, Oxford University Clinical Research Unit, and †Children's Hospital 2 (CH2), Ho Chi Minh City, Vietnam; ‡Department of Medical Microbiology, Academic Medical Centre, Amsterdam, The Netherlands; §Centre for Tropical Medicine, Oxford University, Oxford, United Kingdom; and ¶The Department of Medicine, The University of Cambridge, Cambridge, United Kingdom.
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Address for correspondence: Stephen Baker, PhD, The Hospital for Tropical Diseases, 764 Vo Van Kiet, Quan 5, Ho Chi Minh City, Vietnam. E-mail: sbaker@oucru.org
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a regime used in diarrheal therapy in hospitals in Vietnam, would be superior to placebo in reducing the time from the first dose of study medication to the start of the first 24-hour period without diarrhea.[12]

MATERIALS AND METHODS

Study Population and Setting

We conducted a double-blind, randomized, placebo-controlled trial recruiting participants with acute watery diarrhea at Children's Hospital 2 in Ho Chi Minh City, Vietnam. This 1400-bed hospital serves the local community and acts as tertiary referral center for children with severe infectious diseases and noncommunicable diseases in southern Vietnam. A full description of the methods has been published in the study protocol previously.[12] Briefly, children (between 9 and 60 months of age) hospitalized with acute watery diarrhea were screened for entry into the trial by study staff who had been appropriately trained in the trial procedures and had received good clinical practice (GCP) certification. Acute watery diarrhea was defined as the passage of loose or watery stools (taking the shape of the container) at least three times in a 24-hour period that did not contain blood or mucus with a history of less than 3 days. These inclusion criteria are comparable to those defined in the Cochran review,[8] "infants and children with 3 watery stools/day without visible blood or mucus (duration not stated)". The reason for targeting these patients was to (1) avoid children who may progress to more severe disease manifestations, (2) avoid recruiting children who would receive empirical standard-of-care antimicrobial on admission to hospital, (3) quantify probiotics are standard of care for this presentation in this location, and (4) to quantify viral loads in those infected with either norovirus and rotavirus, of which acute watery diarrhea is the most common presentation of these infections. Patients were excluded if (1) they had at least one episode of diarrhea in the month before admission, (2) they were known to have short bowel syndrome or chronic (inflammatory) gastrointestinal disease, (3) they were immunocompromised or immunosuppressed, (4) they were on prolonged steroid therapy, (5) or they were diagnosed as being severely dehydrated, to avoid recruiting those with a more severe manifestation of diarrhea.

A medical monitor oversaw the safety of the trial. Written informed consent to participate in the study was required from a parent or an adult guardian of all patients. The study protocol was reviewed and approved by the Ethical Committee of Children's Hospital 2 and the Oxford Tropical Research Ethics Committee (OxTREC) of the United Kingdom. The trial was registered at Clinical Trial.gov, number SRCTN88101063. Consort checklist provided in Supplemental Digital Content 5, http://links.lww.com/INF/C803.

Study Treatments and Quality Control

All patients received the standard of care according to the National Guidelines for the management of infectious diarrheal diseases in children, which included oral rehydration solution and zinc, but typically not antimicrobials for acute watery diarrhea. However, participants were not excluded when prescribed antimicrobials, and all therapies were recorded in a standardized case report form (CRF). Participants received either 2 sachets of 1×10^8 CFUs of *L. acidophilus* twice daily (ie, 4×10^8/day) or 2 sachets of identical tasting placebo (maltodextrin excipient only) dissolved in 10 mL of water. The study medications were purchased from, and manufactured by, Imexpharm pharmaceutical company (Cao Lanh, Vietnam) according to good manufacturing practices-World Health Organization (GMP-WHO) regulations; the appearance of the probiotic and placebo sachets and their contents were indistinguishable. The treatment regimens were identical in both groups: doses every 12 hours for 5 days. An additional dose was given (up to 2 extra doses in 4 hours) to participants who vomited within 30 minutes of taking the study medication.

