Considerations for developing an intervention plan using probiotics to manage diarrhea in children from low and middle-income countries

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List of Abbreviations

CFU	Colony forming units
DALYs	Disability-adjusted life years
EE	Environmental enteropathy
ESPGHAN	European Society of Paediatric Gastroenterology, Hepatology and Nutrition
FAO	Food and Agriculture Organization
GDP	Gross domestic product
GRAS	Generally recognized as safe
L.	Lactobacillus
LMIC	Low and middle-income countries
ORL	Oral rehydration salts
RUTF	Ready-to-Use Therapeutic Foods
S.	Saccharomyces
UNICEF	United Nations Children's Fund
WASH	Water, Sanitation and Hygiene
WHO	World Health Organization

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Executive Summary

Diarrhea is a leading cause of malnutrition in children under five years old and is the second leading cause of death in this age group. The compelling scientific evidence from developed countries that has led to the inclusion of probiotics in the European guidelines is not enough to generalize to other settings. Since studies targeted to children in developing countries have thrown mixed results. This report aims to explain some of the discrepancies among trials by the assessment of the operational considerations for developing an intervention plan using probiotics to manage diarrhea in children in low and middle-income countries.

A total of twenty randomized controlled trials were included, of which fifteen reported some improvement in subjects that received probiotics compared to controls. The most common organism evaluated was *L. rhamnosus GG*, doses varied between 10^6-10^{12} colony forming units, most products needed to be dissolved before administration and treatment period varied between five days and one year. None of the trials included reported any adverse effects from the use of probiotics. The heterogenicity of the operational variables among trials highlights the need for the standardization of the methodology regarding strain, dose, duration of treatment and administration method.

The implementation of a program including both WASH improvement and probiotic supplementation requires further investigation since it has a possibly high impact at community level. The utilization of probiotics as a public health tool integrated response to benefit populations, which carry the highest burden of diarrhea could be key to interrupt the vicious diarrhea-malnutrition circle.

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I. Introduction

According to the World Health Organization, malnutrition contributes to nearly one-third of the infant mortality worldwide (1). The nutritional status of a population is influenced by a myriad of complex factors among which poor access to food, substandard sanitary conditions, a high incidence of diarrhea and communicable diseases play an important part.

Diarrhea is a leading cause of malnutrition in children under five years old and is the second cause of death in this age group. The situation is of particular concern in low-income countries where children experience on average three episodes of diarrhea every year in their first three years of life (2). Evidence has also shown that a higher burden of diarrhea adversely affects a child's nutritional status which is associated with stunted growth in early childhood and has a negative impact on further development (3).

Moreover, undernourished children have weakened immunity, underlying clinical conditions, and concomitant diseases; making them more susceptible to infections leading to severe and prolonged episodes of diarrhea (4). Another cause of child undernutrition is environmental enteropathy (EE), a subclinical disorder of the small intestine caused by the ingestion of fecal bacteria (5) prolonging the episodes of diarrhea and impairing proper intestinal barrier functions (6). This highlights the importance of developing safe, efficient and multifactorial intervention strategies to address malnutrition and enteric infections as major public health challenges.

In children, particularly those in vulnerable socio-economic environments, the risk of life-threatening malnutrition as a result of enteropathies is high (4). Improvements in socioeconomic development with corresponding increases in maternal education, falling fertility rates, and improved living conditions (with reduced crowding) are important contributors to reductions in child mortality. However, to reduce childhood diarrhea interventions are needed that directly lower disease transmission and severity, and promote access to life-saving treatment once a child becomes sick (7). Evidence suggests the importance of implementing nutrition sensitive interventions since even when scaling up to 90% coverage of the proven nutrition specific interventions there is only a reduction of 35% in diarrhea-specific mortality and 20% in stunting (8).

The WHO & UNICEF Action Plan has set ambitious goals about diarrhea in children under five years for the world to achieve by 2025: a 75% reduction in the incidence compared to 2010 levels and end all preventable childhood deaths due to this disease. It also aims for a 40% reduction in the global number of children under five who are stunted compared to 2010 levels (9).Therefore, it is of great interest to establish an intervention strategy aimed at decrease prevalence, illness duration, the probability of reinfection and it’s devastating effects.

The current recommendations for diarrhea treatment include replacement of fluid and electrolytes losses to prevent or reverse dehydration through the use of low concentration oral rehydration salts (ORS) and zinc supplementation. Nevertheless, supply issues are one of the challenges to scaling up the coverage of this treatment in some countries (9). Therefore,

strategies that focus on prevention seem more encouraging and dietary modulation could be a feasible alternative.

In recent years, an increasing body of evidence has shown that adding probiotics to the rehydration and treatment plan may be beneficial for pediatric patients, providing a viable avenue for disease management with low associated risk (10,11). FAO & WHO define probiotics as "Live microorganisms which, when administered in adequate amounts, confer a health benefit on the host"(12). A Cochrane review analyzed 63 studies (56 pediatric trials) including 8,014 participants and concluded that the average effect of probiotics was a decrease of the mean duration of diarrhea by 24.76 h (10). The positive effects on prevention and treatment of diarrhea in children could be attributable to the capacity of probiotics to improve the indigenous microflora properties, hamper the growth of diarrheal pathogens, and boost immune response in patients (13). However, efficacy and safety from a probiotic should not be extrapolated to others since effects are strain-specific (11).

The European Society of Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) recognizes the benefit of some strains and recommends their use in previously healthy children (excludes children with chronic disorders or immunodeficiency) (14). Nevertheless, the WHO does not (yet) include the use of probiotics in their recommendations. But then again, studies targeted to children in low and middle-income countries (LMIC) have thrown mixed results (1,15), few trials have been developed in community settings and there is insufficient evidence to extrapolate for global recommendations. However, the choice of probiotic strain, treatment duration, age group, and other operational variables could eventually explain some of the discrepancies among trials.

Even if the effects of probiotics are modest at an individual level, it could mean large beneficial effects in a community-based approach (16) when combined with education programs and access to better water, sanitation, and hygiene (WASH). Therefore, an accurate intervention designed for settings in LMICs could have a significant impact on the health systems and diarrhea-associated costs. However, little consensus exists about approaches to scale up coverage and about delivery strategies to reduce disparities and provide equitable access to marginalized populations (7).

The utilization of probiotics as a public health tool integrated response to benefit populations, which carry the highest burden of diarrhea could be key to interrupt the vicious diarrhea-malnutrition circle. This project aims to analyze if probiotics could be a viable intervention to address diarrhea in children from LMICs by evaluating the operational variables that could influence the outcomes of the trials performed.

II. Objectives

Overall

Assess the operational considerations for developing an intervention plan using probiotics to manage diarrhea in children from low and middle-income countries.

Specific Objectives

- Review the available evidence regarding the efficacy of probiotics as a measure to lower the incidence of diarrhea in children in LMIC.
- Analyze possible operational considerations that could involve an intervention using probiotics (safety concerns, administration method, strain and dose of probiotics, quality assurance methods, cost-effectiveness, acceptability, monitoring, and evaluation).
- Identify gaps in the research that would provide further evidence required to develop effective interventions.

III. Methodology

A literature review was performed to gather the existing evidence regarding the efficacy of probiotics in diarrhea treatment. To identify potentially relevant studies to be included, the electronic databases MEDLINE and Cochrane Library were searched. The keywords of interest were “probiotics”, “enteropathy” “diarrhoea” and “diarrhea”; in different combinations. Only studies from peer-reviewed journals were included. Publications in languages other than English and Spanish were excluded.

To be eligible for inclusion, a study must be a randomized controlled trial of any duration published between the year 2000 and 30 April 2017 performed in humans. Articles comparing strains of probiotics, single or combined, administered in any form, in children under five years of age were considered. The trials were not restricted to a certain strain; however, interventions where specific probiotic organisms were not identified were not considered. The outcomes of interest were mean diarrhea duration, effects on disease symptoms, stool frequency and adverse reactions to treatment. In order to be included, the study had to report results in at least one of the outcomes of interest.

Countries were categorized according to the World Bank classification (17) and only studies performed in low and middle-income countries were included. Records of excluded articles in this stage were saved for future reference. Search was not constrained to a specific diarrhea etiology since is not usually investigated before an intervention in clinical or public health practice. An additional search was performed within bibliographies of reviews and trials to detect other articles fulfilling the above criteria.

From the studies that met the described criteria the following information was extracted: study characteristics (design, duration of treatment, sample size and country), patient characteristics (age, and nutrition status, outcome results) and operational considerations (product safety, administration method, strain, dose, supply chain management, cost effectiveness analysis and acceptability).

A comparison of operational variables between different studies was performed in order to develop a series of recommendations for conducting an intervention of this nature, as well as to identify possible research gaps that could contribute to support the feasibility of the treatment of enteropathies with the use of probiotics.

