Effect of Probiotics on Diarrhea in Children With Severe Acute Malnutrition: A Randomized Controlled Study in Uganda

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ABSTRACT

Objectives: The aim of the study was to assess the effect of probiotics on diarrhea during in- and outpatient treatment of children with severe acute malnutrition (SAM).

Methods: A randomized, double-blind, placebo-controlled study was conducted involving 400 children admitted with SAM. Patients received 1 daily dose of a blend of Bifidobacterium animalis subsp lactis and Lactobacillus rhamnosus (10 billion colony-forming units, 50:50) or placebo during hospitalization followed by an 8- to 12-week outpatient treatment period, depending on patients’ recovery rate. All outcomes were reported for in- and outpatient treatment separately. The primary outcome was number of days with diarrhea during hospitalization. Secondary outcomes included other diarrhea outcomes, pneumonia, weight gain, and recovery.

Results: There was no difference in number of days with diarrhea between the probiotic (n = 200) and placebo (n = 200) groups during inpatient treatment (adjusted difference = 0.2 days, 95% confidence interval −0.8 to 1.2, P = 0.69); however, during outpatient treatment, probiotics reduced days with diarrhea (adjusted difference = 2.2 days 95% confidence interval −3.5 to −0.3, P = 0.025). There were no effects of probiotics on diarrhea incidence and severity or pneumonia, weight gain or recovery during in- or outpatient treatment. Twenty-six patients died in the probiotic versus 20 in the placebo group (P = 0.38).

Conclusions: Bifidobacterium animalis subsp lactis and Lactobacillus rhamnosus had no effect on diarrhea in children with SAM during hospitalization, but reduced the number of days with diarrhea in outpatient treatment by 26%. Probiotics may have a role in follow-up of hospitalized children with SAM or in community-based treatment of malnourished children, but further studies are needed to confirm this.

Key Words: diarrhea, low-income country, probiotic, severe acute malnutrition, young children

What Is Known

• Severe acute malnutrition is responsible for 0.5 to 1 million child deaths annually and diarrhea is a common morbidity associated with mortality. Probiotics reduce the duration of acute diarrhea by 1 day in well-nourished or mildly malnourished children and reduce the risk of antibiotic-associated diarrhea.

What Is New

• The probiotics Lactobacillus rhamnosus and Bifidobacterium animalis subsp lactis had no effect on diarrhea during inpatient treatment of children with severe acute malnutrition, but reduced days with diarrhea during outpatient treatment by 26%.

• The study results do not support using probiotics for inpatient treatment of children with severe acute malnutrition, but may have a role in outpatient treatment.

Susceptible and case fatality rates in many sub-Saharan hospitals are often above 20% (3). Diarrhea is a major complication to SAM associated with increased morbidity, longer hospitalization, and death (4,5).

Meta-analyses have shown that probiotics reduce the duration of acute infectious diarrhea by approximately 1 day and reduce the risk of acute diarrhea lasting 4 days or more (6). The dosage and the strains used were, however, different. Investigation of strain-specific effects have led to recommendation of 2 strains, Lactobacillus rhamnosus (LGG) and Saccharomyces boulardii, in

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the treatment of acute gastroenteritis in children (7). Both strains seem to reduce duration of acute diarrhea by 1 day and the risk of having diarrhea on day 3 or 4 by approximately 50% (7). Probiotics may also reduce the risk of antibiotic-associated diarrhea and the duration of persistent diarrhea (8–10).

Pneumonia is the most frequent infectious cause of mortality in children below 5 years and the risk of dying if severely malnourished is several folds higher (11). Studies on the effects of probiotics on pneumonia are scarce; however, a number of studies have indicated that probiotics may reduce the risk of upper respiratory infections (12). Furthermore, a few probiotic studies have shown a small improvement in growth (13).

Some of the studies mentioned above included mildly to moderately malnourished children from low-income countries, but most studies were conducted in well-nourished children from high-income countries. The large ProNUT study (an intervention study testing probiotics and prebiotics supplied in Plumpy’Nut for children with SAM) investigated the effects of a mixture of pro- and prebiotics in Malawian children with SAM (14). There was no effect on nutritional cure, or on death, weight gain, or time to cure, but a trend toward lower mortality (relative risk [RR] = 0.65, \(P = 0.06\)) among patients receiving pro- and prebiotics in the outpatient period was observed.