For quality control purposes, we performed bacterial culture (for enumeration and identification) on a random selection of sachets (n=5) containing placebo and probiotic before the study initiation and at 3-monthly intervals (5 occasions in total). At all time points, the probiotic sachets contained $>1 \times 10^8$ CFUs of *L. acidophilus* only (the specified contents of the sachet); all placebo sachets were sterile. To determine the identity of the *L. acidophilus* in the sachets, we performed whole genome sequencing on the contents of the sachets. Genomic DNA was extracted using the Wizard Genomic DNA Purification Kit (Promega, WI), and 2 μg of genomic DNA was subjected to whole genome sequencing on an Illumina MiSeq 2500platform, following the manufacturer's recommendations to generate 300 bp paired-end reads, as previously described.[13] A de novo assembly was created using SPAdes v·3.7.1 using the "careful" option to optimize error correction; the genome sequence was submitted to Genbank under the accession number SRR4240524. An additional 12 complete *L. acidophilus* genome sequences were retrieved from public database and were aligned together with the aforementioned assembly using Mauve. Locally collinear blocks were trimmed and concatenated, and invariant sites and gaps were removed to produce a 1292-bp single nucleotide polymorphism (SNP) alignment. A maximum likelihood phylogeny was inferred from this alignment using PhyML with 100 bootstrap replicates under the GTR substitution model. By phylogenetic analysis, the strain was confirmed to be *L. acidophilus* La-14 and closely related to a previously sequenced strain (Supplemental Digital Content 1, http://links.lww.com/INF/C799).[14] Reads were also mapped to the reference sequence of *L. acidophilus* La-14 (accession number NC_021181) using SMALT (version 0.7.4). Candidate SNPs were called against the reference sequence using SAMtools, and low-quality SNPs were filtered based on these criteria: consensus quality <50, mapping quality <30, ratio of SNPs to reads at a position <75%, read depth <4, strand bias <0.001, mapping bias <0.001 or tail bias <0.001. As a result, 4 consensus SNPs and 1 intergenic deletion (2 bp) were identified in the *L. acidophilus* strain used in this study compared with the reference, indicating low genetic divergence between the 2.

Randomization, Concealment of Allocation and Blinding

Patients were randomly assigned to receive oral *L. acidophilus* (probiotic) or placebo (1:1) according to a computer-generated randomization list using block randomization with variable blocks of length 4 and 6. A study pharmacist prepared visually matched sachets in identical, sequentially numbered treatment packs according to the randomization list for dispensation in sequential order as participants were recruited. All participants, enrolling physicians and investigators were blinded to the treatment allocations. Attending physicians were responsible for enrolling the participants and ensuring that the study medications were given from the appropriate treatment pack. Daily monitoring of all enrolled inpatients by one of the investigators ensured the uniform management and accurate recording of clinical data in individual study notes.

Investigations and Follow-Up

Routine hematology and biochemistry tests were performed on admission to evaluate the severity of dehydration and disease. Multiplex real-time polymerase chain reaction (PCR) were performed on all fecal samples collected on admission to detect *Shigella*, *Salmonella*, *Campylobacter coli* and *Campylobacter jejuni*.[15] Additionally, quantitative real-time PCR was performed on the daily and follow-up fecal samples to diagnose norovirus and rotavirus and to calculate their viral loads.[16] Furthermore, fecal samples taken on admission, on discharge or on the last day of follow up (1 or 2 days after finishing the treatment course) and at outpatient follow-up visits (7 or 8 days after finishing the treatment course for those

children whose parent/guardians agreed to return) were subjected to metagenomic DNA extraction and PCR-amplified to quantify the concentration of *L. acidophilus* (target copies/mL of feces).[17,18]

Clinical Outcomes

Patients were assessed twice daily until discharge for clinical progress, diarrhea, vomiting, study medication compliance and adverse events, and the study staff collected daily fecal samples. On discharge and at follow-up, assessments were performed and fecal samples were collected.

The primary outcome was the time from the first dose of study medication to the start of the first 24-hour period without diarrhea as assessed by the treating physician or the participant's parent/guardian. Secondary end points included the total duration of diarrhea, the total duration of hospitalization, stool frequency in the first 3 days after enrollment, treatment failure (defined as no resolution of diarrhea during the 5-day treatment course, severe symptoms for which treatment was stopped, the requirement for additional antidiarrheal treatments), the daily rotavirus and norovirus viral loads in patients with a PCR amplification–positive fecal samples and all adverse events. Additional exploratory end-points were the recurrence of diarrhea (defined as a new diarrhea episode since the initial episode as assessed at the day 14(+3) follow-up visit) and the vomiting frequency in the first 3 days after enrollment.

Statistical Analysis and Sample Size Calculation

Data from Children's Hospital 2 identified a median (interquartile range) duration of hospitalization of 5 (3–6) days (mean and SD of $\log_{10}$-duration of 0.61 and 0.27) in our target population[7] and an approximate normal distribution of the log-transformed data. As we had limited pre-existing data on overall length of diarrheal illness (prehospitalized and hospitalized) and as children are usually discharged at the time of resolution of diarrhea, we used variability of the length of hospitalization as the basis of the sample size calculation. The trial was designed with the hypothesis that *L. acidophilus* was superior to placebo for acute watery diarrhea and was powered to detect a relative 20% decrease in the duration of diarrhea (measured in hours) of $4 \times 10^{\geq 8}$ CFUs of probiotics compared with placebo, corresponding to an absolute effect size of approximately 24 hours.[8] For 80% power at the 2-sided 5% significance level, a total of 123 participants per arm were required. To account for potential inadequacies in assumptions and some loss to follow-up, the sample size was increased by 22%. Thus, a total sample size of 300 participants, 150 in each arm, was recruited.