Grey literature was considered for project reports, statistics, guidelines, recommendations from international organisms and technical reports to support findings and formulation of recommendations.

IV. Results and Discussion

The electronic literature search generated 471 registries, 73 were identified as potentially relevant studies from which a total of fifteen papers met the inclusion criteria and qualified for analysis. The exclusion of trials was mainly due to irrelevant location or age of the population (n= 45) and due to being published in a language other than English or Spanish (n= 5). Eight trials could not be retrieved. A list of the excluded trials is included in Annex 1.

Five additional trials were retrieved from the bibliographies of reviews and trials. A total of twenty randomized controlled trials were included. Most included studies were double blinded, placebo controlled and published in English, with the exception of one open labeled (18). The ages of the children enrolled in the trials ranged from birth to five years and with different nutritional status. Trials were performed in low and middle-income countries according to the World Bank classification (17), being India de most common setting (n=11). Table I summarizes the characteristics of the included trials and each intervention is described in Table II. No study specifically identified any of the participants as suffering from antibiotic related or travelers' diarrhea and just one included EE in their outcome of measure.

1. Scientific evidence regarding the use of probiotics

A meta-analysis performed in 2006, showed probiotics to significantly reduce antibiotic-associated diarrhea by 52%, the risk of travelers' diarrhea by 8% and acute diarrhea of diverse causes by 34%. But then again, this study identified only one trial performed in a developing country, which makes results unsuitable for extrapolation (13). The increased interest to understand the role of probiotics at a global level is evident considering the amount of publication in LMIC that have been published since then.

Fifteen of the trials retrieved showed some improvement in subjects that received probiotics compared with controls in mean diarrhea duration (18–29) and average number of stools (18–24). Five trials reported a decrease in hospital stay (19,21,23,24,29); while others failed to see any change in this matter (18,25,26). Nevertheless, this last indicator might not be directly associated with intervention results since in some cases caregivers could have influenced the discharge date if they feared their child would need to be readmitted to hospital and lived far away from the facility (26).

Five studies failed to find any statistical difference of improvement in the intervention group (15,30–33). For most of these, operational variables could have influenced the result and will be discussed later on.

The beneficial effects of probiotics could be dependent on the patient's health status. Sarker *et al.* attributed their negative results to the subjects severe illness compared to other similar studies (27). In a study performed in Uganda, the difference in outcome between inpatient (no significant change) and outpatient (reduction of mean diarrhea duration by 2.2 days) could also be explained by the compromised ability to respond to treatment by more critically ill patients

(34). These differences could suggest that the severity of illness in the patients could be a good predictor of efficacy of the probiotic during an intervention (27). Galpin *et al*. also suggested to give the probiotics before the onset of EE to children under one year of age, since it might prevent the development of the condition and their trial showed no effect in the treatment outcome (15).

The variation in the effectiveness of treatment and the broad spectrum of illness severity within a population could be key variables to consider probiotics as a preventive measure over a therapeutic treatment. A trial performed by Villarruel *et al.* reported significantly less number of stools among the patients receiving the probiotic within the first 48 hours of the onset of diarrhea than those who were administered the product later (35). Moreover, the time needed by probiotics for multiplication and colonization could be also related to the patient's recovery. Basu *et al.* reported a significant reduction in the duration and frequency of diarrhea only after the fourth day of therapy (23). Further supporting the need for probiotic treatment initiation at early stages of the disease for improved efficacy (36).

Unlike the hospital care settings, the community-based studies have shown a consistent favorable trend on the use of probiotics in the prevention of diarrhea (38,39). Sur *et al.* documented the occurrence of first episodes of diarrhea and found a protective effect of probiotics of 14% after 12 weeks of supplementation (38). Congruent with a preview study performed in Peruvian children that showed 13% fewer diarrheal episodes after a 15 months intervention(40). While Sazawal *et al.* reported a 10% lower rate of diarrhea episodes during one year in children older than 24 months. But did not find a statistically significant reduction in the children aged 12 to 24 months (39).

This difference in age group could be attributed to different flora composition or a mature cellular immune system (shifting from extended Th2 at birth to having more Th1 by the second year). That is why Sazawal *et al*. hypothesized that the effect of probiotics could be limited in infants due to intrinsic limitations to produce interferon and other Th1 interleukins (39).

A meta-analysis that compared the efficacy of probiotics in preventing acute diarrhea according to age group, observed an effect size significantly higher among children when compared with adults, the overall reduction was 57% and 26% respectively (13). While Ritchie and Romanuk failed to report any significant difference when analyzing impact in several gastrointestinal diseases (41). The analysis of the age-stratified outcomes of the studies could help determine the most effective time to start an intervention with probiotics but most trials do not publish these data.

The negative results in the Hegar *et al.* study were attributed to the possible minimization of the beneficial effects of probiotics in conjunction to the zinc supplementation(30). But yet again, probiotics were effective in other trials that also included this mineral (18,25,28,39). To understand the synergetic effect of these treatments could be key given the potential of both ingredients in diarrhea prevention.

Some reviews have hypothesized that the different outcomes among studies performed in developed and developing countries could be attributed to the prevalence of malnutrition

among the participants (31,42). The understanding of this interaction would contribute to make an effective intervention since both malnutrition and diarrhea are closely linked. Malnutrition predisposes children to a greater incidence of diarrhea and diarrhea exacerbates nutritional vulnerability (6).

Nevertheless, the nutritional status is difficult to compare between studies since different classifications are used for diagnosis (Table II). The use of standardized WHO classification should be encouraged in trial proposals to assess the relationship of malnutrition and the efficacy of probiotics. Sazawal *et al*, performed a stratified analysis according to the nutritional status and a significant protective effect of probiotics among malnourished children were reported (OR= 0.88; p: 0.002) but failed to document this in non-malnourished children (OR= 1.12, p: 0.08). Other studies have also associated the effectiveness of probiotics to the lower nutritional status of the participants (43) or reported favorable outcomes even when 80% of the population was malnourished (23). Suggesting that malnutrition may not be a barrier for probiotic effectiveness, but rather could be considered favorable.

Regarding the change in anthropometric measures, even though the impact of diarrheal episodes on weight and height has been clearly documented (6), most of the trials included failed to see any difference during the intervention period. Just one study described a higher weight gain (g/kg body weight per day)in inpatients with probiotic therapy but not in the overall intervention (34). While others trials did not report a difference between intervention and control group (15,24,25,28,31,34,38,39).

Table I. Accepted studies characteristics, arranged by publication date

Country	Design	Participants age	Sample size	Participants Nutritional Status	Reference
Malawi	Randomized, double-blind, placebo-controlled trial	3 - 5 years	161	Excluded children with severe acute malnutrition	**Galpin *et al.*** (15)
Bangladesh	Randomized, double-blind, placebo-controlled trial	4 - 24 months	230	Well-nourished or moderate malnutrition[1]	**Sarker *et al.*** (27)
Indonesia	Randomized, double-blind, placebo- controlled trial	< 1 year	58	Well-nourished[2]	**Agustina *et al.***(25)
India	Randomized, double-blind, placebo-controlled trial	4.1 ± 1.8 years	235	90% were malnourished[3]	**Basu et al** (23)
India	Randomized, double-blind, controlled trial	< 2 years	662	69.20% malnourished[3]	**Basu *et al.* a** (32)
India	Randomized, double-blind, placebo-controlled trial	6 mo – 2 years	224	Well-nourished or moderate malnutrition [4]	**Dubey et al** (22)
India	Randomized, double-blind, placebo-controlled trial	3 mo- 3 years	80	Not reported	**Narayanappa *et al.*** (20)
India	Randomized, double-blind, placebo-controlled trial	< 3 years	559	60.11% were malnourished[3]	**Basu et al** (21)
India	Randomized, double-blind, placebo-controlled trial	<36 months	210	Moderately malnourished (criteria not detailed)	**Misra *et al.*** (31)
India	Randomized, double-blind, community-based trial	1–3 years	624	32% Normal. 16%Wasted and Stunted. 5% Wasted. 47% Stunted. [5]	**Sazawal *et al.*** (39)
Bolivia	Randomized, double-blind placebo- controlled trial	1 - 23 months	64	Well-nourished or moderate malnutrition[6]	**Grandy *et al.*** (26)
India	Randomized, double-blind, placebo- controlled trial	1–5 years	3758	23.5% normal. 31.3% mild undernourished. 31.3% moderate undernourished. 13.1% severe undernourished [7]	**Sur *et al.*** (38)
India	Randomized, double-blind, placebo-controlled trial	6 - 24 months	148	Excluded severe malnourished (criteria not detailed)	**Dutta *et al.*** (33)
Egypt	Randomized, double-blind, placebo-controlled trial	3 - 59 months	108	Well-nourished or moderate malnutrition [8]	**Riaz *et al.*** (28)
India	Open-label randomized controlled trial	6 mo to 5 years	200	Well-nourished or moderate malnutrition [6]	**Aggarwal *et al.*** (18)
Indonesia	Randomized, double-blind, placebo- controlled trial	6 – 36 months	112	Well-nourished [9]	**Hegar *et al.*** (30)
Egypt	Randomized, double-blind, clinical trial	1 - 23 months	50	Well-nourished or moderate malnutrition [6]	**Abou *et al.*** (19)
Egypt	Randomized control follow-up clinical trial	< 2 years	65	Not reported	**El-Gendy *et al.*** (24)
Uganda	Randomized, double-blind, placebo-controlled trial	6 - 59 months	400	Severe Acute Malnutrition[10]	**Grenov *et al.***(34)
India	Randomized, double-blind, placebo-controlled trial	3 mo - 5 years	60	Well-nourished or moderate malnutrition [6]	**Das *et al.*** (29)

[1] ≥65% weight for age by the standard of the National Centre for Health Statistics of Bangladesh.

