The American Journal of Clinical Nutrition 117 (2023) 976–984

The American Journal of CLINICAL NUTRITION

journal homepage: www.journals.elsevier.com/the-american-journal-of-clinical-nutrition

Original Research Article

Probiotics for children with uncomplicated severe acute malnutrition (PruSAM study): A randomized controlled trial in the Democratic Republic of Congo

Richard Mbusa Kambale [1,2,3,4,5,*], Joseph Ntagerwa Ntagazibwa [2,3], Joe Bwija Kasengi [2,3], Adrien Burume Zigashane [2,3], Isia Nancy Francisca [2,3], Benjamin Ntaligeza Mashukano [2,3], Gaylord Amani Ngaboyeka [6], Esto Bahizire [2,5,7,8], Francis Zech [1], Laure B. Bindels [9,†], Dimitri Van der Linden [1,10,†]

[1] *Institute of Experimental and Clinical Research, Université catholique de Louvain, Brussels, Belgium;* [2] *Faculty of Medicine, Université catholique de Bukavu, Bukavu, Democratic Republic of Congo;* [3] *General Pediatrics, Pediatric Department, Hôpital Provincial Général de Référence de Bukavu, Bukavu, Democratic Republic of Congo;* [4] *Institut Supérieur des Techniques Médicales, Bukavu, Democratic Republic of Congo;* [5] *Center for Tropical Diseases and Global Health, Université catholique de Bukavu, Bukavu, Democratic Republic of the Congo;* [6] *Ecole Régionale de Santé Publique, Université catholique de Bukavu, Bukavu, Democratic Republic of Congo;* [7] *Center for Research in Natural Sciences of Lwiro, Bukavu, Democratic Republic of the Congo;* [8] *Department of Medical Microbiology, University of Nairobi, Nairobi, Kenya;* [9] *Metabolism and Nutrition Research Group, Louvain Drug Research Institute, Université catholique de Louvain, Brussels, Belgium;* [10] *Pediatric Infectious Diseases, Specialized Pediatrics Service, Pediatric Department, Cliniques universitaires Saint Luc, Brussels, Belgium*

A B S T R A C T

Background: Severe acute malnutrition (SAM) contributes to nearly 1 million deaths annually worldwide, with diarrhea and pneumonia being the common morbidity associated with mortality.

Objectives: To assess the effect of probiotics on diarrhea, pneumonia, and nutritional recovery in children with uncomplicated SAM.

Methods: A randomized, double-blind, placebo-controlled study was conducted involving 400 children with uncomplicated SAM randomly assigned to ready-to-use therapeutic food (RUTF) either with ($n = 200$) or without ($n = 200$) probiotics. Patients received 1 mL daily dose of a blend of *Lacticasebacillus rhamnosus* GG and *Limosilactobacillus reuteri* DSM 17938 (dosage, 2 billion colony-forming units; 50:50) or placebo during 1 mo. They were simultaneously fed with the RUTF for 6 to 12 wk, depending on patients' recovery rates. The primary outcome was the duration of diarrhea. Secondary outcomes included diarrheal and pneumonic incidence, nutritional recovery, and transfer to inpatient care rate.

Results: For children with diarrhea, the number of days of disease was lower in the probiotic group (4.11; 95% CI: 3.37, 4.51) than that in the placebo group (6.68; 95% CI: 6.26, 7.13; $P < 0.001$). For children aged 16 mo or older, the risk of diarrhea was lower in the probiotic group (75.6%; 95% CI: 66.2, 82.9) than that in the placebo group (95.0%; 95% CI: 88.2, 97.9; $P < 0.001$), but no significant difference of the risk for the youngest. In the probiotic group, nutritional recovery happened earlier: at the 6th wk, 40.6% of the infants were waiting for nutritional recovery, contrasting with 68.7% of infants in the placebo group; but the nutritional recovery rate at the 12th wk was similar between the groups. Probiotics had no effect on pneumonic incidence and transfer to inpatient care.

Conclusions: This trial supports using probiotics for the treatment of children with uncomplicated SAM. Its effect on diarrhea could positively affect nutritional programs in resource-limited settings.

This trial was registered https://pactr.samrc.ac.za as PACTR202108842939734.

Keywords: diarrhea, probiotics, low-income country, uncomplicated severe acute malnutrition, infants

Abbreviations used: CMAM, Community-based Management of Acute Malnutrition; DRC, Democratic Republic of Congo; ProbiSAM, Probiotics for Severe Acute Malnutrition; PRONUT, Probiotics and prebiotics for severe acute malnutrition; PruSAM, Probiotics for uncomplicated severe acute malnutrition; RUTF, ready-to-use therapeutic food; SAM, severe acute malnutrition.

* Corresponding author.
E-mail address: richkambale7@gmail.com (R.M. Kambale).

† LBB and DVdL contributed equally to this work.

https://doi.org/10.1016/j.ajcnut.2023.01.019
Received 18 July 2022; Received in revised form 25 January 2023; Accepted 27 January 2023
Available online 19 April 2023

Introduction

Severe acute malnutrition (SAM) affects 14 million children aged younger than 5 y worldwide and directly contributes to nearly 1 million deaths annually [1,2]. It is also an important underlying contributor to many other child deaths, especially those due to diarrhea and pneumonia [2]. These 2 are the leading complications of SAM associated with increased morbidity, longer hospitalization, and mortality [3–5]. Long-term effects of SAM include high postdischarge mortality after inpatient management; and poorer growth, body composition, and physical and cognitive function; and low probability of attaining a high level of education [6–10].

SAM in children is classified as uncomplicated or complicated. Uncomplicated SAM refers to the child who has no clinically obvious acute infections or other medical complications and has a good appetite. Complicated SAM refers to child who has clinical features of infection or metabolic and electrolyte disturbance, severe edema, or poor appetite [11].

Malnutrition is due to a complex set of determinants, such as inadequate food intake and infections [12]. Recently, a number of metagenomic studies in undernourished children from Bangladesh, India, and sub-Saharan Africa provided evidence that microbiota immaturity was involved in development or maintenance of acute malnutrition [13–18]. The growing evidence for a causal role of microbial dysbiosis in child malnutrition warrants intervention studies using microbiota-targeted therapies to prevent or treat undernutrition. Probiotics, that is, live microorganisms that confer a health benefit to the host when administered in adequate amounts [19], provide a potential opportunity to promote a healthy gut microbiota. In well-nourished children with acute diarrhea, recent meta-analyses evidence suggests that probiotics are effective in reducing the duration of hospitalization [20]. Investigations of strain-specific effects have led to recommendation of 3 main strains: *Lacticasebacillus rhamnosus* GG, *Saccharomyces boulardii,* and *Limosilactobacillus reuteri* DSM 17938 [21]. In children with undernutrition, our recent meta-analysis has shown that probiotics could reduce significantly the hospital stay duration by approximately 3 d [22].