All statistical analyses were predefined in a detailed statistical analysis plan, which was finalized before the trial was unblinded (Supplemental Digital Content 6, http://links.lww.com/INF/C804). All randomized participants were included in the main analysis population following the intention-to-treat principle. The primary outcome, the time from the first dose of study medication to the start of the first 24-hour diarrhea-free period, was compared between the study groups based on a lognormal accelerated failure time regression model. Children withdrawn or lost to follow-up before cessation of diarrhea were treated as right-censored at the time of withdrawal or loss to follow-up. Homogeneity of the treatment effect was assessed in predefined subgroups.

The secondary and exploratory outcomes were compared between the treatment groups based on logistic regression for binary data (treatment failure and recurrence of diarrhea), quasi-Poisson regression for count data (stool frequency and vomiting frequency) and the lognormal accelerated failure time model for time-to-event data (total duration of diarrhea and duration of hospitalization), with treatment as the only covariate. Viral load measurements were summarized by the area under the curve (AUC) of $\log_{10}$-transformed viral load measurements between enrollment (day 1) and day 7 and compared using linear regression models with adjustment for the respective baseline $\log_{10}$-viral load. Log_{10}-transformed *L. acidophilus* bacteria load changes between enrollment and days 7 and 14, respectively, were compared in the same way. All analyses were performed using the statistical software R version 3.1.1.[19]

RESULTS

Baseline Characteristics and Patient Recruitment

Between October 2014 and September 2015, 303 patients with acute watery diarrhea were screened for enrollment into this trial (Fig. 1). Three hundred of these patients (150 in each study

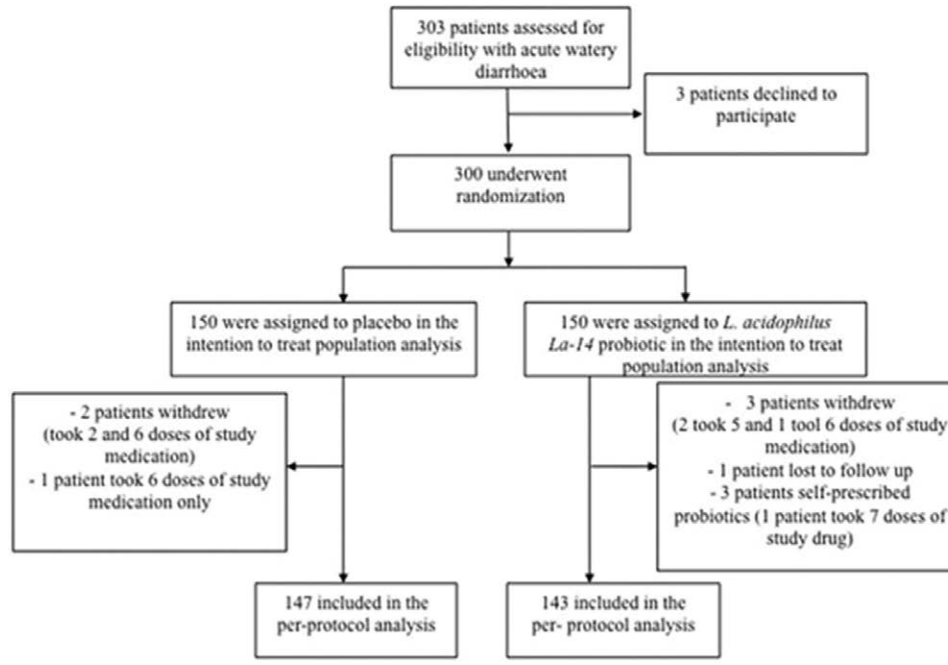

FIGURE 1. Consort flow diagram for trial screening and randomization.

arm) met the inclusion criteria and were randomly assigned to receive a best-selling Vietnamese brand of probiotic consisting of *L. acidophilus* only or placebo. Over 14 days of study follow-up, 2 patients in the placebo arm and 3 patients in the probiotic arm withdrew from the study after receiving a maximum of 6 doses of study drug. One additional subject in the placebo arm received only 6 doses of study drug, and 1 patient in the probiotic group was lost to follow-up. The parents/guardians of 3 children randomized to the probiotic group gave alternative probiotics in addition to the study treatment, leaving 290 (147 in the placebo arm and 143 in the probiotic arm) in the per-protocol population. In total, 5 subjects in each arm received less than the scheduled 10 doses of study treatment.