*Bifidobacterium animalis* subsp *lactis* (BB-12) and LGG are among the most widely consumed and studied probiotic strains. Earlier studies have indicated that these strains reduce diarrhea and upper respiratory infections in well-nourished children (15–18) and that LGG may reduce diarrhea in mildly to moderately undernourished children (19–21). We aimed at assessing the effect of a combination of BB-12 and LGG on diarrhea, pneumonia, and growth in children admitted with SAM.

**METHODS**

**Design and Ethics**

The ProbiSAM study was a randomized, double-blind, placebo-controlled, 2-arm parallel-group study in children with SAM.

The study was conducted at Mwanamugimu Nutrition Unit (MNU), Department of Pediatrics and Child Health, Mulago National Referral Hospital, Kampala, Uganda. The mortality rate at the unit is approximately 20%.

The study was performed in accordance with the principles in the Declaration of Helsinki. Ethical approval was obtained from the Makerere University School of Medicine Research Ethics Committee in Uganda and a consultancy approval was given by The National Committee of Health Research Ethics in Denmark. Oral and written information about the study was provided and written; informed consent was obtained from all caregivers before enrolment in the study. An independent data safety monitoring board (DSMB) was established to monitor patient safety throughout the study.

**Participants**

Children of age 6 to 59 months admitted with SAM (mid-upper-arm-circumference <11.5 cm or weight-for-height/weight-for-length z score (WHZ/WLZ) < -3 or bipedal pitting edema) were eligible. Patients with shock or severe respiratory difficulty at admission, weight below 4.0 kg, obvious disability or significant congenital or malignant disease and patients admitted with SAM the previous 6 months were excluded.

**Randomization, Allocation Concealment, and Blinding**

The study products were labeled with a 4-digit number. There were 4 different 4-digit numbers, 2 for placebo and 2 for probiotics. Only the study supply coordinator at Chr. Hansen A/S had access to the blinding code. The randomization list was generated by a person not involved in the study (http://www.randomization.com, accessed February 6, 2014). Randomization to probiotic or placebo treatments was performed in a 1:1:1:1 ratio in blocks of 4 and 8 in random order. The randomization list was kept by the head of MNU and only made available to staff responsible for pre-packaging of study products. These staff members were not involved in patient enrolment or treatment. Subject allocation was performed by assigning eligible subjects to the first available randomization number in consecutive order. All participants, caregivers, investigators, and staff involved in the study were blinded until the database was locked. The appearance, taste, and smell of the products were identical, except for the 4-digit number.

**Intervention and Procedures**

Patients received 1 sachet of study product daily in addition to standard treatment of SAM. The study product was administered from hospital admission to discharge and throughout an outpatient treatment period of minimum 8 weeks and maximum 12 weeks, depending on the nutritional recovery of each child. Each sachet contained 1 g of white powder: maltodextrin with or without a combination of the 2 probiotic strains BB-12 and LGG (dosage 10 billion colony-forming units [CFU], 50:50). Study products were manufactured by Chr. Hansen A/S, Hørsholm, Denmark.

During hospitalization, study personnel administered, registered, and supervised consumption of study products, which were provided together with the morning food. Standard treatment of SAM was given in compliance with World Health Organization (WHO) recommendations (22) and the Ugandan national protocol (23). Standard treatment involved a stabilization phase where children received F-75 formula (Nutriset, Malaunay, France) followed by a rehabilitation phase with step-wise transitioning to ready-to-use-therapeutic-food (RUTF; Plumpy’Nut, Nutriset) or F-100 formula (Nutriset) if RUTF was not tolerated well. A commercial soy-based, lactose-free infant formula (Isomil, Abbott, Chicago, IL) was used if patients were suspected to have lactose intolerance based on acidic stool pH and profuse diarrhea with >10 stools per day. Breast-feeding was encouraged throughout the study. Study staff measured weight daily and assessed children daily for...
vital signs, signs of pneumonia (respiratory rate, chest in-drawing, respiratory auscultation, oxygen saturation), degree of edema and dehydration, and other signs of illness or adverse events. Triple measurements of weight and length/height were performed weekly and a thorough physical examination and anthropometry was performed at admission and discharge. Antibiotics were administered as part of standard treatment for minimum 5 days. Ampicillin and gentamycin were first-line antibiotics, and second- and third-line antibiotics included chloramphenicol, ceftriaxone, clocaxillin, and ciprofloxacin.