[2] Weight for height Z-score of more than -2 SD based on the WHO - U.S. National Center for Health Statistic (NCHS)

[3] Body weight less than 80% of the expected weight for that age according to Indian Academy of Pediatrics classification

[4] Body weight higher than 60% of standard according to National Centre for Health Statistics (NCHS)

[5] Stunting: height for age <−2 Z-scores. Wasting: weight for height<−2 Z-scores. Underweight: weight for age <−2 Z-scores

[6] Weight for Height at or higher than -3SD according to WHO growth chart

[7] Weight-for-age Z score. Normal (≥-1) Mild undernourishment (≥-2&<-1) Moderate undernourishment (≥-3&<-2) Severe undernourishment (<-3).

[8] Weight for age higher than 60% and weight for height higher than 70% not specified parameters.

[9] Weight >P3 no classification criteria mentioned

[10] Mid-upper-arm-circumference <11.5 cm or weight-for-height/weight-for-Length z score ≤3 or bipedal pitting edema

Table II. Intervention characteristics of accepted studies arranged by publication date

Treatment	Outcome	Reference
Capsules containing *L. rhamnosus GG* 5^{10} CFU twice a day for 30 days. Content was sprinkled on maize porridge at ambient temperature.	No significant difference in diarrhea duration 0.4 ± 1.42 vs 0.5 ± 1.6 (p=0.67)	**Galpin *et al.*** (15)
*L. paracasei ST1*1 5 × 10^9 CFU Powder mixed with ORS twice daily for 5 days	Undifferentiated diarrhea: no difference in duration 123.8 ± 46.0 vs 126.1 ± 44.8 (p=0.71) Rotavirus-infected cases: no difference in duration 127.1 ± 43.4 vs 126.5 ± 37.0 (p=0.94) Non-rotavirus cases: reduction in stool frequency 27.9 ± 17.0 vs 42.5 ± 26.0 (p=0.02) and a higher proportion of cases resolve diarrhea within 6 days 76% vs 49% (p=.03)	**Sarker *et al.*** (27)
Low lactose infant formula with *L. rhamnosus LMG P-22799* 5 x 10^8 CFU, prebiotic (inulin) and dietary fiber (soya polysaccharides) and micronutrients (zinc and iron) supplement. Given for 7 days maximum	Shorten diarrhea duration by one day, 1.63 vs 2.45 days (p<0.05) No difference in length of hospital stay 3.19 ± 1.62 vs 3.70 ± 1.43 (p=0.12)	**Agustina *et al.*** (25)
Powder containing *L. rhamnosus GG* 60 x10^6 CFU dissolved in 100 mL of ORS twice a day for 7 days	Reduction in the stools frequency after the fourth day, duration of diarrhea 5.3±2.1 vs 9.2±2.8 (p<0.05) and hospital stay 7.3±1.6 vs 15.5±1.5 (p<0.05)	**Basu *et al*. 2007a** (23)
Powder containing *L. rhamnosus GG* 60 x10^6 CFU dissolved in 100 mL of ORS twice a day for 7 days	No significant difference in diarrhea duration (6.8 ± 2.1 vs. 6.6 ± 2.3 days) or mean hospital stay (9.3 ± 1.3 vs. 9.2 ± 1.3 days)	**Basu *et al.* 2007b** (32)
4 strains of Lactobacilli (*L. acidophilus, L paracasei, L. bulgaricus, L. plantarum*), 3 strains of Bifidobacteria (*Bifidobacterium breve, Bifidobacterium infantis, Bifidobacterium longum*), 1 strain of *Streptococcus thermophiles*. Sachet with of 90 billion bacteria mixed with breast milk, formula milk, oral rehydration salts, or water, administered daily for 4 days.	Reduction in duration and stool frequency. Overall recovery rates were significantly better 101 vs 44 (p<0.001)	**Dubey *et al.*** (22)
Sachets with *Lacto Bacillus Sporegens*-50 million and prebiotics (*Strep Faeecalis, Clostridium Butyricum, Bacillus Mesentericus*) reconstituted with 20ml of water, three times a day for 14 days	Reduction of around one day in mean duration of diarrhea 4.35 ± 1.252 vs 5.45 ± 1.694 (p=0.001) and reduction in daily stool frequency	**Narayanappa *et al.*** (20)
Powder containing *L. rhamnosus GG* either x10^{10} or 10x^{12} CFU dissolved in 100 mL of ORS twice a day for minimum 7 days	Frequency, duration of diarrhea 7.23±1.27 vs 5.02±1.32 vs 5.12±1.16 (p=0.00) and hospital stay 9.75±2.06 vs 6.21±1.24 vs 6.24±1.07 (p=0.00) were significantly lower in both the intervention groups compared with the controls. There was no significant difference between the two intervention groups.	**Basu *et al.* 2009** (21)
Capsules to be opened and given with liquid or semi-solid foods with *L. rhammosus GG* 1x10^9 CFU for 10 days	No significant difference between groups, stool frequency on day 3 (p=0.95), day 6 (p=0.53), day 10 (p=0.93), duration of diarrhea (p=0.2), relative risk of diarrhea on day 3 (p=0.63) and day after day 3 (p=0.11)	**Misra *et al.*** (31)
Reconstituted fortified milk powder with *Bifidobacterium lactis HN019* 1.9x10^7 CFU and 2.4 g/day of prebiotic oligosaccharide for 1 year	Prevalence of severe illness was 16% lower (95% CI: 5 to 26%; p=0.004), 6% reduction diarrhea rate (95% CI: −1 to 12%; p=0.08) Children aged 12 to 24 months: no significant difference diarrhea rate (OR 0.99; 95% CI: 0.89 to 1.11, p=0.91) Children aged >24 months: 10% lower diarrhea rate (OR 0.90; 95% CI: 00.83 to 0.98, p=0.02) Fewer diarrhea episodes among malnourished children compared with controls (OR 0.88; 95% CI: 0.81 to 0.96, p=0.002). No difference among non-malnourished children.	**Sazawal *et al.*** (39)