Probiotic effects have also been investigated in 2 large trials among children with SAM in Malawi [23] and Uganda [24], and no benefits of probiotic treatment on clinical outcomes or nutritional recovery were reported. Nevertheless, these studies demonstrated that probiotics were safe to use in children with immunodeficient SAM. Indeed, no cases of probiotic-related bacteremia were identified. Moreover, they showed trends toward reduced outpatient mortality and outpatient days with diarrhea in the probiotic group, suggesting possible positive role of probiotics in community-based management of children with SAM. Several bacterial species, such as *Lactobacillus* strains, have been previously proposed as probiotic candidates as an alternative to fecal transplantation to address children with SAM [25].

In this study, we aimed to assess the effect of a combination of probiotics, *Lacticasebacillus rhamnosus* GG and *Limosilactobacillus reuteri* DSM 17938, on diarrhea, pneumonia, and nutritional recovery in children treated for uncomplicated SAM in the community.

Methods

Study design and participants

The Probiotics for Uncomplicated Severe Acute Malnutrition (PruSAM) trial was a double-blind, individually randomized placebo-controlled trial. It was conducted in the Kadutu Health Zone, in the South Kivu Province, eastern Democratic Republic of Congo (DRC). Kadutu Health Zone has 450,000 people, including 85,000 children aged younger than 5 y, with 24 health centers and 5 referral hospitals. Furthermore, it encompasses 5 outpatient therapeutic centers where children with uncomplicated SAM receive routine medical care and a take-home ration of RUTF once a fortnight, and 1 intensive therapeutic and feeding unit where all children with complicated SAM are managed. Both centers receive children of the same age category. The prevalence of acute malnutrition in this region was estimated to be 3% in 2018. The PruSAM trial was conducted in 2 of the 5 outpatient therapeutic centers. Selection of these 2 trial centers was based on demographic, epidemiologic, accessibility, and logistical criteria.

Children were eligible for inclusion if they were aged between 6 and 24 mo; had a diagnosis of SAM [mid–upper arm circumference of $<$115 mm, weight-for-length *z*-score (WAZ) ≤ -3, and/or grade 1 or 2 nutritional edema]; passed an appetite test performed as per the national protocol [26]; and had no medical complications and their parent or guardian provided written informed consent. Exclusion criteria were a known allergy to RUTF, congenital or acquired disorders affecting growth, and grade 3 nutritional edema. Children were enrolled after their parent or guardian provided written informed consent. Although these ineligible children were excluded from the study, they were compassionately enrolled in the standard RUTF therapeutic feeding program, unless there is another contraindication such as peanut allergy. Those with complicated SAM were referred for inpatient care. After stabilization, they were returned to standard RUTF therapeutic feeding program.

Randomization, allocation concealment, and blinding

Children were randomly assigned in a 1:1 ratio, and in computer-generated blocks of 6, to receive probiotics or placebo. The randomization codes were created with a computerized random-number generator according to site; they were kept inside opaque, sealed, consecutively numbered envelopes and opened by a study team physician in a numerical order. The codes based on the block randomization table were provided to the study team by a designated person through telephone. All participants, caregivers, investigators, and staff involved in the study were blinded until the database was locked.

Intervention, monitoring, and follow-up

Treatment followed DRC national guidelines [26], which are based on standard international WHO [27] and Community-based Management of Acute Malnutrition (CMAM) guidelines [28]. Patients received 1 mL of study product daily in single dose, in addition to the standard treatment of uncomplicated SAM. This 1 mL was picked directly from the vial using a 1-mL graduated pipette provided in the box and administered before RUTF. Each milliliter of study product contained coconut oil with or without a combination of the 2 probiotic strains, at a dosage of 1 billion colony-forming units of each bacterium. *Lactobacillus* strains were chosen because they are one of the most widely used probiotic strains, which have been studied extensively in clinical trials and human intervention studies. Study product was administered for 1 mo, and the first dose was given at inclusion, at the centers. Probiotic products were manufactured by Ineldea, whereas the placebo products containing coconut oil were bottled locally in vials similar to those of probiotics. The appearance, taste, and smell of the products were indistinguishable, so patients were blind to their group allocation. Four very small letters printed on the right corner of the label was the only way to identify the correct group. According to the manufacturer's instructions, the product should be stored at ambient

temperature not exceeding 30 °C. In South Kivu, the mean temperature is between 20–25 °C. Probiotics were transported to the DRC in a single batch at an ambient temperature and were stored at an ambient temperature in South Kivu (Bukavu). Study products were received in Bukavu in August, 2021. The test to see how many colony-forming units were still viable on arrival was not performed. Regular quality control checks performed in December, 2021, and March, 2022, showed that we prescribed more than 10^6/mL of live probiotic bacteria at the time of consumption according to recommendations [29].

Clinical history and examination, anthropometry, blood glucose and hemoglobin levels, rapid malaria tests, and HIV testing using 2 rapid antibody tests (Determine and Unigold) were systematically undertaken at admission. If malarial test showed positive results, an artemisinin-based combination therapy was prescribed. HIV-positive patients received further care according to national protocols, which included referral for antiretroviral medication. Children with suspected tuberculosis were referred to the hospital for diagnosis and management.

After admission into the study, all children received a 5-d course of amoxicillin, a single dose of vitamin A, an anthelmintic, a 2-wk ration of RUTF, and health and nutrition advice. The RUTF ration was calculated to provide 200 kcal/kg of bodyweight/d. Children with SAM normally need 15 kg of RUTF (1 carton) given over a period of 6 to 8 wk for recovery from malnutrition [30]. Considering the length of stay in the CMAM program in DRC (12 wk maximum) and the risk of RUTF sharing within and outside the household, 2 cartons of RUTF were planned per child. All these medications were directly administered to the child by the caregivers at admission, supervised by the nurse. Follow-ups were planned biweekly throughout a period of 12 wk, depending on the nutritional recovery of each child. Breastfeeding was encouraged throughout the study.

Routine procedures in each follow-up appointment included taking anthropometric measurements and vital signs and assessing and managing of signs of pneumonia, diarrhea, dehydration, presence of appetite, other signs of illness, and adverse events. Caregivers were given a stool diary in which they ticked every time their child passed stool. Stool frequency and consistency were checked at follow-up visits. Based on a photograph scale, stool was categorized as watery, abnormally loose, loose, or normal. A repeat 2-wk rations of RUTF was provided at the same dosage rate. RUTF were administered for 8–12 wk depending on each child's nutritional recovery.

Caregivers were contacted by telephone once a week to ask about the health progress of their infants and to remind them about visit dates. In case of missed follow-up, a home visit was performed to inquire about reasons for failure to return for a follow-up visit. Study product compliance was estimated using the following criteria: *1*) verbal report from the caregiver; *2*) examination of the amount of intervention remaining in the medication bottle; *3*) the returned empty or unused RUFT sachets; and *4*) differences between visit dates and number of sachets supplied. Infants were considered compliant to the study products if they fulfilled the abovementioned 4 criteria during the whole study period.