Diarrhea data were collected using a stool diary in which caregivers ticked every time their child passed stool. Each stool was categorized as watery, abnormally loose, loose, or normal according to a photo scale. Caregivers were thoroughly trained in how to use the diary and nutritionists supported and evaluated caregivers’ ability to score stool consistency and fill out the stool diary correctly. Vomiting, fever, and consumption of the study product were also recorded in the diary. Development and validation of the stool diary is described elsewhere (24). Stool frequency and consistency scoring had a high validity, good reliability, and high sensitivity.

During outpatient treatment, children received RUTF at 200 kcal/kg bodyweight per day. Follow-up visits were scheduled every second week to assess the children with respect to anthropometry, medical history, physical examination, follow-up on stool diary data, and to deliver new supplies of RUTF, study products, and stool diaries. The study staff did not attempt to fill the diary together with the caregiver if data were missing. Caregivers were contacted by phone once a week to ask about the status of their child and to remind them about study procedures and visit dates. If caregivers missed a scheduled follow-up visit, they were again contacted by phone or a home visit was done to assess reasons for failure to return for a follow-up visit. Study product compliance during outpatient care was estimated based on ticks in stool diaries, returned empty or unused sachets, and differences between visit dates and number of sachets supplied.

**Outcomes**

The primary outcome was duration of diarrhea during hospitalization. Duration was defined as “number of days with diarrhea” of each patient. Diarrhea was defined as ≥ 3 loose or watery stools per 24 hours (25) based on stool diary data. A diarrhea episode started when the diarrhea definition was fulfilled and was considered to have ceased when the child passed < 3 loose or watery stools per day. If diarrhea reappeared after < 48 hours, it was considered part of the same diarrhea episode, but only days with ≥ 3 loose or watery stools were counted as diarrhea days. The protocol was amended during the clinical study and approved by the ethical committee. In the original protocol, the primary outcome was phrased as “duration of diarrhea episodes” without any specification of in- or outpatient treatment. We considered the total number of days with diarrhea of each child to be clinically more important than the duration of each episode. In addition, we decided to split analyses in in- and outpatient treatment as the populations and data collection were different in the 2 periods with more critically ill patients and closer monitoring of patients during inpatient treatment.

Secondary outcomes were, first, number of days with diarrhea during outpatient treatment and incidence and severity of diarrhea during inpatient and outpatient treatment. Diarrhea incidence was defined as the proportion of children with minimum 1 day of diarrhea. Severity was defined as the Vesikari score for inpatients and a modified Vesikari score for outpatients (26). The Vesikari score is a multidomain diarrhea episode severity score that includes grading of stool frequency, diarrhea duration, vomiting frequency and duration, temperature, dehydration, and need of hospitalization. Dehydration or temperature during outpatient treatment was not assessed. To be conservative, children were considered not to be dehydrated and if the caregiver ticked “fever” in the stool diary, it was considered to be the lowest fever score on the Vesikari scale. The Vesikari scale categorizes diarrhea episodes according to the following ranges: ≤ 7 mild, 7 to 10 moderate, and ≥ 11 severe. Second, pneumonia incidence, duration and severity for inpatients, and pneumonia incidence for outpatients. Pneumonia was diagnosed according to clinical assessment by a pediatrician. Severity of pneumonia was categorized as “mild-moderate” or severe. Duration and severity could not be assessed during outpatient treatment as children were only observed at follow-up visits every second week. Third, weight gain (g/kg bodyweight per day) for in- and outpatients, respectively, and recovery defined as WHZ/WLZ > -2 at study termination. Fourth, other outcomes included days with fever or vomiting during in- and outpatient treatment and duration of hospitalization.