Treatment	Outcome	Reference
Probiotics dissolved in 20 ml of water given for 5 days twice daily: *GB group: S. boulardii* 4 x10^{10} CFU *GARLB group: L. acidophilus* 6.625 x$10^{7,}$ CFU *L. rhamnosus* 3.625x10^{7} CFU, *Bifidobacterium longum* 8.75x10^{6} CFU, *S. boulardii* 1.375 x10^{7} CFU	GB group 31.4% decreased in mean diarrhea duration compared with controls 58 (41) vs 84.5 (94) (p=0.04) GARLB group no statistical difference compared with controls 60 (40) vs 84.5 (94) (p=0.06) There was no effect on duration of hospitalization (p = 0.31) GB and GARLB merged: 24-hour reduction in mean diarrhea duration compared with controls 84.5(94) vs 60(40.5) (p = 0.025)	**Grandy *et al.*** (26)
Probiotic drink with *L. casei Shirota* 6.5 × 10^{9} CFU daily for 12 weeks and 12 weeks' follow-up	Protective efficacy for diarrhea prevention was 14% (95% CI: 4–23, p<0.01)	**Sur *et al.*** (38)
Two tablets contained 60 x10^{6} CFU *L. sporogens* (B. coagulans) dispersed in 15 ml of water two times a day until recovery or for 5 days (240 million spores daily)	No statistically significant difference was found in stool frequency 10.8 ± 8.3 vs 14.1 ± 10.3 (p = 0.05), nor diarrhea duration 34.0 ± 20.4 vs 36.5 ± 21.4 (p = 0.5)	**Dutta *et al*** (33)
S. boullardi in packets of 250mg mixed with puffed rice powder twice daily for 5 days	No statistically significant difference was found in stool frequency Duration of post-intervention diarrhea was shorter 52.08±24.57 h vs 64.04±30.43 h (95% CI=−28.13 to −5.43, p=0.031) Shorter time of appearance of the first semi formed stool 39.48±23.09 h vs 54.13±28.21 h (95% CI −25.4 to −3.87, p=0.008)	**Riaz *et al.*** (28)
Capsules of *L. casei GG* 10 x10^{9} CFU daily for five days dissolved in milk	Reduction of median duration of diarrhea by 18 hours [60 (54-72) h vs. 78 (72-90) h; p<0.001] Faster improvement in stool consistency [36 (30-36) h vs. 42 (36-48) h; p<0.001] Reduction in average number of stools, difference of 1.91 per day No significant reduction of hospital stay [95% CI: -0.97 to 25.26]	**Aggarwal *et al.*** (18)
Capsules with *L. rhamnosus R0011* 1.9×10^{9} CFU and *L. acidophilus R0052* 0.1×10^{9} CFU daily diluted in a tablespoon of water for 7 days.	No statistical difference in the daily stool frequency 5.0 vs. 5.5 (p=0.795) or median diarrhea duration 68.5 vs 61.5 (p=0.596)	**Hegar *et al.*** (30)
Milk formula with *Bifidobacterium lactis* 14.5 x 10^{6} CFU/100 ml daily for 7 days	Reduction of mean diarrhea duration 3.12 ± 0.92 vs 4.10 ± 0.94 (p = 0.02) Reduction of stool frequency 3.96 ± 0.62 vs. 4.46 ± 0.85 (p = 0.04). Shorter hospital stay: 32% discharged before 2 days vs 44% (p = 0.04)	**Abou El-Soud *et al.*** (19)
One sachet twice daily of 10x10^{9} CFU lyophilized heat-killed *L. acidophilus* added to water or yogurt. Given together with a prebiotic formula containing galactooligosaccharides. Duration was variable: from admission until discharge	Reduction of diarrhea duration 4.5 ± 1.4 vs 6.1 ± 1.4 (p= 001) Reduction of stool frequency 1.32 ± 0.61 vs 2.63 ± 1.25 (p=0.001) Shorter hospital stay 5.5 ± 1.9 vs 7.0 ± 1.8 (p=0.01)	**El-Gendy *et al.*** (24)
1g powder Sachet of maltodextrin with *Bifidobacterium animalis subsp lactis* and *L. rhamnosus GG* (10 x 10^{9}CFU, 50:50) administrated between 8 to 12 weeks	No difference in duration of hospital stay +0.1 [95% CI: -1.7 to 1.9, p=0.93] No difference in diarrhea duration in inpatient treatment +0.2 days [95% CI: -0.8 to 1.2, p=0.69], but a reduction during outpatient treatment -2.2 days [95% CI: -3.5 to -0.3, p=0.025] The proportion of patients with diarrhea for 20 or more days was reduced	**Grenov *et al.*** (34)
Sachets of *S. boulardii* 500mg/day mixed with 15 ml of normal drinking water for 5 days	Shorter median diarrhea duration by about 29 h [95% CI: -41.2 to -16.8] Hospital stay reduced by about 17 hours [95% CI: -33.46 to -0.54]	**Das *et al.*** (29)

2. Operational Considerations

2.1 Product safety concerns

Probiotics are considered "generally recognized as safe" (GRAS) products (44) and ESPGHAN has only advised against the use of one strain (*Enterococcus faecium SF68*)in children due to safety concerns from *in vivo* data (14).

In accordance with the substantial body of evidence that has demonstrated the good safety profile of probiotics (36), none of the trials included reported any adverse effects from the use of probiotics. Three studies presented severe medical complications (respiratory failure, severe pneumonia, shock, dehydration, and septicemia) but there were no differences between intervention and control groups that could allocate these outcomes to the probiotic intake (21,32,34). Grenov *et al.* and El-Gendy *et al.* reported forty-six and two deaths respectively among the intervention groups, but all related to complications not attributable to the use of probiotics (24,34).

This suggests that probiotics could be considered as part of a public health intervention in LMIC that include children with different baseline characteristics. There are some theoretical adverse risks (for example; transmigration impact on gastrointestinal physiology, adverse immunologic effect and the potential for antibiotic resistance transfer within the gastrointestinal tract) that should be considered especially in vulnerable populations (45). But few cases have been documented and the inherent health risks are poorly understood. For example, the risk of Lactobacillus infection is estimated at about one case per 10 million people over a century of probiotic consumption in France and the risk of lactobacillemia at less than one case per million individuals (46).

Which is why, even though probiotics have been reported safe in many clinical trials, interventions should include proven strains and doses, consider the method of administration, assure quality in the consumer´s hand and implement an active surveillance system to identify possible adverse effects (45,46).

2.2 Strain and dosage

Scientific evidence points to the fact that the beneficial effects of probiotics are strain dependent and dose-dependent(10,36,47). Yet again, the inconsistencies among outcomes in the included trials are not surprising since trials methodologies include probiotics that have not been recommended by experts or there is still insufficient evidence for their use.

Currently, only *L. rhamnosus GG* and *S. boulardii* have a strong recommendation in the management of children with acute gastroenteritis in addition to rehydration therapy (11,48). Seven trials included one of these probiotics (15,21,23,28,29,31,32), being *L. rhamnosus GG* the most common organism evaluated. Doses among studies varied between 10^6-10^{12} CFU and duration of administration between five days and one year.

The two studies including *S. boulardii* reported a positive outcome in the intervention group (28,29), although Riaz *et al*, described a decrease in diarrhea duration only in the post-

intervention phase. The beneficial effect of *S. boulardii is* consistent with a meta-analysis performed without setting restriction (49).

Regarding the studies using *L. rhamnosus GG,* dosage seems to be key to determine the outcome. The optimal dose of the probiotic has not been verified with pharmacokinetic studies (31), but the threshold dose found to be most effective in a meta-analysis was 10 billion colony forming units per day (50).

Basu *et al.* hypothesized that the lack of effectiveness of their intervention could have been due the low dose of 60 $x10^6$ CFU used. But yet, is not possible to assume that a higher dose could have improved colonization and showed improvements in diarrhea outcome (32). And it does not explain why another paper by the same author analyzing children persistent diarrhea, using that dose, did report an improvement in duration of diarrhea and stool frequency (23). A study performed in the same country by Misra *et al* failed to report any improvement using $1x10^9$ CFU (31); while other trial using a higher dose (10^{10} and 10^{12}CFU) was effective to decrease by two days the average diarrhea duration and by three days the hospital stay (21). That is why is fair to consider that other operational variable could have influenced the inconsistencies among studies using the same strain but they are not detailed in the papers.

The studies that used different strains of *L. rhamnosus* also incorporated other products to the treatment. Hegar *et al.* combined *L. rhamnosus ROO11* with *L. acidophilus ROO52*. This last one, experts sustain that there is insufficient evidence to make a recommendation yet (48) and consequently failed to see any improvement in the intervention group (30). While, Agustina *et al.* combined *L. rhamnosus LMG P-22799* (5 x 10^8 CFU) with the prebiotic inulin, soy polysaccharides as dietary fiber and micronutrients, under the concept that could provide substrate growth and establish water binding capacity and fecal mass in the colon. Nevertheless, it is not clear if the positive results (shorten in diarrhea duration by one day and a significantly better recovery in the intervention group) can be attributable to a single ingredient or to the synergy between them (25).

The other 13 studies used probiotic strains with not yet sufficient evidence to make a recommendation (48). Positive outcomes were reported using the species *L. casei* (18,38), *Bifidobacterium lactis* (19,39), *L. acidophilus* (24) and *L. paracasei* (27). While, *L. sporenes* at 240 million spores daily did not show any statistical difference (33).

The findings of studies using a combination of probiotics are even more difficult to generalize. Grandy *et al.* compared the use of a single probiotic *(S. boulardii*) against a group receiving a combination of them. Even though the mean diarrhea duration was reduced compared to oral rehydration solution alone, the decrease was significant only for the single species group. Nevertheless, the dosage of each of the probiotics present in the group receiving a combination of strains could have been insufficient, since the individual contribution is below the amount described as effective (26). Grenov *et al.* also failed to report any benefit when combining *Bifidobacterium animalis subsplactis* and *L. rhamnosus GG* (34). While Dubey by the incorporation of eight different strains reported a reduction in the duration of diarrhea, stool frequency, and overall better recovery rates (22). The effectiveness of a single strain vs multiple strains has yet to be determined.

It is unclear which strain should be recommended to obtain the best results. Due to the heterogenicity of the trials performed in LMIC, it is not possible to attribute differences in outcome merely to strain selection. Besides, studies analyzing the dose-response effect of probiotics in human intervention are inconsistent and discordant. There has been convincing data on dose dependence relationship observed in antibiotic-associated diarrhea, but results cannot be extrapolated to other etiologies (51).

In addition, the effect of only one dose or partial ingestions should be further researched. It is hypothesized that the administration of a single dose could result in a better gut colonization instead of using several doses throughout the day. This could explain the negative results of the randomized controlled trial performed in Malawi that assessed the effect of probiotics for severe acute malnutrition (PRONUT study). In spite of efforts to perform regular quality checks to assure the correct CFU in each package, patients ingested them in divided amounts spread throughout the day (52).