Infants identified at any point in the process who did not demonstrate an appropriate appetite, or had medical complications, or remained severely malnourished after 4 follow-up visits were referred for inpatient care. These infants were followed up until 12 wk to correctly allocate severity of hospital course or death to each study group.

Outcomes

The primary outcome was the number of days with diarrhea. Based on stool diary data, diarrhea was defined as the passage of 3 or more loose or liquid stools per 24 h [31]. A diarrhea episode started when the diarrhea definition was fulfilled, and ceased when the child passed <3 loose or watery stools per day. If diarrhea re-appeared after <48 h, it was considered part of the same diarrhea episode, but only days with ≥3 loose or watery stools were counted as diarrhea days.

Secondary outcomes were, first, diarrheal incidence, defined as the proportion of infants with minimum 1 d of diarrhea, and second, pneumonic incidence, defined as the proportion of infants with pneumonia. Pneumonia was defined as the combination of signs, such as fever, tachypnea, focal crackles, or decreased breath sounds together, with characteristic radiologic abnormalities [31]. Third outcome was nutritional recovery. Infants were considered to have recovered when they were without edema, had a WAZ of −2 or higher, and mid–upper arm circumference of ≥125 mm at 2 consecutive follow-up visits with a maximum of 15 d between the visits. Finally, other outcomes such as weight gain, time to recovery, transfer to inpatient care, death rate, and RUTF and probiotic safety were assessed. Transfer to inpatient care included infants referred to inpatient care owing to medical complications.

Statistical analysis

Sample size

We calculated that a sample of 178 children in each group would provide the study with an 80% power at a 5% significance level to detect a 1-d reduction in number of days with diarrhea. Allowing for a 10% rate of loss to follow-up, 200 infants were recruited per arm. Assuming that the standard deviation of days with diarrhea in outpatient therapeutic center was 3 d, it would be possible to detect a 1-d reduction in days with diarrhea, which is similar to what is found in meta-analyses [22].

Data management and statistical analysis

Data were double-entered in EpiData v.3.1 (EpiData) and analyzed using SPSS 25.0 (SPSS). WHO Anthro plus 1.0.4 (WHO) was used to calculate anthropometric *z*-scores by applying 2007 WHO References. In each study trial, the age, sex, presence or absence of bilateral pitting edema, and anthropometric measurements of infants were imported into WHO Anthro plus software, which then calculated WAZ, length-for-age *z*-score, and weight-for-length *z*-score and identified outliers: <−6 and >5 for WAZ, <−6 and >6 for length-for-age *z*-score, and <−5 and >5 for weight-for-length *z*-score. These values were excluded and the infants subjected to measurements again. Household Food Insecurity Access Scale was constructed according to Food and Nutrition Technical Assistance indicator guide [32]. Except for the end points, all comparisons between the probiotic and placebo groups were performed using nonparametric tests: χ^2 test (with Cook correction) for dichotomous variables, Mann–Whitney rank test for continuous variables, and Kolmogorov–Smirnov test for continuous and ordinal variables. The number of days with diarrhea was modeled by a zero-inflated γ-Poisson regression. Presence or absence of diarrheal predisposition and number of days of diarrhea, if any, were simultaneously analyzed by the maximum likelihood method. The weight gain achieved at nutritional recovery was modeled using a γ distribution regression for the weight gain achieved, if any. Data regarding the time to reach nutritional recovery were truncated by the limitation of the time of observation and were thus analyzed by a survival statistical calculation method. A Rényi test was performed to assess the gap between the time curves waiting for nutritional recovery of the probiotic and placebo groups. To assess the influence of continuous variables on the time to reach nutritional recovery, the Kvaloy rank test

was used. CIs were measured at 95%. The regression results were transformations of logarithmic or logistic values, giving asymmetric CI values. This trial was registered at a Pan African Clinical Trials Registry as PACTR202108842939734.

Ethics

The study was performed in accordance with the principles in the Declaration of Helsinki of 1975 as revised in 1983. An independent Data Safety Monitoring Board, including 1 statistician and 1 pediatrician, regularly monitored serious adverse events during the study. Children were enrolled after verbal and written consent had been provided by their caregivers. Caregivers were made aware of their right to withdraw from the study at any time. Ethical approval was granted by the Université catholique de Bukavu Ethics Committee (Bukavu/DRC, UCB-CIES 01-05/2020) and the Comité d'Ethique Hospitalo-Universitaire of the Université catholique de Louvain and Cliniques Universitaires Saint-Luc (Brussels/Belgium, 04-01-2021).

Results

Between August 2021 and May 2022, 694 children with SAM were assessed for eligibility in the 2 trial centers. After the exclusion of ineligible children, 400 children were randomly assigned to a study group. The total loss to follow-up was 7 in the probiotic group and 9 in the placebo group. Finally, 384 children (193 in the probiotic group and 191 in the placebo group) were included in the final analysis (Figure 1).

Baseline sociodemographic, anthropometric, and clinical characteristics were similar in both groups (Table 1).The median age was 16 mo (IQR: 11–20 mo), 212 (53%) infants were boys, 288 (72%) were currently breastfed, and 236 (59%) had edematous malnutrition (kwashiorkor). Forty-six (11.5%) infants showed positivity for malaria, and 7 (1.8%) were HIV seropositive. Moreover, 327 (81.8%) caregivers were from households with a low socioeconomic level, 231 (57.8%) had a low education level, 163 (40.8%) were currently pregnant, and 52 (13%) were undernourished.

Compliance to study product consumption was 98% and 96% for probiotics and placebo, respectively. Study products and RUTF were tolerated equally well in both groups. No cases of allergy or anaphylaxis were identified. During follow-up, 3 infants died: 1 infant in the probiotic group and 1 in the placebo group died at home due to unknown cause; and 1 infant in the placebo group died in hospital of septic shock. All were HIV-negative children.

Table 2 summarizes main study outcomes. Compared with that of the placebo group, the number of days with diarrhea was multiplied by