Safety outcomes included mortality and other adverse events. Due to the high background morbidity and mortality in the study population, only medical conditions that were judged by a study pediatrician to be uncommon in this population were recorded as other adverse events.

**Statistical Analysis**

**Sample Size Calculation**

To have 80% power at 5% significance level to detect a 0.3 SD reduction in number of days with diarrhea, 178 children were needed per study arm. To account for loss to follow-up, 200 children were recruited per arm. Assuming that the SD of days with diarrhea at MNU was 3 days, it would be possible to detect a 1-day reduction in days with diarrhea, which is similar to what is found in meta-analyses (6).

**Statistical Analyses**

Data were double-entered in EpDta v.3.1 (EpiData, Odense, Denmark) and analyzed using statistical software R version 3.1.1 (2014-07-10) (27).

The primary outcome and the secondary outcomes of diarrhea severity, weight gain, hospitalization, fever, and vomiting were analyzed using linear-mixed models with subject-specific random effects. Remaining secondary outcomes were analyzed either using logistic regression (mixed-effects models) incidence of diarrhea and pneumonia, fever, recovery) or log-linear Poisson models with adjustment for overdispersion (diarrhea days in outpatients, pneumonia duration, and severity inpatients). All models were adjusted for age, sex, human immunodeficiency virus (HIV) status, baseline edema, and WHZ/WLZ. Mortality was analyzed using a Cox-regression adjusted for sex and age only as HIV data were missing for a number of patients that died. In addition, analyses of inpatient data included adjustment for duration of hospitalization and baseline diarrhea or pneumonia. All analyses of diarrhea inpatient data were repeated with additional adjustment for Isomil treatment and reduction in days with diarrhea during outpatient treatment was repeated with adjustment for duration of outpatient treatment. Effect modification was also investigated for age, sex, HIV status, duration of hospitalization and baseline edema, WHZ/WLZ, and diarrhea. Model checking was based on residuals and predicted random effects, which were evaluated visually using (cumulated) residual plots and normal probability plots.
Intention-to-treat analysis was performed on all patients with any available data related to the specific outcome, assuming that drop-outs occurred at random. Intermittent missing values in inpatient diarrhea data were imputed to obtain complete episodes, which were needed for the primary outcome and for calculation of the Vesikari score. Specifically, deterministic hot-deck imputation was used: gaps in stool diaries were imputed using data from multiple matched patients with complete data for the same days of hospitalization as the gap occurred and an identical diarrhea pattern before/after the gap as the patient having the gap (28). For the primary outcome, a per-protocol analysis was also carried out and a subgroup analysis was performed to investigate if the subgroup of children who were discharged and analyzed in the outpatient phase showed a different result in the primary outcome than the total population.

At predefined, regular intervals, the DSMB monitored safety with specific focus on mortality by evaluating unblinded individual case fatality reports, serious adverse events, and the reasons of patients lost to follow-up. The study was registered at www.isrctn.com as ISRCTN16454889.

RESULTS

Of 757 children screened, 400 children were randomized to receive probiotics (n = 200) or placebo (n = 200) (Fig. 1). Patients were recruited between March 10, 2014 and July 8, 2015 and followed until October 2015.

Baseline characteristics were comparable between the 2 study groups (Table 1). Mean age was 17.0 months, 58% were boys, 66% had edematous malnutrition, 14% were HIV seropositive, and 34% had an HIV-positive mother. The total loss to follow-up, including deceased patients, was 18% (n = 73/400) and 12% (n = 38/327) during in- and outpatient treatment, respectively. The numbers were equally distributed between the 2 study groups. No patients were discharged to outpatient treatment with edema, but 44% still had SAM with no medical complications (see Supplemental Digital Content 1, Table, http://links.lww.com/MPG/A882).

The mean duration of hospitalization was 181 ± 9.2 days and most patients (>80%) were discharged from outpatient treatment after 8 weeks. The number of patients included in intention-to-treat diarrhea analyses during in- and outpatient treatment was n = 369 (probiotics n = 187, placebo n = 182) and n = 289 (probiotics n = 147, placebo n = 145), respectively. The main reasons for patients not being included in the statistical models were lack of data on HIV status or lack of stool diaries in the outpatient period.