The demographic and baseline characteristics of patients were balanced between the 2 treatment groups in the intention to treat population (Table 1). The median age of the children was 16 months, and approximately one third were female. The inclusion criteria made it more likely that we would enroll those with a viral infection than a bacterial infection, and this was the case as 56/150 (37%) and 64/150 (43%) of the fecal samples were PCR amplification positive for rotavirus and 38/150 (25%) and 30/150 (20%) of the fecal samples were PCR amplification positive for norovirus in the placebo arm and probiotic arm, respectively. The proportion of bacterial infections (for the pathogens screened by PCR amplification: *Salmonella*, *Campylobacter*, and *Shigella*) was also comparable between the 2 groups. Finally, hematology or biochemical parameters were similar in both groups.

Primary Outcome and Subgroup Analyses

In the intention to treat population, the median time from the first dose of study medication to the start of the first 24-hour diarrhea-free period was 43 hours (interquartile range [IQR], 15–66 hours) and 35 hours (IQR, 20–68 hours) in the placebo and the probiotic group, respectively. Despite an 8-hour difference between the median times to cessation of diarrhea, the overall distribution of the primary end point was similar in both groups, and a statistical comparison did not reach significance ($P = 0.62$; Table 2 and Fig. 2). There was also no evidence for probiotic efficacy in the per-protocol population or in any of the predefined subgroups according to age, prior treatment or pathogen (Table 2).

Secondary Outcomes and Adverse Events

Analyses of the secondary end points are shown in Table 3. There were no significant differences in the secondary outcomes or exploratory outcomes between the probiotic group and the placebo group. Specifically, the median total duration of diarrhea was

TABLE 1. Baseline Characteristics by Treatment Group in the Intention to Treat Population

Characteristic	Placebo (N=150)	Probiotic (N=150)
Demographics, history and clinical examination		
Age (months), median (IQR)	15.5 (12.5–21.5)	15.6 (11.8–21.3)
Sex (female)	49/150 (33%)	52/150 (35%)
Weight (kg), median (IQR)	11.1 (9.0–12.0)	11.2 (9.0–12.0)
Temperature (°C), median (IQR)	37.8 (37.1–38.3)	37.8 (37.2–38.5)
Pulse (beats/min), median (IQR)	121.0 (121.0–128.0)	124.0 (121.0–128.0)
Duration of diarrhea before enrollment (hours), median (IQR)	33 (20–53)	36 (24–51)
Prior treatment with antibiotics in the previous month		
Yes	47/150 (31%)	38/150 (25%)
No	90/150 (60%)	100/150 (67%)
Unknown	13/150 (9%)	12/150 (8%)
Prior treatment with probiotic in the previous week		
Yes	75/150 (50%)	82/150 (55%)
No	59/150 (39%)	52/150 (35%)
Unknown	16/150 (11%)	16/150 (11%)
Microbiology		
Rotavirus*	56/150 (37%)	64/150 (43%)
Norovirus†	38/150 (25%)	30/150 (20%)
Campylobacter	18/150 (12%)	11/150 (7%)
C. Coli	3/18 (17%)	0/11 (0%)
C. jejuni	15/18 (83%)	11/11 (100%)
Shigella	20/150 (13%)	17/150 (11%)
Salmonella	21/150 (14%)	14/150 (9%)
Hematology and biochemistry		
Hematocrit (%), median (IQR)	38.8 (36.5–41.6)	38.5 (36.4–41.2)
White blood cell (K/μL), median (IQR)	11.1 (8.0–12.8)	11.3 (8.0–12.2)
Neutrophils (%), median (IQR)	53.6 (41.2–67.7)	51.8 (34.7–68.4)
Lymphocytes (%), median (IQR)	34.1 (22.5–46.9)	37.0 (23.3–53.5)
Eosinophils (%), median (IQR)	1.2 (1.0–1.7)	1.3 (1.0–1.9)
Platelet (K/μL), median (IQR)	317.9 (255.4–386.3)	322.0 (273.6–389.8)
Sodium (Na+) (mEq/L), median (IQR)	133.0 (131.0–136.0)	134.0 (131.0–135.0)
Potassium (K+) (mEq/L), median (IQR)	3.8 (3.5–4.1)	3.8 (3.5–4.1)
Urea (g/L), median (IQR)	1.3 (1.2–1.4)	1.3 (1.2–1.4)
Creatinine (mg/L), median (IQR)	4.4 (4.0–5.1)	4.6 (4.2–5.0)

*Placebo arm included 41 rotavirus monoinfections, 2 rotavirus and norovirus co-infections, 12 rotavirus and bacterial co-infections and 1 rotavirus and norovirus and bacterial co-infections. Probiotic arm included 52 rotavirus monoinfections, 1 rotavirus and norovirus co-infection, 10 rotavirus and bacterial co-infections and 1 rotavirus and norovirus and bacterial co-infection.