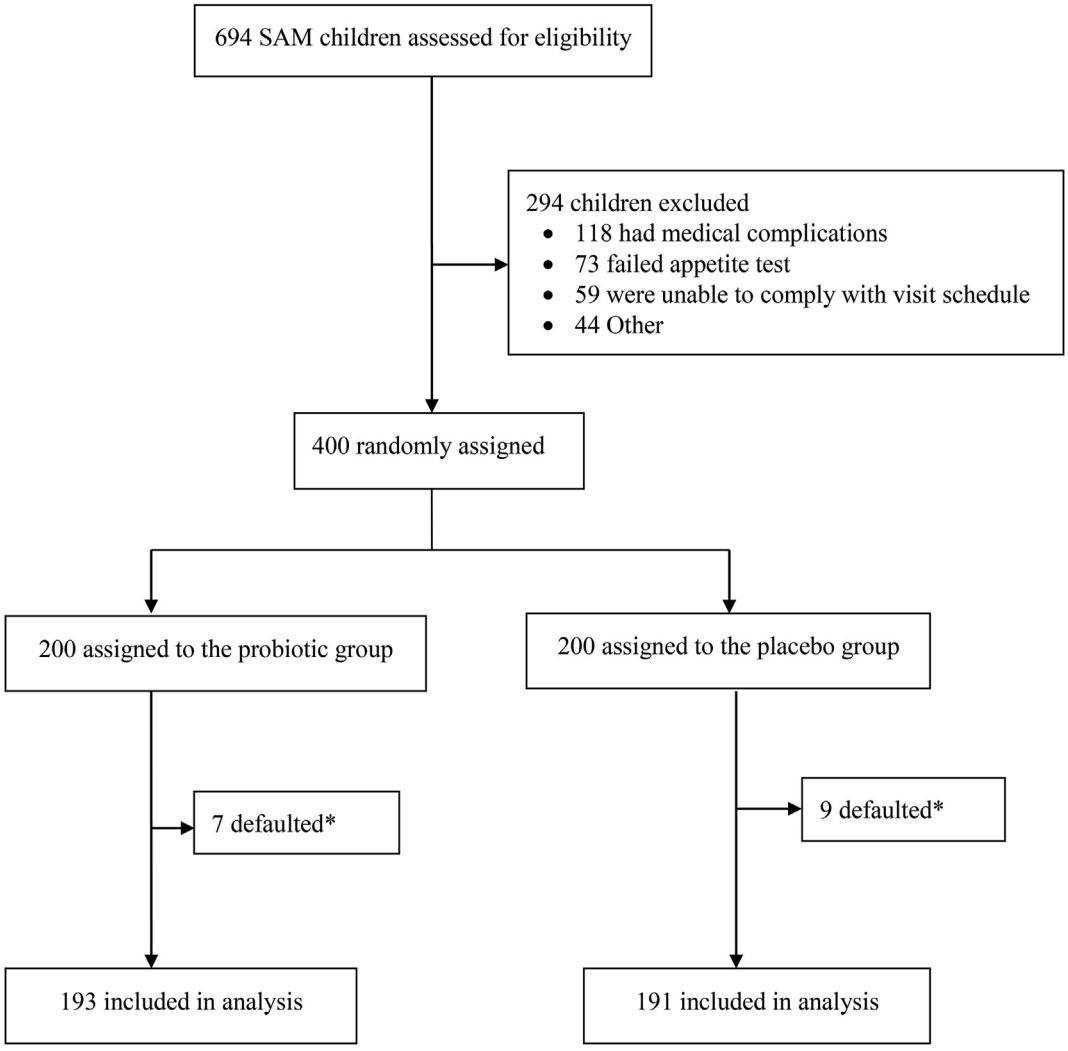

FIGURE 1. Trial profile. SAM, severe acute malnutrition. *Default was defined as 3 or more consecutive missed weekly visits. Children who defaulted did not recover and withdrew from trial follow-up before 12 wk.

TABLE 1
Baseline characteristics

	Probiotics ($n = 200$)	Placebo ($n = 200$)
Sociodemographic characteristics		
Age (y)	16 (12–20)	16 (11–20)
Male	107 (53.5)	105 (52.5)
Vaginal birth	182 (91.0)	185 (92.5)
Birth weight (g)	3035 (2900–3600)	3200 (2940–3700)
Born at full-term	194 (97.0)	195 (97.5)
Currently breastfed	142 (71.0)	146 (73.0)
Age of introduction of CFs (months)	5 (3–6)	5 (4-6)
No. siblings	3 (2–5)	3 (3–6)
Child lives with both parents	151 (75.5)	148 (74.0)
Mother education primary school or lower	116 (58.0)	115 (57.5)
Mother with undernutrition	25 (12.5)	27 (13.5)
Mother currently pregnant	83 (41.5)	80 (40.0)
Household size	7 (5–9)	7 (5–9)
Household with low socioeconomic level	162 (81.0)	165 (82.5)
Anthropometric characteristics		
Weight (kg)	6.7 (6.0–7.4)	6.6 (5.7–7.2)
Length (cm)	70 (67–73)	69.5 (65–73.1)
MUAC (mm)	118 (110–125)	117 (110–123)
Head circumference (cm)	45 (43–46)	45 (43–46)
Weight-for-age *z*-score	−3.6 (−4.2 to −3.1)	−3.6 (−4.4 to −3.1)
Length-for-age *z*-score	−3.3 (−4.2 to −2.5)	−3.6 (−4.3 to −2.6)
Weight-for-length *z*-score	−2.6 (−3.3 to −1.9)	−2.5 (−3.3 to −1.7)
Medical characteristics at admission		
Fever	12 (6.0)	11 (5.5)
Cough	138 (68.0)	133 (66.5)
Diarrhea	88 (44.0)	91 (45.5)
Vomiting	45 (22.5)	49 (24.5)
Had a malaria	22 (11.0)	24 (12.0)
Hemoglobin (g/dL)	9.1 (8.4–10.2)	9.0 (8.0–10.1)
HIV-positive children (serology)	4 (2.0)	3 (1.5)
Kwashiorkor (edematous malnutrition)	116 (58.0)	120 (60.0)

Values are given as *n* (%) or median (IQR).
CF, complementary food; MUAC mid–upper arm circumference.

TABLE 2
The outcomes of infants with SAM randomly assigned to probiotics or placebo

	Probiotics ($n = 193$)	Placebo ($n = 191$)	*P*
Average duration of diarrhea, if any[1] (d)			
Whole population[2]	4.11 (3.37, 4.51)	6.68 (6.26, 7.13)	<0.001
Half youngest infants[3]	3.44 (2.88; 4.09)	6.48 (5.76, 7.29)	<0.001
Half oldest infants[4]	4.45 (4.00, 4.96)	6.77 (6.26, 7.33)	<0.001
Risk of diarrhea (%)			
Whole population	61.6 (54.4, 68.2)	70.8 (63.9, 76.6)	0.063
Half youngest infants	45.9 (35.6, 56.3)	45.8 (36.0, 55.6)	0.99
Half oldest infants	75.6 (66.2, 82.9)	95.0 (88.2, 97.9)	<0.001
Still waiting for nutritional recovery at week 12[5] (%)	17.8 (12.6, 23.7)	19.6 (11.1, 25.7)	0.58
Still waiting for nutritional recovery at year 6 (%)			
Whole population	40.6 (33.4, 47.6)	68.7 (61.4, 75.0)	<0.001
Infants with diarrhea	33.7 (24.1, 43.5)	70.0 (59.4, 78.3)	<0.001
Infants without diarrhea	34.2 (23.7, 45.1)	46.3 (32.7, 58.8)	0.22
Weight gain, if any (whole population)[6,7] (g/kg/d)	4.34 (4.09, 4.56)	4.01 (3.37, 4.51)	0.069
Frequency of pneumonia (%)	7.8 (4.4, 12.5)	9.4 (5.7, 14.5)	0.57
Transfer to inpatient care (%)	6.2 (3.3, 10.6)	5.2 (2.5, 9.4)	0.69