Compliance to study product consumption was 98% for both groups during inpatient treatment based on study staff registrations. During the outpatient phase, compliance was estimated at 93% and 96% for probiotics and placebo, respectively, based on comparison of the number of study products delivered and the number of days between follow-up visits.

There was no difference in the primary outcome between the probiotic and placebo groups with an adjusted difference of mean number of days with diarrhea of +0.2 days (95% confidence interval [CI] 0.8 to 1.2, P = 0.69) (Table 2). During outpatient treatment, number of days with diarrhea was lower in the probiotic compared with the placebo group with an adjusted difference of
TABLE 1. Baseline characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Probiotics (n = 200)</th>
<th>Placebo (n = 200)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (mo ± SD)</td>
<td>17.5 ± 8.5</td>
<td>16.5 ± 8.4</td>
</tr>
<tr>
<td>Sex (male, %)</td>
<td>115 (58%)</td>
<td>115 (58%)</td>
</tr>
<tr>
<td>Weight (kg ± SD)</td>
<td>7.0 ± 1.7</td>
<td>6.9 ± 1.7</td>
</tr>
<tr>
<td>Height or length or height (cm ± SD)</td>
<td>71.1 ± 6.6</td>
<td>71.0 ± 6.1</td>
</tr>
<tr>
<td>MUAC (cm ± SD)</td>
<td>11.6 ± 1.4</td>
<td>11.5 ± 1.5</td>
</tr>
<tr>
<td>Height-length-for-age z score</td>
<td>−3.2 ± 1.4</td>
<td>−3.0 ± 1.4</td>
</tr>
<tr>
<td>Weight-for-age z score</td>
<td>−3.5 ± 1.3</td>
<td>−3.5 ± 1.3</td>
</tr>
<tr>
<td>Weight-for-height/length z score</td>
<td>−2.6 ± 1.5</td>
<td>−2.7 ± 1.6</td>
</tr>
<tr>
<td>Edema (n, %)</td>
<td>132 (66%)</td>
<td>132 (66%)</td>
</tr>
<tr>
<td>HIV-positive children (n, %)</td>
<td>28 (14%)</td>
<td>24 (13%)</td>
</tr>
<tr>
<td>HIV-positive mothers (n, %)</td>
<td>56 (34%)</td>
<td>53 (34%)</td>
</tr>
<tr>
<td>Presenting symptoms at admission n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fever</td>
<td>101 (50%)</td>
<td>110 (55%)</td>
</tr>
<tr>
<td>Cough</td>
<td>131 (66%)</td>
<td>131 (66%)</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>118 (59%)</td>
<td>126 (63%)</td>
</tr>
<tr>
<td>Vomiting</td>
<td>84 (42%)</td>
<td>87 (44%)</td>
</tr>
<tr>
<td>Medical treatment</td>
<td>182 (91%)</td>
<td>184 (93%)</td>
</tr>
<tr>
<td>Household n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Child lives with mother</td>
<td>160 (80%)</td>
<td>157 (79%)</td>
</tr>
<tr>
<td>Mother education primary school or lower</td>
<td>94 (47%)</td>
<td>117 (59%)</td>
</tr>
</tbody>
</table>

HIV = human immunodeficiency virus; MUAC = mid-upper-arm circumference; SD = standard deviation.

2.2 days (95% CI −3.5 to −0.3, P = 0.025). Supplemental Digital Content 2, Figure, http://links.lww.com/MPG/A883, shows the distribution of diarrhea days in the probiotic versus the placebo group during outpatient care. As seen, the proportion of patients with diarrhea for 20 or more days was reduced in the probiotic group.

The incidence of diarrhea was 89% versus 85% in the probiotic versus placebo group during hospitalization, odds ratio (OR) 1.6 (95% CI 0.8 to 3.3, P = 0.17) and 70% versus 76% in the outpatient period, OR 0.7 (95% CI 0.4 to 1.2, P = 0.17). The severity of diarrhea episodes measured by the Vesikari score was comparable between the study groups during in- and outpatient treatment. Episodes observed during inpatient treatment were more severe (592 episodes, mean score 10.0 (5–20)) compared with outpatient episodes (752 episodes, mean score 4.3 (1–13)).