†Placebo arm included 25 norovirus monoinfections, 2 rotavirus and norovirus co-infections, 10 norovirus and bacterial co-infections and 1 rotavirus and norovirus and bacterial co-infection. Probiotic arm included 22 norovirus monoinfections, 1 rotavirus and norovirus co-infection, 6 norovirus and bacterial co-infections and 1 rotavirus and norovirus and bacterial co-infection.

IQR indicates interquartile range.

TABLE 2. Summary of the Primary Outcome in All Patients and in Predefined Subgroups

	Placebo (N=150)		Probiotic (N=150)		Comparison	
Subgroup	N	Median (IQR) (Hours)	N	Median (IQR) (Hours)	Acceleration Factor (95% CI); *P* Value	Test for Effect Heterogeneity (*P* Value)
All patients (intention-to-treat)	150	43 (15–66)	150	35 (20–68)	1.09 (0.78–1.51); *P* = 0.62	
Per-protocol population	147	43 (15–66)	143	33 (20–68)	1.09 (0.79–1.52); *P* = 0.60	
Age						0.2
0–12 (months)	32	56 (27–91)	40	43 (18–85)	0.76 (0.40–1.46); *P* = 0.41	
13–24 (months)	85	43 (6–70)	77	34 (21–68)	1.18 (0.75–1.87); *P* = 0.47	
25–36 (months)	22	42 (22–56)	21	28 (14–53)	0.75 (0.33–1.73); *P* = 0.50	
37–60 (months)	11	21 (1–30)	12	22 (21–62)	2.94 (0.96–8.95); *P* = 0.058	
Prior treatment with antibiotics in the past month						0.42
Yes	47	48 (23–95)	38	45 (22–66)	1.34 (0.78–2.29); *P* = 0.29	
No	90	42 (15–61)	100	28 (14–68)	0.96 (0.63–1.45); *P* = 0.83	
Unknown	13	22 (3–41)	12	49 (12–70)	1.95 (0.47–8.07); *P* = 0.36	
Prior treatment with probiotics in the past week						0.47
Yes	75	45 (19–70)	82	28 (17–62)	0.90 (0.59–1.37); *P* = 0.63	
No	59	47 (12–68)	52	45 (22–78)	1.29 (0.73–2.29); *P* = 0.39	
Unknown	16	24 (6–42)	16	54 (4–125)	1.56 (0.50–4.90); *P* = 0.44	
Pathogen						0.17
Rotavirus	56	48 (18–66)	64	45 (21–76)	1.07 (0.62–1.85); *P* = 0.81	
Norovirus	35	24 (5–64)	28	42 (26–76)	2.1 (1.01–4.37); *P* = 0.047	
Infected by other bacteria	24	45 (23–64)	18	24 (20–64)	0.74 (0.34–1.61); *P* = 0.44	
Unknown	35	46 (19–86)	40	27 (17–45)	0.78 (0.42–1.46); *P* = 0.43	

N refers to the number of subjects in each subgroup. Median and interquartile range (IQR) of the primary outcome were calculated for each randomized treatment group separately using Kaplan–Meier estimation. Comparisons between groups were based on a parametric lognormal accelerated failure time regression models, with treatment as the only covariate. The acceleration factor refers to the estimated relative difference between the duration in the two arms. Values <1 refer to a faster estimated diarrhea clearance in the probiotics arm. Heterogeneity was tested with a likelihood ratio test for an interaction between treatment and each subgrouping variable.

CI indicates confidence interval.

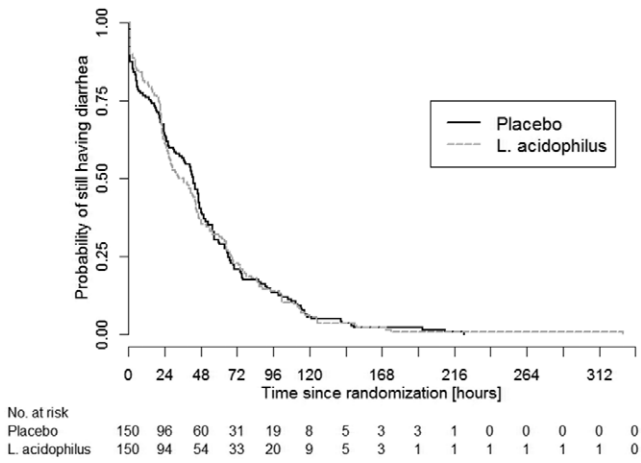

FIGURE 2. Kaplan–Meier curve of the primary outcome by treatment group. Curves showing the probability of still having diarrhea, that is, the probability of not yet having reached the onset of the first 24-hour diarrhea-free period (*y* axis), against the time since randomization (*x* axis) by treatment arm in the intention-to-treat population. *Lactobacillus acidophilus* (broken line) and placebo (solid line).

identical at 76 hours (Supplemental Digital Content 2, http://links.lww.com/INF/C800), and the median duration of the hospitalization was 78 hours (IQR, 53–104 hours) and 79 hours (IQR, 54–104 hours) in the placebo and the probiotic group, respectively (Table 3). Treatment failure occurred in only 11 individuals in the placebo group and 10 in the probiotic group. There was no difference in the number of episodes of diarrhea or vomiting between treatment arms, and recurrence of diarrhea occurred in 12% of subjects in each group.