Nevertheless, since there is sufficient body of evidence incentivizing the use of *L. rhamnosus GG* and *S. boulardii* and a tendency for preference of 10^9 or 10^{10} dosage administrated at once, future studies should consider using these parameters and focus on optimizing other operational variables that could influence the intervention.

2.3 Administration Method

Besides strain and dose, the choice of administration method of the probiotics could play an essential role in the intervention outcome since it influences acceptance and stability of the products. Sur *et al.* recognized the administration method as part of the exclusion criteria of the study since children younger than one year would have difficulty in swallowing the 65ml drink proposed at one time (38). In this sense, administration methods that contain smaller volumes could help cover a bigger sample of the population. Most of the studies used products that needed to be dissolved before administration (15,18,20–24,26,27,29–34,39) while the rest describe a ready to use drink (19,38) or formula (25).

Interventions that delivered drinks could be challenging for the supply chain management, in terms of volume, safety and storage issues at large scale and household level. These concerns are highlighted in community-based interventions or outpatient management. The use of capsules, tablets or sachets could make handling and distribution easier than ready to consume products, but at the same time could generate new threats since quality water supply in developing countries tends to be an issue. Studies to assess the probiotic viability in different levels of water quality should be considered. Yet again, integrating them to food could help the incorporation of probiotics into a public health strategy without being seen as a medicine.

Misra *et al.* and Galpin *et al*. approach to incorporate the capsule content to semi-solid foods (15,31) might facilitate the consumption across the different age groups and increase acceptability. Plus it does not rely on the availability of a special separate ingredient to dissolve the capsule, like in the case of Aggarwal *et al* trial and the addition of milk(18). But then again,

food composition (fat content, type of proteins, sugar, and pH) could affect probiotic growth and survival in food (53).

For this reason, a promising approach could be the inclusion of the active ingredients to ready-to-use therapeutic food (RUTF) were the composition can be monitored, good manufacturing practices assured and shelf-life conditions established. However, a trial performed in Malawi with acute malnourished children failed to see any difference in the group receiving RUTF plus a combination of probiotics. One barrier with this administration method could be the suboptimal consumption caused by sharing of the product, a possibility that cannot be ruled out from this study. And also, the fragmented ingestion of dosage during the day as discussed before (52). In this sense, choosing a product that allows the consumption of the probiotics in a single dose could improve the acceptability and adherence to treatment.

2.4 Supply management (quality assurance)

The supply management for the use of probiotics should focus in assuring that the products maintain their viable cell count since it could determine the outcome. Few of the studies included mentioned something about the supply management of the probiotic during the trial. Maintenance of the cold chain was the most mentioned matter (27,30,31,38). Hegar *et al.* acknowledge the challenge to transport and store the products at the suggested temperature by the label (4 °C). Nevertheless, they did not attribute their negative results to improper management since the strains used have proven to not change efficacy after stored for one month at 28–32 °C. Hence, storage of the probiotics in the patient's home for seven days most likely did not decrease the viability of the bacteria in the capsule (30).

To assure that the temperature of the product is safe through the intervention could be especially challenging in tropical countries. And measures taken in certain studies seem unlikely to be feasible in a setting outside a clinical trial. For example, Sur *et al* for their study community health workers delivered the probiotic drink daily to the community in carrier boxes with ice packs. And were all stored at 4–10 °C in monitored cold rooms (38).

A study analyzed the survival trends of *L. rhamnosus GG* inoculated in peanut butter under refrigeration (4 °C), ambient (25 °C), and abusive (37 °C) storage conditions. The rate of probiotic survival was inversely related to storage temperature and storage time. The initial dose of probiotic used was 10^7 CFU/g and after the intervention counts reported to be at least 10^6 CFU/g in products stored at 4 °C for 48 weeks and at 25°C for 27 weeks (53). Which suggests that a higher level of probiotics needs to be inoculated in the initial formulation for the final product to contain the required dosages. Therefore, community-based interventions should consider performing similar tests to account for the possible loss of CFU according to the setting and storage conditions in LMIC.

Performing quality checks, as part of an intervention protocol, could be key to control the supply chain, as performed by Sarker *et al.* At the end of the study, they analyzed the leftover sachets to assure that the viable bacterial count was the same that at the beginning (27). However, it could be useful to perform this analysis in different stages of the intervention in a

scheduled matter as part of the monitoring process to understand how probiotic strains respond to all conditions that it may be exposed during a clinical investigation. None of the other included studies had evidence to demonstrate that the product was stable for the duration of the trial.

Interventions should incorporate probiotics that are easy to prepare, with a reliable production process, a long shelf life over a wide range of temperatures and resistant to high moisture. For procurement of the product, validated shipping containers and reliable distribution companies should be used to assure the quality is maintained in all stages of the supply chain. Random testing of samples in storage units and at the study participants household would provide confidence that the product was held appropriately.

2.5 Cost-effectiveness analysis

To our knowledge, there is no published study that describes the cost effectiveness of an intervention using probiotics in LMIC–to prevent or treat diarrhea cases. And very few have been performed in other settings using children and ORS therapy as a reference.

A study performed in the ambulatory care in Belgium assessed the cost-effectiveness of using *S. boulardii* together with conventional treatment (oral rehydration therapy and intravenous fluids). For the children receiving probiotics, the duration of diarrhea was reduced by one day and the need of additional medication during the treatment was significantly lower compared with placebo. Hence, even though the treatment with probiotics increased the initial cost of the intervention when add-on medication and extra consultations were taken into account, it resulted in a reduction of 25% in health care cost (54).

Another study performed in day school children in Mexico showed similar results. By daily administration of *L. reuteri DSM 17938* for three months there was a reduction in the frequency and duration of diarrheal episodes. Plus, it showed a significant reduction in secondary costs since the number of doctor visits, antibiotic use, absenteeism from day school and parental absenteeism from work were significantly reduced (55).

However, these encouraging findings cannot be extrapolated to other settings. Since costs and diarrhea prevalence would vary across places. A cost-effectiveness analysis should be carried at a country or sub-regional level to assist policymakers to reach decisions based on a local analysis.

The analysis should consider using as reference the recommended WHO guidelines, which includes continual feeding of the child during the episode and maintaining hydration with ORS and compare it with that therapy plus probiotics. A second analysis should be carried using as control group ORS plus zinc. The interpretation of the results should be done with the WHO threshold recommendation, which establishes that an intervention can be considered cost-effective if each DALY avoided, costs less than three times the per-capita GDP (56).

The outcome could be measured by disability-adjusted life years (DALYs) and for costs should consider intervention activity (e.g. administration, planning, supervision), supplies (probiotic

and supply chain costs), human resources (hours invested in treatment by health professionals and costs related to training) and other indirect costs.

But for this study to be performed, it is required to generate stronger evidence regarding the optimal administration method, type of probiotic and dosage of strain needed. And according to the willingness to pay determine the cost effectiveness of the use of probiotics.

2.6 Acceptability

None of the studies included reported any problem with acceptability of the therapy nor significant dropouts in the intervention group. These finding contrast with a randomized controlled trial undertaken in childcare centers in Australia called the CUPDAY study. This study included 496 children and reported a 20% reduction in the number of days lost due to diarrheal disease in the intervention group. The children received a milk product containing both probiotics and prebiotics over a period of 5 months. Yet, more than one-third dropped out of the study in the first week, being the most common reason the dislike of the milk product among the infants. Nevertheless, it was mentioned by the mothers, that this was the most socially acceptable reason for not wishing to complete all of the records associated with the trial (57). To properly assess the feasibility of an intervention using probiotics, evaluation of acceptability should go beyond the tolerability of product by the children but also consider the perception and burden presumed by the caregivers since influences the adherence to treatment.

A qualitative analysis is recommended in future interventions to identify possible contextual or cultural factors that positively or negatively affect behavior related to treatment and perception of diarrhea in children in these settings. Similar to the *Guide to conducting formative research:* "*Introducing Zinc in a Diarrhoeal Disease Control Programme"* (58); interventions to understand and improve the local knowledge of diarrhea are needed to effectively include probiotics at a public health level.

Therefore, it is vital to determine local terms and practices related to diarrhea to develop key messages for the population and test them. The message must be delivered alongside with the importance of ORS to treat dehydration and not be perceived as a substitution. Besides, when used as a preventable measure probiotics need to be promoted as a product to improve gut health and not as an alternative to nutritious food.

Reactions towards the use of probiotics and method of administration need to be assessed and tailored to each setting, to assure is appropriate for the children and well accepted by the caregivers. Behavioral trials, individual interviews, and focus groups could be useful at this stage. The review and evaluation of this data could help determine whether any changes need to be made in the way the intervention are carried out or in the messages that are presented to caregivers (58).