Value given in parentheses are 95% CIl.
[1] The number of days with diarrhea was modeled by a zero-inflated γ-Poisson regression. Presence or absence of diarrhea predisposition and number of days of diarrhea were simultaneously analyzed by the maximum likelihood method.
[2] With probiotics, the mean of placebo is multiplied by 0.615×/1.059 (95% CI: 0.550, 0.689).
[3] With probiotics, the mean of placebo is multiplied by 0.530×/1.114 (95% CI: 0.430, 0.655). Half youngest infants were defined as younger than the median (16 mo); $n = 184$.
[4] With probiotics, the mean of placebo is multiplied by 0.657×/1.071 (95% CI: 0.575, 0.752). Half oldest infants were defined as equal or above the median; $n = 200$.
[5] Data regarding the time to reach nutritional recovery were truncated by the limitation of the time of observation and were thus analyzed by a survival statistical calculation method. A Rényi test was performed to assess the gap between the time curves waiting for nutritional recovery of the probiotics and placebo groups.
[6] The weight gain achieved at nutritional recovery was modeled using a γ distribution regression for the weight gain achieved, if any.
[7] With probiotics, the mean of placebo is multiplied by 1.083×/1.043 (95% CI: 0.996, 1.117).

0.615 (95% CI: 0.550, 0.689) in the probiotic groups, corresponding to a reduction of 38.5% (95% CI: 31.1, 45.0), regardless of age ($P < 0.001$). There was a trend toward a decreased risk of diarrhea in the probiotic group, but the bivariate analysis, including age and probiotics usage, showed a significant interaction, so that the probiotics had more influence on the risk for the oldest infants ($P < 0.001$). This observation required separated regressions by the age level. For children aged 16 mo (the median) or older, probiotics decreased the risk of diarrhea by 19.3% ($P = 0.006$) and, in case of diarrhea, decreased the duration by 34.3% ($P < 0.001$). For the youngest, the risk was not modified by the probiotics, but in case of diarrhea, the duration was reduced by 47.0% ($P < 0.001$). The magnitude of the effect on the duration of diarrhea was not significantly different in these 2 groups ($P = 0.099$), in contrast with the difference between the 2 groups regarding the risk ($P = 0.003$). With equal treatment, age increased the risk of diarrhea ($P < 0.001$) with only a slight increase in duration.

Overall, 292 (76.0%) infants enrolled in the trial recovered from SAM. There was no significant difference between groups in the frequency of nutritional recovery at 12 wk ($P = 0.77$), but the curves of time hindsight while waiting for nutritional recovery were different ($P < 0.001$, Rényi test). In the probiotic group, nutritional recovery happened earlier for a large proportion of infants (Figure 2): At the 6th wk, 40.2% of infants were waiting for nutritional recovery, contrasting with 68.7% of infants in the placebo group. Logistic regressions showed a significant interaction between treatment allocation and presence of diarrhea on the percentage reaching nutritional recovery at 6 and 8 wk. The effect of probiotics was restricted to infants with diarrhea. In these children, probiotic use was associated with an earlier nutritional recovery ($P = 0.010$, Rényi test), whereas for infants without diarrhea, this effect was not demonstrated ($P = 0.43$, Rényi test). The age was not associated with weight gain ($P = 0.26$) nor with earlier nutritional recovery ($P = 0.35$, Kvaloy test). The weight gain,

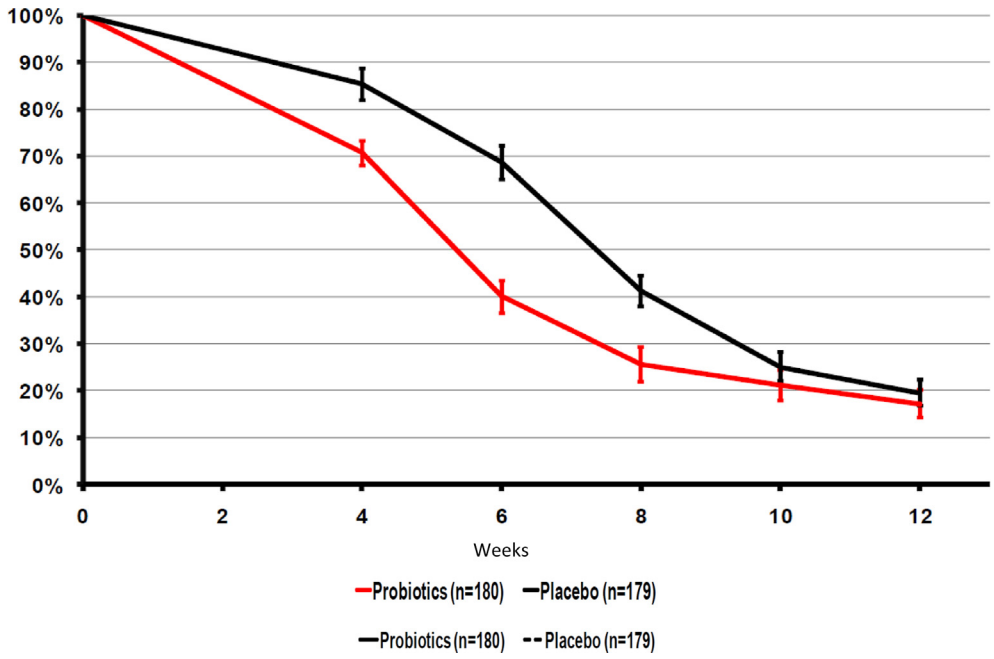

FIGURE 2. Time before nutritional recovery.

the risk of transfer to inpatient care, and the risk of death were similar between the placebo and probiotic groups.

Discussion

This trial investigated the efficacy of probiotics on diarrhea, pneumonia, and nutritional recovery in infants with uncomplicated SAM in the CMAM. Probiotics reduced the number of days corresponding to 39% of the mean number of days with diarrhea.

In the Probiotics for Severe Acute Malnutrition (ProbiSAM) study [24], a randomized, double-blind, placebo-controlled trial in Uganda to assess the effect of probiotics on diarrhea during inpatient and outpatient treatment of children with SAM, the probiotics had no effect on diarrhea during inpatient treatment but a significant 26% reduction in days with diarrhea during the outpatient treatment. An earlier double-blind efficacy randomized controlled trial in Malawi [Probiotics and prebiotics for severe acute malnutrition (PRONUT) study] [23] to assess the clinical and nutritional efficacy of a probiotics during inpatient and outpatient treatment of children with SAM, reported trends toward less severe diarrhea in outpatient treatment in the probiotic group.