Pneumonia incidence, duration, and severity were not different between the study groups during inpatient treatment. The incidence during outpatient treatment was 5% (n = 8) in the probiotic group and 10% (n = 16) in the placebo group, but the difference was insignificant (OR 0.5, 95% CI 0.2 to 1.3, P = 0.17). Nutritional recovery, total weight gain (g/kg bodyweight per day), fever, vomiting, and duration of hospitalization did not differ significantly between the groups.

Forty-six patients died during the course of the study; 39 (23 probiotics, 16 placebo) during hospitalization and 7 (3 probiotics, 4 placebo) during outpatient treatment. There was no difference between the probiotic and placebo groups during the entire study (hazard ratio [HR] = 1.3, 95% CI 0.7 to 2.3, P = 0.38). Other adverse events were not reported.

The per-protocol analysis (probiotics n = 176, placebo n = 169) resulted in an adjusted difference of the primary outcome of +0.2 days (95% CI −0.8 to 1.2, P = 0.68) and analysis of the subpopulation of inpatients that were included in the outpatient analysis showed an adjusted difference of +0.1 days (95% CI −1.1 to 1.2 days, P = 0.91). There was no effect on modification of the primary outcome by any of the covariates (data not shown). Days with diarrhea during outpatient treatment resulted in similar results after adjustment for duration of outpatient treatment (data not shown).

DISCUSSION

Probiotics did not reduce number of days with diarrhea during hospitalization, whereas days with diarrhea were reduced by 2.2 days corresponding to 26% of the mean number of days with diarrhea in the outpatient phase. The different results may be explained by more severe illness and a compromised gut barrier in children during hospitalization. Children admitted to MNU were often ill with multiple life-threatening conditions and it may have affected their ability to respond to probiotic treatment. More specifically, their gut function may have been impaired resulting in poor adhesion of the probiotics to the mucosa.

Antibiotic use was also different during in- and outpatient treatment. Broad-spectrum antibiotics were administered intravenously as part of standard treatment during hospitalization whereas oral antibiotics were used only when children developed respiratory or other infections during outpatient treatment. Both oral and intravenously administered antibiotics are known to cause diarrhea in some patients probably by disturbing the gut microbiota and reducing the colonization resistance to pathogens. The 2 probiotic strains were sensitive to most of the used antibiotics and the antibiotics may therefore have influenced the effectiveness of the probiotics. On the contrary, probiotic studies including LGG have been shown to reduce the risk of antibiotic-associated diarrhea after administration of broad-spectrum antibiotics (29).

Finally, diarrhea was more severe with higher stool frequency, more dehydration, fever, and vomiting during inpatient compared to outpatient treatment. Meta-analyses of probiotics in general (6) or LGG alone (30) have indicated that the effect on acute diarrhea is higher in community-based than inpatient studies. The results, however, vary and a study with LGG in hospitalized children with acute diarrhea showed effect in patients with profuse diarrhea (19).

The ProNUT study investigated a combination of pre- and probiotics in children with SAM and also observed differences during in- and outpatient periods (14). They found more vomiting, severe diarrhea (>6 stools per day) and cough, and a small, nonsignificant increase in mortality in inpatients, whereas they reported a trend toward reduced mortality and fewer cases of severe diarrhea in outpatients receiving pro- and prebiotics. The increase in severe diarrhea among inpatients was suggested to be related to the intake of prebiotics.

Based on our results, it is not possible to conclude if the effect in outpatients is dependent on probiotic administration initiated before discharge. However, studies on treatment or prevention of diarrhea either started probiotic treatment together with or maximum 2 days before exposure to the cause of diarrhea (15,16,31,32).