2.7 Monitoring and evaluation

The intervention indicators should use the "SMART" criteria, suggesting that would be: specific, measurable, attainable, relevant and time-bound (59). Proposed indicators are described in Table III.

A mix of both quantitative and qualitative indicators is suggested to extend the intervention evaluation and help understand the possible treatment limitations in each setting. Data for the quantitative indicators would be collected by the program coordination team and health workers. While for the qualitative indicator it would rely on the information provided by caregivers and gather by health workers.

Table III. Proposed indicators for monitoring and evaluating the intervention

Task	Purpose	Indicator
Program implementation monitoring	Product quality assurance	Viable bacterial count of product in different stages of the intervention
	Human resources capacity building	-Training duration -Hours and frequency of supervision
	Equity and coverage assurance	-Proportion of at-risk children receiving the intervention
Intervention outcome evaluation	Health outcomes determination	- Mean diarrhea duration - Children weight change - Number of episodes - Number of patients admitted to health facility - Inpatient treatment duration
	Community perception description	- Caregivers level of perceived risk of the child contracting diarrhea - Caregivers level of perception of the benefits of treatment - Caregivers perception of the administration method used (acceptability).

3. Research Gaps

Regardless of the increasing body of evidence being developed in LMIC, there are still some gaps that need to be addressed before a recommendation at a global level can be made. Replication and consistency are two of the features missing to translate the evidence-based interventions into effective policies.

There is a need for study designs explicitly performed to compare community-based prevention protocols since most of the studies identified were therapeutic and hospital based. By directing probiotics as a preventive tool instead of therapeutically, could help intervene earlier in the epidemiological chain.

In addition, future hospital-based randomized control trials might consider incorporating measurement of biomarkers alongside clinical outcomes. Better knowledge about immune markers and changes in the microbiota and gut barrier function can contribute to the understanding of the possible working mechanisms of probiotics in infectious diseases.

Studies in vulnerable groups are lacking; interventions in children with chronic disorders or immunodeficiency need to be performed to evaluate the efficacy and safety of probiotics in at-risk populations. There is also a need for better-aligned study designs and methodology that allows reaching strain, dose and duration standardization. Studies in LMIC should focus on utilizing already proven safe and effective probiotics to optimize the rest of the operational variables and pharmacokinetic studies should be developed to determine the correct dose. We recommend variables as diarrhea definition, nutritional status classification and measure of outcomes to be homogenous among studies to facilitate analysis and the formulation of effective generalizable recommendations.

It would be interesting for future studies to consider stratifying the result analysis according to nutritional status and access to WASH to further contribute to the design of a holistic intervention and consider the potential of the use of probiotics in different environmental conditions.

Moreover, the interaction of probiotics with zinc therapy and other medications needs to be addressed. Even though this report did not focus in a particular diarrhea etiology, further studies need to consider the role of probiotics in norovirus acute gastroenteritis and the validity of including them together with the rotavirus vaccine in settings with a high burden of disease. From a public health approach this could be crucial since the efficacy of oral rotavirus vaccines is lower in some developing world countries (60) and probiotics supplementation could improve vaccine performance and maximize the effects (61).

Country-specific reports need to examine the acceptability and cost-effectiveness of using probiotics at different doses and administration methods. From a qualitative point of view, there is a need to understand the caregiver's perception of diarrhea and probiotics in each setting since various interventions rely on their report of the outcome, this could influence the data collection. Plus, culture appropriate health education and public health messages need to be tailored to seek changes in preventive diarrhea behaviors.

4. Integrated Approach of a Nutritional-Sensitive Intervention

Reviews have shown that nutrition-specific interventions are likely to be insufficient by themselves and programs that include a multi-sectorial approach seem to be more successful (62). The studies included in this report were all nutrition-specific and, to our knowledge, there are no interventions performed with probiotics that have combined other strategy in conjunction at LMIC.

The most frequently incorporated plans to reduce stunting are nutrition education, growth monitoring and promotion, immunization, WASH, and social safety net programs (cash transfer, social insurance, universal health care). There is no specific combination of programs that have been recommended to address malnutrition and the design could vary from one country to another (62).

Investments in WASH coverage and maintenance translate in improved nutrition outcomes. Hand washing with soap, improved sanitation, and water supply reduce diarrhea by 40%, 28%, and 34% respectively(63). Hence, for the scope of this study, the combination of probiotics with WASH could be potentially beneficial.

As part of a nutrition-sensitive intervention, WASH programs could interrupt specific pathways for fecal-oral transmission while probiotics could contribute to support a healthy microbiota, and theoretically potentiate a reduction in the risk of diarrhea in children. In addition, unsanitary practices have been related to the occurrence and persistence of EE (15).

It is stated that for WASH programs to be more nutritional-sensitive, interventions should go beyond the evaluation of clinical disease appearance (for example, diarrhea) and instead also consider nutritional outcomes (stunting, anemia). Consequently, evaluations should be planned with enough time frames to permit changes in nutritional outcomes. Also, target the first 1,000 days after conception since it is the time that has been identified as critical for development (64) and providentially could go jointly with the hypothesis that probiotics could be more effective before the progression of the condition.

This combination of strategies seems increasingly pertinent considering the rise in evidence regarding the involvement of EE in nutritional status. EE is characterized by a gut and systemic inflammation, alterations in gut barrier function, and absorptive capacity. There are still gaps in the knowledge about the effect of the condition on gut physiology, but the significant public health implications (undernutrition, stunting, reduced immune response to oral vaccines, and cognitive development deficiencies) justify an increase in research investment (65).

Poor WASH practices have been associated with the prevalence of this condition (15) and probiotics have the potential to participate in EE modulation (66). Jointly they could tackle the underlying causes of malnutrition without waiting for the onset of symptoms especially considering that a child with EE might not present diarrhea. Therefore, a strategy that combines WASH improvement and probiotics supplementation are among the approaches considered viable to prevent EE (65).

The lack of encouraging results from the first reported trial using probiotic for the treatment of EE further highlighted the need for better understanding of the pathogenesis of this condition to identify and test potential therapeutic strategies. This intervention failed to see any change in the intestinal integrity of the group receiving *L. rhamnosus GG* 5 x 10^{10} CFU for 30 days (15).

Nevertheless, the authors highlighted the need to perform trials with larger doses or different strain combinations and not reject the potential of probiotics altogether. Also, future interventions could consider targeting the population group under one year old, as including probiotics before the onset of the condition could help prevent it (15). And supplementation at early stages of the disease has proven increased effectiveness of treatment for other pathologies (36). Further studies to understand the timeline of EE development could be key to design effective intervention strategies.

The implementation of a program including both WASH improvement and probiotic supplementation would require strong political commitment and multi-sectorial collaboration since it involves a diverse range of experts. Nevertheless, since this nutrition-sensitive intervention is focused on prevention and early child development the investment could be significantly cost effective in a long-term.

5. Limitations

This report is an effort to analyze the operational variables that should be considered when implementing an intervention using probiotics in children from LMIC. It contributes to translate past experiences and evidence into well-defined interventions and influence the diarrhea management policies later on.

Nevertheless, this review does have limitations. By only including studies from peer-reviewed journals, it may have missed important unpublished data. All the studies were published in English and most were performed in India, which could introduce language and location bias respectively. There is also the possibility of reporting bias since not all the trials described the operational variables included for the study. Besides, the identification of potentially relevant studies, eligibility assessment, and analysis was performed by a single person and not compared with another reviewer. Which also forbids to perform the GRADE assessment of the quality of the evidence (67).

V. Conclusion

The clear link between diarrhea and malnutrition is a defining factor for the increased interest for interventions such as probiotics supplementation. The use of probiotics as a public health strategy in LMIC is an attractive approach because of its safety profile and potential to prevent diarrhea occurrence and progression, particularly in high-disease endemic areas. The compelling scientific evidence from developed countries that has led to the inclusion of probiotics in ESPGHAN guidelines is not enough to generalize to other locations. Since most studies are hospital based, results may not be a fair reflection of cases in different settings and community-based trials are scarce.

The heterogenicity of the operational variables among trials prevents from formulating a policy recommendation based on this report. Nevertheless, this is the first attempt to describe the influence of these variables in studies performed in LMIC and provides recommendations built from the gathered evidence.

There is a need for the standardization of the methodology regarding strain, dose, duration of treatment and administration method. Besides a further understanding of the gut microbiota, evidence of the probiotics interaction with other agents and qualitative studies are needed. The implementation of a program including both WASH improvement and probiotic supplementation requires further investigation since it has a possibly high impact at community level.