To assess if there was any potential effect of *L. acidophilus* on those with a viral infection, we performed quantitative real-time PCR on longitudinal fecal samples from those infected with rotavirus and norovirus (Fig. 3; Supplemental Digital Content 3, http://links.lww.com/INF/C801). There was a substantial reduction in the number of target copies of rotavirus and norovirus over the 14-day follow-up period; the AUC of the viral loads were calculated to assess these dynamics between the 2 study arms. We found that the median AUC of rotavirus load ($\log_{10}$ copies/mL × days) was 63.25 and 63.16 in the placebo and the probiotic group, respectively. The median AUC of norovirus loads ($\log_{10}$ copies/mL × days) were 43.66 and 45.98 in the corresponding groups (Table 3). Finally, we measured the dynamics of *L. acidophilus* colonization over the course of the study follow up. *L. acidophilus* colonization was not distinct between the 2 groups, $\log_{10}$-transformed *L. acidophilus* load change in target copies after 7 and 14 days in both arms were −1.17 and −1.12 ($\log_{10}$ copies/mL) and 1.06 and −1.13 ($\log_{10}$ copies/mL) in the placebo and the probiotic group, respectively (Supplemental Digital Content 4, http://links.lww.com/INF/C802). No adverse events were reported in either of the study groups.

DISCUSSION

The use of probiotics for treating acute diarrhea is contentious, with various studies showing both positive and nonpositive effects. However, as highlighted in a Cochrane review, the study designs, selected probiotics and the target populations in the scientific literature are inconstant, thus, leading to extensive variability in the combined data.[8] We aimed to address many limitations of poor study design in this trial. First, the study was double blinded and placebo controlled using a locally sourced probiotic, a brand that is commonly used in hospitals in Vietnam to treat diarrhea. Second, we assessed the quality of the probiotic by regular quantitative counts and via genome sequencing to identify

TABLE 3. Summary of Secondary and Exploratory Outcomes

Outcome	Placebo (N=150)	Probiotic (N=150)	Comparison Estimate (95% CI); *P* Value
Secondary outcomes			
Total duration of diarrhea			AF of time to diarrhea clearance
Median (IQR) (hours)	76 (54–109)	76 (54–111)	1.02 (0.89–1.17); *P* = 0.75
Treatment failure*			OR of treatment failure
Frequency (%)	11/150 (7%)	10/150 (7%)	0.90 (0.36–2.21); *P* = 0.82
Total stool frequency in the first 3 days			Relative difference in stool frequency
Median (IQR) (count)	7 (3–15)	8 (3–15)	1.05 (0.83–1.32); *P* = 0.68
Rotavirus viral load AUC	N=42†	N=52†	
Median (IQR) (log_{10} copies/mL)	63.99 (57.98–68.87)	63.76 (58.59–67.37)	Adjusted absolute mean difference
Mean (log_{10} copies/mL)	63.25	63.16	−1.27 (−3.68–3.14); *P* = 0.87
Norovirus viral load AUC	N=25†	N=22†	
Median (IQR) (log_{10} copies/mL)	43.29 (39.02–49.82)	44.70 (41.31–51.03)	Adjusted absolute mean difference
Mean (log_{10} copies/mL)	43.66	45.98	2.63 (−1.58 to 6.85); *P* = 0.21
Duration of hospitalization			AF of duration of hospitalization
Median (IQR) (hours)	78 (53–104)	79 (54–104)	0.97 (0.85–1.11); *P* = 0.66
L. acidophilus bacteria load change after 7 days (log_{10} copies/mL)	N=37‡	N=51‡	
Median (IQR) (log_{10} copies/mL)	−1.17 (−1.63 to 1.15)	1.06 (−1.43 to 1.28)	Adjusted absolute mean difference
Mean (log_{10} copies/mL)	−1.18	1.39	0.4 (−1.23 to 1.04); *P* = 0.21
L. acidophilus bacteria load change after 14 days (log_{10} copies/mL)	N=34‡	N=34‡	
Median (IQR) (log_{10} copies/mL)	−1.12 (−1.46 to 1.30)	−1.13 (−1.03 to 1.09)	Adjusted absolute mean difference
Mean (log_{10} copies/mL)	−1.12	−1.36	1.095 (−1.48 to 1.67); *P* = 0.75
Exploratory outcomes			
Recurrence of diarrhea			OR of recurrence of diarrhea
Frequency (%)	18/150 (12.00%)	18/150 (12.00%)	1.00 (0.50–2.02); *P* = 1.00
Total vomiting frequency in the first 3 days			Relative difference in vomiting frequency
Median (IQR) (count)	1 (0–5)	1 (0–5)	1.21 (0.80–1.82); *P* = 0.37