The discrepancy between our findings with ProbiSAM and PRONUT trials may be due to several factors. The first one is the patient population. Compared with the ProbiSAM and PRONUT trials in which children aged 6 to 59 mo and 5 to 168 mo were included, respectively, we included children aged 6 to 24 mo. Indeed, the life cycle contains windows of opportunity in which microbiota-targeted therapies may be more amenable to intervention. The 1000-d period represents a period of microbial assembly in which the microbiome could be targeted to optimize healthy composition [33].

The second is the type of children with SAM: ProbiSAM and PRONUT trials enrolled children with complicated SAM with multiple life-threatening conditions. Furthermore, the PRONUT study included a large proportion (45%) of HIV-infected children. More severe illness and a compromised gut barrier in children during hospitalization may have affected their ability to respond to probiotic treatment. In a follow-up of the ProbiSAM trial, Castro-Mejia et al. [34] investigated gut microbiome evolution during rehabilitation from SAM and the effect of probiotics supplementation on the gut microbiome during inpatient and outpatient treatment of SAM. In addition, they assessed whether gut microbiome composition and development were linked to the observed reduction in days with diarrhea when administered probiotics during the randomized controlled ProbiSAM trial. The findings suggested that gut microbiome diversity and composition change over the course of rehabilitation from SAM and approach the gut microbiome of apparently healthy subjects as treatment progresses. Furthermore, probiotic treatment reduced the cumulative incidence of diarrhea during the outpatient phase, with the strongest effect in children where the administered probiotics could be detected in the gut microbiome [34].

Third one is the trial design. We performed a community-based trial to reduce the risk of possible contamination between the study groups. Indeed, in PRONUT study, freeze-dried symbiotic powder was factory-mixed into RUTF. The possible contamination between the groups by sharing RUTF while patients were living closely together during inpatient care could not be formally excluded.

Finally, a fourth factor worth considering are the antimicrobials used. Indeed, in PRONUT trial, all inpatients received co-trimoxazole, and 50% also had parenteral antibiotics. Children who were HIV seropositive continued on long-term co-trimoxazole prophylaxis afterward discharge, during and after outpatient treatment. However, in vitro testing showed sensitivity to co-trimoxazole in 2 of the 4 probiotic organisms used in this trial. Antibiotics might have decreased the viability of the probiotics and, hence, their efficacy.

This study found that in older infants, probiotic treatment reduced the risk and duration of diarrhea, and in infants with diarrhea, it resulted in a greater percentage of early nutritional recovery and probability of weight gain. The shorter time to reach the nutritional recovery, favorable to probiotics, is an interesting finding for low-income and middle-income countries. Indeed, these countries experience high prevalence of SAM among children aged younger than 5 y (13 million) contrasting with low treatment program coverage, with at least 75% of children with SAM aged 6–59 mo not accessing care [35,36]. The main reason

for low access to SAM treatment is related to CMAM programs considered to be expensive, and therefore largely underfunded, owing to the cost of RUTF [37]. The high cost and large quantity of RUTF administered during nutritional treatment in outpatient therapeutic centers [38] have prompted attempts to optimize product formulation and use. In a noninferiority, randomized controlled trial performed in DRC, Cazes et al. [39] assessed whether a single protocol for moderate and SAM management, using only RUTF and reducing the dose as the child improves [the optimising malnutrition treatment (OptiMA) strategy], could achieve similar or higher individual efficacy, increase coverage, and minimize costs compared with the current programs (standard strategy).The findings suggested that the OptiMA strategy was superior to the standard one regarding favorable outcomes (being alive, not acutely malnourished, and with no further episodes of acute malnutrition throughout the 6-mo observation period), time to nutritional improvement, and treatment cost.

In this study, as in most CMAM programs in DRC, 2 cartons of RUTF ($92) were planned per infant for 12 wk of nutritional treatment. In addition, the cost of probiotics for 1 infant was $36. Our study showed that a larger percentage of infants (>60%) in the probiotic group received only 1 carton of RUTF ($46) because of the short time to reach nutritional recovery in this group (6–8 wk). Finally, the combined cost of RUTF and probiotics in the probiotic group was lower than the cost of RUTF in the CMAM program in DRC ($82 and $92, respectively). The overall cost of RUTF is a major cost driver in malnutrition treatment programs, and the global community is already struggling to pay for all RUTF that is needed. In addition to the individual benefits for children regarding the reduction of the number of days of diarrhea, the probiotic intervention seems to hold major advantages from a programming perspective because it allows more children to access treatment at a lower cost. Thus, the probiotic intervention and the OptiMA strategy seem to be promising strategies to facilitate increased coverage of SAM treatment in a resource-constrained environment.

The WHO [27] and a recent meta-analysis [40] support the use of oral amoxicillin in children with uncomplicated SAM because of the high risk of mortality and the significant reduction of mortality rate among children with uncomplicated SAM treated with antibiotics. Antibiotic-associated diarrhea is a potential consequence of prescribing broad-spectrum antibiotics for the outpatient treatment of children with SAM. Regarding the ambivalence of antibiotics in children with uncomplicated SAM, probiotics seem to be useful in this population because of its protective effect for preventing antibiotic-associated diarrhea, as suggested in a recent meta-analysis [41].

Implementing and scaling up the use of probiotics in low-income and middle-income countries to address childhood malnutrition requires several logistical considerations: The need for vigilant safety monitoring, owing to the effects of malnutrition on the child's immune system; strengthening local manufacturing and storage capacities to ensure product viability despite infrastructure, financial, and climatic challenges; using existing distribution networks to ensure accessibility to those most in need of the treatment; and gaining acceptability by cultures that might not be inclined to add live microbes to dietary and medical practices [42]. Many of these concerns might be circumvented by using locally sourced dietary approaches to target the gut microbiota and associated pathologies. Recent data have provided promising evidence for the potential of microbiota-directed foods to improve child growth. Gehrig et al. [43] conducted a pilot randomized controlled trial to compare the efficacy of microbiota-directed complementary food with the traditional rice-based and lentil-based ready-to-use supplementary food used in the treatment of moderate acute malnutrition. Findings suggested that such microbiota-targeted foods enhanced biomarkers of growth, bone formation, neurodevelopment, and immune function in both experimental animals and undernourished children. Hence, microbiota-targeted therapies pose potential as promising alternatives to standard therapeutic and complementary foods in settings with a high prevalence of child undernutrition.

Our study has several strengths. First, the study was a randomized, double-blind controlled design, with low default rate after admission. Second, the follow-up and the compliance to study products and associated measures were excellent despite the challenging postconflict context. The study was implemented by well-trained and experienced research staff, and home visits were performed by well-involved and committed community health workers. Follow-ups were close and meticulous.