VI. Recommendations

Future studies

Even though there are still some research gaps that need to be addressed, with the available information we can recommend that future studies use probiotics that have been proven efficacious in other clinical trials and are strongly recommended by ESPGHAN (*L. rhamnosus GG* and *S. boulardii*) in a dosage between 10^9 and 10^{10} CFU. Interventions using probiotics we suggest should be aimed at prevention of the condition and choose products in which the manufacturer process has been closely regulated. Different quality assessment controls should be performed during the supply chain to assure the correct dosage is delivered. The administration method might be of small volume and incorporate the probiotic content in one single dosage. The analysis should be stratified by age and nutritional status to determine if there is a response difference and contribute to determine the best introduction time. The trial we suggest should be performed parallel to a qualitative assessment and cost-effectiveness analysis to help determine local feasibility. An intervention conceptual proposal is described in Annex 2.

Industry

The challenges that arise from quality control, supply chain and acceptability of administration method highlight the opportunity for researchers and industrial leaders to work together against one of the biggest health threats for children worldwide. The development of a product including probiotics that is stable at different temperatures, with a long shelve life, and with an easy supply chain could be key for the success of a probiotic intervention in LMIC.

Policy makers

Financing programs that aim to treat the underlying causes of malnutrition, such as WASH and probiotics as a potential preventive measure for diarrhea could maximize returns on investments. Political engagement towards policies that work double-duty is key to address nutritional challenges. Integration of WASH to nutrition-specific interventions is required to achieve the global commitment assumed in the Sustainable Development Goals to end all forms of malnutrition by 2030. The execution of integrated programming would need the collaboration of governments, donors, civil society organizations, and businesses at international and national level.

VII. References

1. World Health Organization. Guideline: Updates on the Management of Severe Acute Malnutrition in Infants and Children [Internet]. World Health Organization. Geneva; 2013 [cited 2016 Nov 20]. Available from: www.who.int/about/licensing/copyright_form/en/index.

2. World Health Organization. Diarrhoeal disease: fact sheet [Internet]. WHO. World Health Organization; 2017 [cited 2017 May 7]. Available from: http://www.who.int/mediacentre/factsheets/fs330/en/

3. Checkley W, Buckley G, Gilman RH, Assis AM, Guerrant RL, Morris SS, et al. Multi-country analysis of the effects of diarrhoea on childhood stunting. Int J Epidemiol [Internet]. 2008 Aug [cited 2016 Nov 20];37(4):816–30. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18567626

4. World Health Organization, United Nations Children's Fund, United States Agency for International Development. Improving nutrition outcomes with better water, sanitation and hygiene: Practical solutions for policy and programmes [Internet]. WHO. Switzerland: World Health Organization; 2015 [cited 2016 Nov 20]. Available from: http://apps.who.int/iris/bitstream/10665/193991/1/9789241565103_eng.pdf?ua=1&ua=1

5. Humphrey JH, Black R, Allen L, Bhutta Z, al. et, Victora C, et al. Child undernutrition, tropical enteropathy, toilets, and handwashing. Lancet (London, England) [Internet]. 2009 Sep 19 [cited 2016 Nov 20];374(9694):1032–5. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19766883

6. Prendergast A, Kelly P. Enteropathies in the developing world: neglected effects on global health. Am J Trop Med Hyg [Internet]. 2012 May [cited 2016 Nov 15];86(5):756–63. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22556071

7. Bhutta ZA, Das JK, Walker N, Rizvi A, Campbell H, Rudan I, et al. Interventions to address deaths from childhood pneumonia and diarrhoea equitably: what works and at what cost? Lancet [Internet]. 2013 Apr [cited 2017 Apr 18];381(9875):1417–29. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0140673613606480

8. Bhutta ZA, Das JK, Rizvi A, Gaffey MF, Walker N, Horton S, et al. Evidence-based interventions for improvement of maternal and child nutrition: what can be done and at what cost? Lancet (London, England) [Internet]. 2013 Aug 3 [cited 2016 Dec 4];382(9890):452–77. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23746776

9. WHO & UNICEF. The Integrated Global Action Plan for the Prevention and Control of Pneumonia and Diarrhoea. 2013 [cited 2017 May 6]; Available from: www.who.int/about/licensing/copyright_form/en/index.

10. Allen SJ, Martinez EG, Gregorio G V, Dans LF. Probiotics for treating acute infectious diarrhoea. In: Allen SJ, editor. Cochrane Database of Systematic Reviews [Internet]. Chichester, UK: John Wiley & Sons, Ltd; 2010 [cited 2016 Nov 20]. Available from: http://doi.wiley.com/10.1002/14651858.CD003048.pub3

11. Guarino A, Ashkenazi S, Gendrel D, Lo Vecchio A, Shamir R, Szajewska H, et al. European

Society for Pediatric Gastroenterology, Hepatology, and Nutrition/European Society for Pediatric Infectious Diseases Evidence-Based Guidelines for the Management of Acute Gastroenteritis in Children in Europe. J Pediatr Gastroenterol Nutr [Internet]. 2014 Jul [cited 2017 Apr 13];59(1):132–52. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24739189

12. Morelli L, Capurso L. FAO/WHO Guidelines on Probiotics. J Clin Gastroenterol [Internet]. 2012 Oct [cited 2017 Apr 7];46:S1–2. Available from: http://content.wkhealth.com/linkback/openurl?sid=WKPTLP:landingpage&an=00004836-201210001-00002

13. Sazawal S, Hiremath G, Dhingra U, Malik P, Deb S, Black RRE, et al. Efficacy of probiotics in prevention of acute diarrhoea: a meta-analysis of masked, randomised, placebo-controlled trials. Lancet Infect Dis [Internet]. 2006 Jun [cited 2016 Nov 20];6(6):374–82. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16728323

14. Szajewska H, Guarino A, Hojsak I, Indrio F, Kolacek S, Shamir R, et al. Use of probiotics for management of acute gastroenteritis: a position paper by the ESPGHAN Working Group for Probiotics and Prebiotics. J Pediatr Gastroenterol Nutr [Internet]. 2014 Apr [cited 2017 May 6];58(4):531–9. Available from: http://content.wkhealth.com/linkback/openurl?sid=WKPTLP:landingpage&an=00005176-201404000-00030

15. Galpin L, Manary MJ, Fleming K, Ou C-N, Ashorn P, Shulman RJ. Effect of Lactobacillus GG on intestinal integrity in Malawian children at risk of tropical enteropathy. Am J Clin Nutr [Internet]. 2005 Nov [cited 2016 Nov 20];82(5):1040–5. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16280436

16. Nair GB, Takeda Y. Health impact of probiotics : vision & opportunities. 1st ed. India: Elsevier; 2012. 176 p.

17. The World Bank. World Bank Country and Lending Groups (Country Classification) [Internet]. 2017 [cited 2016 Dec 1]. Available from: https://datahelpdesk.worldbank.org/knowledgebase/articles/906519

18. Aggarwal S, Upadhyay A, Shah D, Teotia N, Agarwal A, Jaiswal V. Lactobacillus GG for treatment of acute childhood diarrhoea: an open labelled, randomized controlled trial. Indian J Med Res [Internet]. 2014 Mar [cited 2017 Apr 21];139(3):379–85. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24820831

19. Abou El-Soud NH, Said RN, Mosalam DS, Barakat NAM, Sabry MA. Bifidobacterium lactis in Treatment of Children with Acute Diarrhea. A Randomized Double Blind Controlled Trial. Open Access Maced J Med Sci [Internet]. 2015 Aug 7 [cited 2017 Mar 21];3(3):403. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27275258

20. Narayanappa D. Randomized double blinded controlled trial to evaluate the efficacy and safety of Bifilac in patients with acute viral diarrhea. Indian J Pediatr [Internet]. 2008 Jul 21 [cited 2017 Apr 13];75(7):709–13. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18716740

21. Basu S, Paul DK, Ganguly S, Chatterjee M, Chandra PK. Efficacy of High-dose Lactobacillus rhamnosus GG in Controlling Acute Watery Diarrhea in Indian Children. J Clin Gastroenterol [Internet]. 2009 Mar [cited 2017 Apr 6];43(3):208–13. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18813028

22. Dubey AP, Rajeshwari K, Chakravarty A, Famularo G. Use of VSL#3 in the Treatment of Rotavirus Diarrhea in Children. J Clin Gastroenterol [Internet]. 2008 Sep [cited 2017 Apr 12];42:S126–9. Available from: http://content.wkhealth.com/linkback/openurl?sid=WKPTLP:landingpage&an=00004836-200809001-00006

23. Basu S, Chatterjee M, Ganguly S, Chandra PK. Effect of Lactobacillus rhamnosus GG in Persistent Diarrhea in Indian Children. J Clin Gastroenterol [Internet]. 2007 Sep [cited 2017 May 1];41(8):756–60. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17700424

24. El-Gendy F, Hasan S, Shalan G. Effects of supplementation with probiotics and prebiotics in infants with gastroenteritis. Menoufia Med J [Internet]. 2015 [cited 2017 Apr 29];28(2):508. Available from: http://www.mmj.eg.net/text.asp?2015/28/2/508/163910

25. Agustina R, Lukito W, Firmansyah A, Ningsih H, Md S, Murniati D, et al. The effect of early nutritional supplementation with a mixture of probiotic, prebiotic, fiber and micronutrients in infants with acute diarrhea in Indonesia. Asia Pac J Clin Nutr. 2007;16(3):435–42.