Comparisons were based on lognormal accelerated failure time models (total duration of diarrhea, duration of hospitalization), logistic regression (treatment failure, recurrence of diarrhea), quasi-Poisson regression (stool and vomiting frequency) and linear regression with adjustment for baseline log_{10}-viral load or log_{10}-bacterial load (norovirus and rotavirus AUC, *Lactobacillus acidophilus* bacteria load change after 7 days and 14 days). Median (IQR) of total duration of diarrhea and duration of hospitalization were computed based on Kaplan–Meier estimation.

*Treatment failure events were no resolution of diarrhea after 5 days of treatment (7 patients on placebo, 5 on probiotics), requirement for additional antidiarrheal treatment (3 on placebo, 2 on probiotics), or both of these reasons (1 on placebo, 3 on probiotics).

†Longitudinal viral load measurements were only performed in patients without bacterial co-infection (Placebo: 40 rotavirus, 23 norovirus, 2 rotavirus and norovirus; Probiotic: 51 rotavirus, 21 norovirus, 1 rotavirus and norovirus). AUCs could not be computed for 1 patient with rotavirus infection, 1 patient with rotavirus and 3 patients with norovirus withdrew at day 1.

‡Longitudinal *L. acidophilus* bacteria load measurements were only available for patients who agreed to follow-up after discharge (Placebo: 37 after 7 days, 34 after 14 days; Probiotic: 51 after 7 days, 34 after 14 days).

AF indicates acceleration factor; AUC, area under the curve of log_{10}-transformed viral load from day 1 to 7; CI, confidence interval; IQR, interquartile range; OR, odds ratio.

the strain composing the probiotic. Third, we measured opposite end points on a robust sample size in an appropriate population. Finally, we aimed to stratify outcomes by etiologic agent and performed quantitative PCR for norovirus, rotavirus and *L. acidophilus* in the longitudinally collect fecal specimens. Therefore, we suggest that this study provides strong evidence for a lack of efficacy of *L.* acidophilus in treating children with acute watery diarrhea in Asia.

The use of probiotics in Vietnam is common, and they are considered to be safe and cheap; one sachet of the probiotic used in this study cost approximately 1500 Vietnam Dong (<0.10 USD), and they are frequently prescribed in hospitals and in the community for diarrhea. Here, the duration of acute diarrhea (time from the first dose of study medication to the start of the first 24-hour period without diarrhea or total duration of diarrhea) was not statistically different between children who received *L. acidophilus* or placebo, and there was no evidence that this probiotic provided benefit in the overall population or in any of the predefined subgroups. Furthermore, we observed no difference in norovirus or rotavirus viral loads between the two groups. The same observation was true for colonization with *L. acidophilus*, suggesting that oral *L. acidophilus* may even not efficiently colonize the gastrointestinal tract during acute diarrhea.

L. acidophilus La-14 is a common probiotic that has been used in various studies previously[20,21] and has been show to boost immunoglobulin G responses during oral cholera immunization.[21] Furthermore, this strain has been found to intrinsically resistant to an array of antimicrobials and to produce a bacteriocin with antimicrobial activity against *Listeria moncytogenes*.[22] There are no previous studies specifically assessing the potential use of *L. acidophilus* La-14 as a treatment for acute diarrhea, and strain selection may be pivotal. There is some scientific evidence that *L. acidophilus* may have an inhibitory effect on gastrointestinal pathogens; a recent laboratory study conducted in Korea assessed the antiviral activity of probiotics (including *L. acidophilus*) for rotavirus in vero cells.[23] This study found that *L. acidophilus* had the second highest inhibitory effect after *Bifidobacterium longum* and significantly shortened the duration of diarrhea in a limited number of patients.[23] Further, data generated using *L. acidophilus* (strain NCFM) showed that strain selection was important in stimulating rotavirus-specific antibody and B cell responses in gnotobiotic pigs vaccinated with rotavirus vaccine. Our data suggest that further, more physiologic, investigations need to be performed to assess there is a potential mechanism for a clinical impact on diarrhea with *L. acidophilus.*

There is an urgent need for new therapies for diarrhea; extensive antimicrobial resistance in many Gram-negative enteric pathogens means that we are becoming short of alternative options.[24] Probiotics offer an attractive solution and may have an effect if a functional formulation can be identified and