Meta-analyses of probiotic studies with LGG on treatment of acute diarrhea have shown a reduction of diarrhea duration by 1 day (30) and a study on persistent diarrhea reduced duration by 4 days (9). The meta-analyses on acute diarrhea found that LGG seems to have slightly higher effect in studies with a dose of 10 billion CFU/day compared with studies with lower doses and there was a tendency toward lower effect in non-European countries compared with European countries (30). Some older studies with BB-12 found an effect on diarrhea (33,34), but 2 new larger studies in hospitalized and community-based children showed no effect on diarrhea (35,36).
Diarrhea in the outpatient phase was usually mild according to the Vesikari scale, but a number of patients had prolonged periods of diarrhea, especially in the placebo group (Supplemental Digital Content 2, Fig. http://links.lww.com/MPG/A833). This could be associated with presence of environmental enteric dys-function (EED), which involves increased gut permeability, reduced absorptive capacity and inflammation (37). A reduction in the number of children with long diarrhea periods could improve the long-term nutritional status and reduce the risk of hospital admissions in community-based children with SAM.

Regarding respiratory tract infections probiotics are mainly reported to prevent upper respiratory tract infections (12). Both B. longum and L. GG have been reported to reduce upper respiratory tract infection (15–17); however, sometimes with conflicting results (18,35,36).

### Table 2. Results of probiotic treatment on diarrhea, pneumonia and nutritional recovery during in- and outpatient treatment

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Probiotics (N = 200)</th>
<th>Placebo (N = 200)</th>
<th>Adjusted effect size (95% CI) probiotics vs placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diarrhea</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duration, days</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inpatient</td>
<td>6.9 ± 6.0</td>
<td>6.5 ± 6.4</td>
<td>+0.2 (–0.8 to 1.2), P = 0.69</td>
</tr>
<tr>
<td>Outpatient</td>
<td>6.0 ± 8.2</td>
<td>8.5 ± 10.9</td>
<td>–2.2 (–3.5 to –0.3), P = 0.025</td>
</tr>
<tr>
<td>Incidence n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inpatient</td>
<td>177 (89%)</td>
<td>169 (85%)</td>
<td>OR 1.6 (0.8 to 3.3), P = 0.17</td>
</tr>
<tr>
<td>Outpatient</td>
<td>107 (70%)</td>
<td>116 (76%)</td>
<td>OR 0.7 (0.4 to 1.2), P = 0.17</td>
</tr>
<tr>
<td>Severity (score)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Inpatient</td>
<td>2.6 ± 5.5</td>
<td>2.7 ± 5.3</td>
<td>–0.3 (–1.0 to 0.7), P = 0.50</td>
</tr>
<tr>
<td>Outpatient</td>
<td>NA</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Nutritional recovery</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Weight gain (g/kg bodyweight per day)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inpatient</td>
<td>6.5 ± 4.7</td>
<td>6.1 ± 4.2</td>
<td>+0.3 (–0.6 to 1.3), P = 0.49</td>
</tr>
<tr>
<td>Outpatient</td>
<td>3.0 ± 2.1</td>
<td>3.2 ± 2.3</td>
<td>–0.1 (–0.6 to 0.4), P = 0.80</td>
</tr>
<tr>
<td>Total</td>
<td>4.1 ± 2.2</td>
<td>4.2 ± 2.2</td>
<td>+0.02 (–0.5 to 0.5), P = 0.94</td>
</tr>
<tr>
<td>Recovered n (%)</td>
<td>133 (66%)</td>
<td>128 (64%)</td>
<td>OR 1.2 (0.6 to 2.4), P = 0.68</td>
</tr>
<tr>
<td>Other</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Fever, days</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inpatient</td>
<td>7.0 ± 5.1</td>
<td>6.7 ± 4.4</td>
<td>+0.3 (–0.3 to 1.0), P = 0.29</td>
</tr>
<tr>
<td>Outpatient</td>
<td>1.6 ± 2.9</td>
<td>2.0 ± 3.9</td>
<td>–0.5 (–1.3 to 0.2), P = 0.16</td>
</tr>
<tr>
<td>Vomit, days</td>
<td>2.1 ± 3.3</td>
<td>2.0 ± 4.4</td>
<td>–0.02 (–0.7 to 0.7), P &gt; 0.99</td>
</tr>
<tr>
<td>Outpatient</td>
<td>1.2 ± 2.3</td>
<td>1.6 ± 6.0</td>
<td>–0.3 (–1.4 to 0.7), P = 0.53</td>
</tr>
<tr>
<td>Hospitalization, days</td>
<td>18.3 ± 9.1</td>
<td>18.0 ± 9.3</td>
<td>+0.1 (–1.7 to 1.9), P = 0.93</td>
</tr>
</tbody>
</table>

Data are reported as mean ± SD or n (%) unless otherwise indicated.