The study has some limitations. First, data on diarrheal etiology are lacking. Because diarrhea in children with SAM is triggered by infectious (bacterial, viral, fungi, or parasitic) or noninfectious (malabsorption or enteropathy) agents, probiotics are likely to have different effects based on these agents. Second is the lack of metagenomic and culturomic data. This approach could enable the analysis of the gut microbiota evolution during rehabilitation from SAM and the effect of probiotics supplementation on the gut microbiota. Third is the selection bias. Only 384 of the 694 subjects assessed for eligibility were analyzed, and they may have different characteristics from those of the unanalyzed or those lost to follow-up. Nevertheless, we believe that this would not have significantly altered our main conclusions because the characteristics at admission did not differ between the excluded/lost to follow-up and analyzed subjects. Fourth, the multiple secondary outcomes tests might lead to increase in type I error and should therefore be interpreted with caution. Finally, the study interventions were performed by well-trained and supervised medical staff. These features may not be generally representative of standard care provided in many outpatient therapeutic centers. Our findings should be confirmed in studies designed to reflect real-life conditions of nutritional programs.

In conclusion, the current findings support using probiotics for the treatment of children with uncomplicated SAM. Its effect in reducing the number of days of diarrhea and the time to reach nutritional recovery has potential for impact in resource-poor settings. Future research is needed for an economic analysis of scaling up probiotic use nationally and internationally, for exploration of local products (e.g., yogurts and fermented foods), which might have similar benefits, and to support breastfeeding, a free, widely available but much undersupported source of probiotic immunologically active compounds.

We express our sincere gratitude to the data collection team members for their hard work and commitment and for working tirelessly during the data collection phase. We thank all the nutritional and nursing staff of the Kadutu Health Zone for their participation in this survey.

The authors' responsibilities were as follows—RMK, LBB, DVdL: designed the research; DVdL: provided essential materials; RMK, JNN, JBK, ABZ, INF, BNM: conducted the research; EB, GAN, FZ: performed the statistical analysis; RMK: wrote the paper; DVdL, EB, FZ, LBB: critically revised the manuscript; RMK, EB, FZ, LBB, and DVdL: had primary responsibility for the final content; and all authors: read and approved the final version of the manuscript.

Conflict of intrest

The authors report no conflicts of interest.

Data Availability

Data described in the manuscript, code book, and analytic code will be made available on request pending application and approval.

Funding

The study was funded by a grant from AUCAM (Association Universitaire Catholique d'Appui au Monde en développement). The sponsor of the study did not have any role in study design, data collection, data analysis, interpretation of results, or writing of the report. The statistician for the trial and the principal investigators had full access to all data in the study. The principal investigators had final responsibility for the decision to submit for publication.

References

[1] United Nations Children's Fund (UNICEF), World Health Organization, International Bank for Reconstruction and Development/The World Bank. Levels and trends in child malnutrition: key findings of the 2021 edition of the joint child malnutrition estimates, United Nations Children's Fund, New York, 2021.

[2] United Nations Inter-agency Group for Child Mortality Estimation (UN IGME), Levels & trends in child mortality: report 2022, Estimates developed by the United Nations Inter-agency Group for Child Mortality Estimation [Internet], United Nations Children's Fund, New York (2023). Available from: https://www.childmortality.org/wp-content/uploads/2023/01/UN-IGME-Child-Mortality-Report-2022.pdf.

[3] A.H. Irena, M. Mwambazi, V. Mulenga, Diarrhea is a major killer of children with severe acute malnutrition admitted to inpatient set-up in Lusaka, Zambia, Nutr. J. 10 (2011) 110.

[4] A. Talbert, N. Thuo, J. Karisa, C. Chesaro, E. Ohuma, J. Ignas, et al., Diarrhea complicating severe acute malnutrition in Kenyan children: a prospective descriptive study of risk factors and outcome, PloS One 7 (2012), e38321.

[5] M.J. Chisti, M. Tebruegge, La S. Vincente, S.M. Graham, T. Duke, Pneumonia in severely malnourished children in developing countries—mortality risk, etiology and validity of WHO clinical signs: a systematic review, Trop. Med. Int. Health 14 (2009) 1173–1189.

[6] A. Talbert, M. Ngari, E. Bauni, M.K. Mwangome, N. Mturi, M. Otiende, et al., Mortality after inpatient treatment for diarrhea in children: a cohort study, BMC Med 17 (2019) 20.

[7] M.M. Ngari, G. Fegan, M.K. Mwangome, M.J. Ngama, N. Mturi, J.A.G. Scott, et al., Mortality after inpatient treatment for severe pneumonia in children: a cohort study, Pediatr. Perinat. Epidemiol. 31 (2017) 233–242.

[8] M. Kerac, J. Bunn, G. Chagaluka, P. Bahwere, A. Tomkins, S. Collins, et al., Follow-up of post-discharge growth and mortality after treatment for severe acute malnutrition (FuSAM study): a prospective cohort study, PLoS One 9 (2014), e96030.

[9] N. Lelijveld, A. Seal, J.C. Wells, J. Kirkby, C. Opondo, E. Chimwezi, et al., Chronic disease outcomes after severe acute malnutrition in Malawian children (ChroSAM): a cohort study, Lancet Glob. Health. 4 (2016) e654–e662.

[10] P. Mwene-Batu, G. Bisimwa, M. Baguma, J. Chabwine, A. Bapolisi, C. Chimanuka, et al., Long-term effects of severe acute malnutrition during childhood on adult cognitive, academic and behavioral development in African fragile countries: the Lwiro cohort study in Democratic Republic of the Congo, PLoS One 15 (2020), e0244486.

[11] WHO, UNICEF, WFP, UN System Standing Committee on Nutrition, Joint Statement on the Community-Based Management of Severe Acute Malnutrition [Internet] (2007). Available from: http://www.who.int/maternal_child_adolescent/documents/a91065/en/.

[12] UNICEF, Improving child nutrition: the achievable imperative for global progress [Internet], United Nations Children's Fund (2013). Available from: https://data.unicef.org/wp-content/uploads/2015/12/NutritionReport_April2013_Final_29.pdf.

[13] T.S. Ghosh, S.S. Gupta, T. Bhattacharya, D. Yadav, A. Barik, A. Chowdhury, et al., Gut microbiomes of Indian children of varying nutritional status, PLoS One 9 (2014), e95547.

[14] S. Monira, S. Nakamura, K. Gotoh, K. Izutsu, H. Watanabe, N.H. Alam, et al., Gut microbiota of healthy and malnourished children in Bangladesh, Front. Microbiol. 2 (2011) 228.

[15] A. Reyes, L.V. Blanton, S. Cao, G. Zhao, M. Manary, I. Trehan, et al., Gut DNA viromes of Malawian twins discordant for severe acute malnutrition, Proc. Natl. Acad. Sci. U.S.A. 112 (2015) 11941–11946.

[16] S.S. Gupta, M.H. Mohammed, T.S. Ghosh, S. Kanungo, G.B. Nair, S.S. Mande, Metagenome of the gut of a malnourished child, Gut Pathog 3 (2011) 7.

[17] M. Million, A. Diallo, D. Raoult, Gut microbiota and malnutrition, Microb. Pathog. 106 (2017) 127–138.