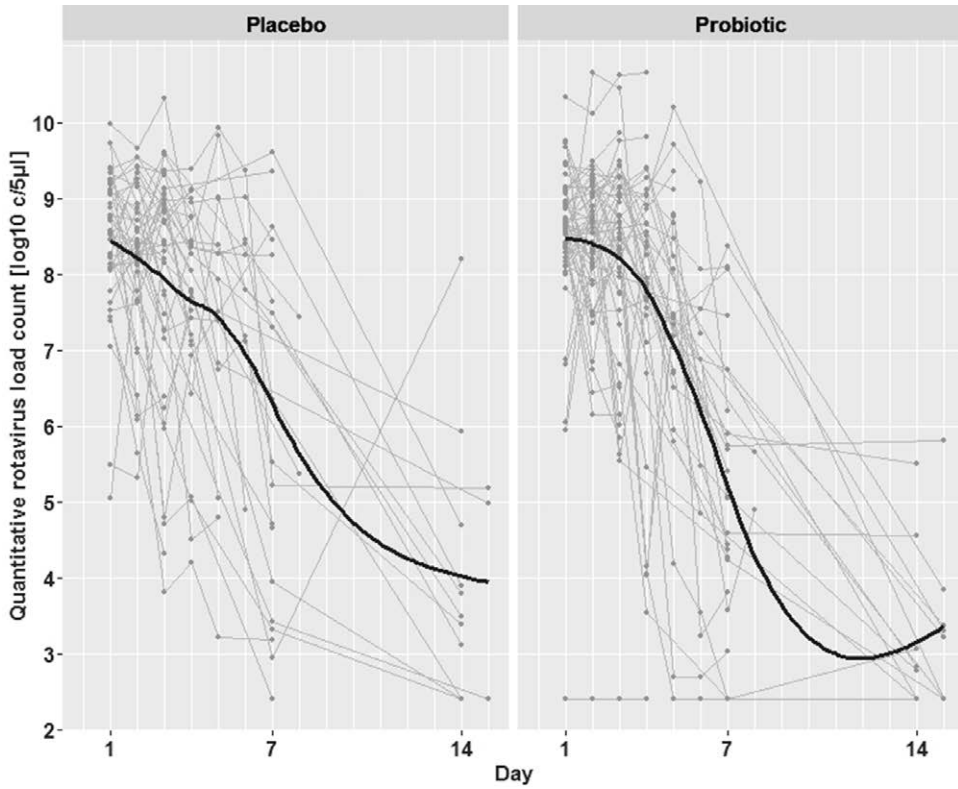

FIGURE 3. Longitudinal viral load measurements of rotavirus by treatment group. Plots show rotavirus viral load measurements by quantitative PCR in $\log_{10}$ target copies per milliliter of feces (*y* axis) against time since randomization (*x* axis) in the two groups. Gray lines refer to individual patient profiles, solid black lines to locally weighted scatterplot smoothing. Longitudinal viral load measurements were collected in patients with confirmed rotavirus infection without bacterial co-infection only.

studies are suitably powered. Pooled data from 4 small RCTs from France, Ecuador, Peru and Thailand found a reduction in mean duration of diarrhea caused by predominantly unknown pathogens in children treated with heat-killed *L. acidophilus* LB.[25] While, similar to our data, a group in India found no difference in the duration of diarrhea, stool frequency and the duration of hospitalization with tyndalized *L. acidophilus* (undefined strain) in acute diarrhea study in young children.[26] Overall, published meta-analyses suggest that diarrheal episodes are shortened by approximately 24 hours with *Lactobacillus rhamnosus GG (LGG)*, *L. acidophilus*, *L. bulgaricus* and *L. reuteri*.[27,28] These findings have led to various guidelines regarding the rational clinical use of probiotics in pediatric acute diarrhea diseases.[29,30] Our data question the conclusion of these findings and suggest that *L. acidophilus may not beneficial for treating acute diarrhea in children in a low-middle income country.*

Our study has limitations, which need to be considered in the context of the presented data. First, the time to cessation of symptoms was assessed by a caregiver or a patient/guardian and may vary according to those recording these data. Second, we were unable to accurately assess the type and duration of antimicrobial given to children before inclusion in this study, which may affect duration and type of symptoms. Notwithstanding these limitations, we performed an adequately powered, double-blind, study under operational conditions with a common available and routinely used probiotic.

In conclusion, we found that *L. acidophilus* did not reduce the time from the first dose of study medication to the start of the first 24-hour period without diarrhea in comparison to placebo. Further, there was no difference between intervention and placebo in the total duration of diarrhea, the total duration of hospitalization, stool frequency during the first 3 days of treatment, treatment failure or daily rotavirus and norovirus fecal loads. Our data add additional evidence regarding the role of probiotics in treating diarrheal disease and suggest that *L. acidophilus* may not have a measurable effect in this setting.

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the experiments contributing to this study. C.T., C.N., N.L., and S.B. wrote the manuscript.

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