CI = confidence interval; OR = odds ratio.

1 Total number of days with diarrhea. Number of subjects analyzed: Inpatients: probiotics n = 187, placebo n = 182. Outpatients: probiotic n = 147, placebo n = 145.

2 Incidence of diarrhea: Number of patients with minimum 1 day of diarrhea.

3 Incidence of pneumonia: Number of patients with minimum 1 day of pneumonia.

4 Pneumonia was diagnosed based on clinical symptoms. Number of subjects analyzed: Inpatients: probiotics, n = 189, placebo n = 183. Outpatients: probiotics, n = 148, placebo, n = 149. Incidence: number of children with minimum 1 day with pneumonia. Severity: sum of daily pneumonia scores (0 = no pneumonia, 1 = pneumonia, 2 = severe pneumonia) during hospitalization. Pneumonia duration and severity were not possible to measure in outpatients as they were only seen by a medical doctor every second week.

5 Weight gain during inpatient treatment was calculated as the difference between discharge weight and the minimum weight during hospitalization and days were total number of hospitalization days. Outpatient weight gain was calculated from discharge to week 8. Total weight gain was the difference between minimum weight during hospitalization and week 8. Number of subjects analyzed: Inpatients probiotics n = 159, placebo n = 155, outpatients and total weight gain: probiotics n = 138, placebo n = 142.

6 Recovery was defined as weight-for-length > –2.
We did not find any difference in weight gain between the probiotic and placebo groups, neither during hospitalization nor during outpatient treatment. The overall evidence regarding effect of probiotics on growth is scarce (38). Onubi performed a systematic review of the effect of probiotics on growth in children and evaluated 12 studies (13). Five studies from low-income countries, including 4 studies with undernourished children, showed a positive effect on weight gain whereas 7 studies from high-income countries did not. Both studies on BB-12 or other strains belonging to the same subspecies BB-12 and LGG were included in the review.

Safety of probiotics in immune compromised and critically ill patients has been discussed mainly due to a concern about risk of probiotic-related sepsis (39). The mortality rate was therefore carefully followed throughout the study period by investigators and the DSMB. In the current study, there was a small, nonsignificantly higher number of patients that died in the probiotic group (26 patients) compared with the placebo group (20 patients) (P = 0.38). The mortality reports showed multiple severe medical complications in most of the children, complicating assessment of the exact cause of death. The most common causes of death, according to the mortality reports, were respiratory failure/severe pneumonia and shock/dehydration related to severe diarrhea. Septicemia was considered to be a direct cause of death in 4 patients in each group and contributed to the underlying cause of death in 6 patients in each group. There were no signs of consistent differences between the groups.

Strengths of the study include a randomized, double-blind controlled design, use of a validated stool diary and thorough training and monitoring of caregivers when they recorded children’s stool pattern. Lack of data on diarrhea etiology is a limitation of the study. In children with SAM, diarrhea can be caused by both infectious and noninfectious agents. This includes infections with bacteria, viruses or parasites and diarrhea due to malabsorption, for example, secondary lactose intolerance and enteropathy. Probiotics are likely to have different effects on these diarrhea etiologies, but this was not assessed. The loss to follow-up may have resulted in lower power, imprecise estimates of effects, and attrition bias. The drop-outs were, however, equally distributed in the probiotic and placebo groups. Finally, the results may not be generalizable to all children with SAM as children below 6 months, children with an admission weight below 4 kg and children in shock or with severe respiratory distress were excluded from the study. Children with any of these criteria belong to the most vulnerable children with SAM.

The current results do not support using probiotics for the treatment of hospitalized children with SAM and severe medical complications. The reduction of days with diarrhea in the outpatient phase, especially among children with long diarrhea duration, may, however, be important in future community-based treatment of children with SAM and may reduce admissions and mortality. But further studies are needed to clarify this potential effect.

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REFERENCES

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