[18] K.H.S. Kristensen, M. Wiese, M.J.H. Rytter, M. Özçam, L.H. Hansen, H. Namusoke, et al., Gut microbiota in children hospitalized with edematous and non-edematous severe acute malnutrition in Uganda, PLoS Negl. Trop. Dis. 10 (2016), e0004369.

[19] Food and Agriculture Organization of the United Nations & World Health Organization, Health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria, 1, FAO, Rome, Italy, 2001, p. 1e34. Available from: http://www.fao.org/3/a-a0512e.pdf.

[20] S. Collinson, A. Deans, A. Padua-Zamora, G.V. Gregorio, C. Li, L.F. Dans, et al., Probiotics for treating acute infectious diarrhea, Cochrane Database Syst. Rev. 12 (2020) CD003048.

[21] A. Guarino, A.L. Vecchio, J.A. Dias, J.A. Berkley, C. Boey, D. Bruzzese, et al., Universal recommendations for the management of acute diarrhea in nonmalnourished children, J. Pediatr. Gastroenterol. Nutr. 67 (2018) 586–593.

[22] R.M. Kambale, F.I. Nancy, G.A. Ngaboyeka, J.B. Kasengi, L.B. Bindels, D. Van der Linden, Effects of probiotics and synbiotics on diarrhea in undernourished children: systematic review with meta-analysis, Clin. Nutr. 40 (2021) 3158–3169.

[23] M. Kerac, J. Bunn, A. Seal, M. Thindwa, A. Tomkins, K. Sadler, et al., Probiotics and prebiotics for severe acute malnutrition (PRONUT study): a double-blind efficacy randomized controlled trial in Malawi, Lancet 374 (2009) 136–144.

[24] B. Grenov, H. Namusoke, B. Lanyero, N. Nabukeera-Barungi, C. Ritz, C. Mølgaard, et al., Effect of probiotics on diarrhea in children with severe acute malnutrition: a randomized controlled study in Uganda, J. Pediatr. Gastroenterol. Nutr. 64 (2017) 396–403.

[25] M. Alou, M. Million, S.I. Traore, D. Mouelhi, S. Khelaifia, D. Bachar, et al., Gut bacteria missing in severe acute malnutrition: can we identify potential probiotics by culturomics? Front. Microbiol. 8 (2017) 899.

[26] Ministère de la Santé Publique, Protocole national de Prise en Charge Intégrée de la Malnutrition Aigüe, PCIMA, République Démocratique du Congo, 2016.

[27] World Health Organization (WHO), Updates on the management of severe acute malnutrition in infants and children [Internet] (2013). Available from: https://apps.who.int/iris/bitstream/handle/10665/95584/9789241506328_eng.pdf?ua=1.

[28] Valid, International. Community-based Therapeutic Care (CTC). A field manual [Internet]. Available from: http://www.validinternational.org.

[29] T.D. Boylston, C.G. Vinderolab, H.B. Ghoddusi, J.A. Reinheimer, Incorporation of bifidobacteria into cheeses: challenges and rewards, Int. Dairy J. 14 (2004) 375–387.

[30] WHO, World Food Programme, UN System Standing Committee on Nutrition, UNICEF, Community-based management of severe acute malnutrition [Internet] (2007). Available from: https://apps.who.int/iris/handle/10665/44295.

[31] World Health Organization, WHO recommendations on the management of diarrhea and pneumonia in HIV-infected infants and children [Internet], World Health Organization, Geneva (2010). Available from: https://www.who.int/publications/i/item/9789241548083.

[32] J. Coates, A. Swindale, P. Bilinsky, Household Food Insecurity Access Scale (HFIAS) for measurement of food access: indicator guide, Version 3 [Internet] (2007) th ed. FANTA Project. Available from: https://www.fantaproject.org/sites/default/files/resources/HFIAS_ENG_v3_Aug07.pdf.

[33] R.C. Robertson, A.R. Manges, B.B. Finlay, A.J. Prendergast, The human microbiome and child growth—first 1000 days and beyond, Trends Microbiol 27 (2019) 131–147.

[34] J.L. Castro-Mejia, S.O. Ferrall, Ł. Krych, E.O. Mahony, H. Namusoke, B. Lanyero, et al., Restitution of gut microbiota in Ugandan children administered with probiotics (*Lactobacillus rhamnosus* GG and *Bifidobacterium animalis* subsp. lactis BB-12) during treatment for severe acute malnutrition, Gut Microbes (2020) 1–13.

[35] The state of acute malnutrition. Where are we in treating children with SAM? [Internet], No Wasted Lives Coalition, London (2019). Available from: https://acutemalnutrition.org/en/countries.
[36] M. Shekar, J. Kakietek, J.D. Eberwein, D. Walters, An investment framework for nutrition: reaching the global targets for stunting, anemia, breastfeeding, and wasting [Internet], World Bank Group, Washington, DC (2017). Available from: https://www.worldbank.org/en/topic/nutrition/publication/aninvestment-framework-for-nutrition-reaching-the-global-targets-for-stunting-anemia-breastfeedingwasting.
[37] E. Tadesse, Y. Berhane, A. Hjern, P. Olsson, E.-C. Ekström, Perceptions of usage and unintended consequences of provision of ready-to-use therapeutic food for management of severe acute child malnutrition. A qualitative study in Southern Ethiopia, Health Policy Plan 30 (2015) 1334–1341.
[38] S. Isanaka, N.A. Menzies, J. Sayyad, M. Ayoola, R.F. Grais, S. Doyon, Cost analysis of the treatment of severe acute malnutrition in West Africa, Matern, Child Nutr 13 (2017), e12398.
[39] C. Cazes, K. Phelan, V. Hubert, H. Boubacar, L.I. Bozama, G.T. Sakubu, et al., Simplifying and optimising the management of uncomplicated acute malnutrition in children aged 6–59 months in the Democratic Republic of the Congo (OptiMA-DRC): a non-inferiority, randomized controlled trial, Lancet Glob. Health 10 (2022) e510–e520.
[40] M. Million, J.C. Lagier, D. Raoult, Meta-analysis on efficacy of amoxicillin in uncomplicated severe acute malnutrition, Microb Pathog 106 (2017) 76–77.
[41] Q. Guo, J.Z. Goldenberg, C. Humphrey, R. El Dib, B.C. Johnston, Probiotics for the prevention of pediatric antibiotic-associated diarrhea, Cochrane Database Syst. Rev. 4 (2019) CD004827.
[42] P.T. Edwards, P.C. Kashyap, G.A. Preidis, Microbiota on biotics: probiotics, prebiotics, and synbiotics to optimize growth and metabolism, Am. J. Physiol. Gastrointest. Liver Physiol. 319 (2020) G382–G390.
[43] J.L. Gehrig, S Venkatesh, H-W. Chang, M.C. Hibberd, V.L. Kung, J. Cheng, et al., Effects of microbiota-directed foods in gnotobiotic animals and undernourished children, Science 365 (6449) (2019) eaau4732.