26. Grandy G, Medina M, Soria R, Terán CG, Araya M. Probiotics in the treatment of acute rotavirus diarrhoea. A randomized, double-blind, controlled trial using two different probiotic preparations in Bolivian children. BMC Infect Dis [Internet]. 2010 Dec 25 [cited 2017 Feb 22];10(1):253. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20735858

27. Sarker SA, Sultana S, Fuchs GJ, Alam NH, Azim T, Brüssow H, et al. Lactobacillus paracasei Strain ST11 Has No Effect on Rotavirus but Ameliorates the Outcome of Nonrotavirus Diarrhea in Children From Bangladesh. Pediatrics [Internet]. 2005 Aug 1 [cited 2017 Apr 6];116(2):e221–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15995003

28. Riaz M, Alam S, Malik A, Ali SM. Efficacy and Safety of Saccharomyces boulardii in Acute Childhood Diarrhea: A Double Blind Randomised Controlled Trial. Indian J Pediatr [Internet]. 2012 Apr 14 [cited 2017 May 2];79(4):478–82. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21997865

29. Das S, Gupta PK, Das RR. Efficacy and Safety of Saccharomyces boulardii in Acute Rotavirus Diarrhea: Double Blind Randomized Controlled Trial from a Developing Country. J Trop Pediatr [Internet]. 2016 Jun 9 [cited 2017 May 1];62(6):fmw032. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27283364

30. Hegar B, Waspada IMI, Gunardi H, Vandenplas Y. A Double Blind Randomized Trial Showing Probiotics to be Ineffective in Acute Diarrhea in Indonesian Children. Indian J Pediatr [Internet]. 2015 May 29 [cited 2017 Feb 22];82(5):410–4. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24777621

31. Misra S, Sabui TK, Pal NK. A Randomized Controlled Trial to Evaluate the Efficacy of Lactobacillus GG in Infantile Diarrhea. J Pediatr [Internet]. 2009 [cited 2017 Apr 12];155(1):129–32. Available from: http://www.sciencedirect.com/science/article/pii/S0022347609000547

32. Basu S, Chatterjee M, Ganguly S, Chandra PK. Efficacy of Lactobacillus rhamnosus GG in acute watery diarrhoea of Indian children: A randomised controlled trial. J Paediatr Child

Health [Internet]. 2007 Dec [cited 2017 Apr 6];43(12):837–42. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17803667

33. Dutta P, Mitra U, Dutta S, Rajendran K, Saha TK, Chatterjee MK. Randomised controlled clinical trial of Lactobacillus sporogenes (Bacillus coagulans), used as probiotic in clinical practice, on acute watery diarrhoea in children. Trop Med Int Heal [Internet]. 2011 May [cited 2017 May 1];16(5):555–61. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21332891

34. Grenov B, Namusoke H, Lanyero B, Nabukeera-Barungi N, Ritz C, Mølgaard C, et al. Effect of Probiotics on Diarrhea in Children With Severe Acute Malnutrition: A Randomized Controlled Study in Uganda. J Pediatr Gastroenterol Nutr [Internet]. 2017 Mar [cited 2017 Apr 3];64(3):396–403. Available from: http://www.ncbi.nlm.nih.gov/pubmed/28079729

35. Villarruel G, Rubio DM, Lopez F, Cintioni J, Gurevech R, Romero G, et al. Saccharomyces boulardii in acute childhood diarrhoea: a randomized, placebo-controlled study. Acta Paediatr [Internet]. 2007 Apr [cited 2017 May 2];96(4):538–41. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17306006

36. Hajela N, Nair GB, Ganguly NK. Are probiotics a feasible intervention for prevention of diarrhoea in the developing world? Gut Pathog [Internet]. 2010 Aug 29 [cited 2017 Mar 23];2(1):10. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20799986

37. Billoo AG, Memon MA, Khaskheli SA, Murtaza G, Iqbal K, Saeed Shekhani M, et al. Role of a probiotic (Saccharomyces boulardii) in management and prevention of diarrhoea. World J Gastroenterol [Internet]. 2006 Jul 28 [cited 2017 Apr 6];12(28):4557–60. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16874872

38. Sur D, Manna B, Niyogi SK, Ramamurthy T, Palit A, Nomoto K, et al. Role of probiotic in preventing acute diarrhoea in children: a community-based, randomized, double-blind placebo-controlled field trial in an urban slum. Epidemiol Infect [Internet]. 2011 Jun [cited 2016 Nov 20];139(6):919–26. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20670468

39. Sazawal S, Dhingra U, Hiremath G, Sarkar A, Dhingra P, Dutta A, et al. Prebiotic and Probiotic Fortified Milk in Prevention of Morbidities among Children: Community-Based, Randomized, Double-Blind, Controlled Trial. Belizan JM, editor. PLoS One [Internet]. 2010 Aug 13 [cited 2017 Feb 18];5(8):e12164. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20730056

40. Oberhelman RA, Gilman RH, Sheen P, Taylor DN, Black RE, Cabrera L, et al. A placebo-controlled trial of Lactobacillus GG to prevent diarrhea in undernourished Peruvian children. J Pediatr [Internet]. 1999 Jan [cited 2017 Feb 18];134(1):15–20. Available from: http://www.ncbi.nlm.nih.gov/pubmed/9880443

41. Ritchie ML, Romanuk TN. A Meta-Analysis of Probiotic Efficacy for Gastrointestinal Diseases. Heimesaat MM, editor. PLoS One [Internet]. 2012 Apr 18 [cited 2016 Nov 20];7(4):e34938. Available from: http://dx.plos.org/10.1371/journal.pone.0034938

42. Guarino A, Dupont C, Gorelov A V, Gottrand F, Lee JK, Lin Z, et al. The management of acute diarrhea in children in developed and developing areas: from evidence base to clinical practice. Expert Opin Pharmacother [Internet]. 2012 Jan 22 [cited 2017 Feb 18];13(1):17–26. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22106840

43. Agustina R, Kok FJ, van de Rest O, Fahmida U, Firmansyah A, Lukito W, et al. Randomized Trial of Probiotics and Calcium on Diarrhea and Respiratory Tract Infections in Indonesian Children. Pediatrics [Internet]. 2012 May 1 [cited 2017 Feb 18];129(5):e1155–64. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22492764

44. Cruchet S, Furnes R, Maruy A, Hebel E, Palacios J, Medina F, et al. The use of probiotics in pediatric gastroenterology: a review of the literature and recommendations by Latin-American experts. Paediatr Drugs [Internet]. 2015 Jun [cited 2017 May 6];17(3):199–216. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25799959

45. Snydman DR. The Safety of Probiotics. Clin Infect Dis [Internet]. 2008 Feb [cited 2017 Apr 12];46(s2):S104–11. Available from: https://academic.oup.com/cid/article-lookup/doi/10.1086/523331

46. Sanders ME, Akkermans LMA, Haller D, Hammerman C, Heimbach J, Hörmannsperger G, et al. Safety assessment of probiotics for human use. Gut Microbes [Internet]. 2010 [cited 2017 May 15];1(3):164–85. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21327023

47. Szajewska H, SKórka A, Ruszczynski M, Gieruszczak-Bialek D. Meta-analysis: Lactobacillus GG for treating acute diarrhoea in children. Aliment Pharmacol Ther [Internet]. 2007 Feb 12 [cited 2017 Apr 21];25(8):871–81. Available from: http://doi.wiley.com/10.1111/j.1365-2036.2007.03282.x

48. Szajewska H. What is new on the term probiotics and the role of probiotics for the management of acute gastroenteritis. Benef Microbes [Internet]. 2015 Jan [cited 2017 May 16];6(2):185–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25245574

49. Szajewska H, Skórka A, Dylag M. Meta-analysis: Saccharomyces boulardii for treating acute diarrhoea in children. Aliment Pharmacol Ther [Internet]. 2006 Dec 19 [cited 2017 May 16];25(3):257–64. Available from: http://doi.wiley.com/10.1111/j.1365-2036.2006.03202.x

50. Szajewska H, Mrukowicz JZ. Probiotics in the Treatment and Prevention of Acute Infectious Diarrhea in Infants and Children: A Systematic Review of Published Randomized, Double-Blind, Placebo-Controlled Trials. J Pediatr Gastroenterol Nutr [Internet]. 2001 [cited 2017 Apr 13];(33):S17–S25. Available from: https://fqresearch.org/pdf_files/probiotics-treatment-prevention-acute-diarrhea.pdf

51. Ouwehand AC. A review of dose-responses of probiotics in human studies. Benef Microbes [Internet]. 2017 Apr 26 [cited 2017 May 16];8(2):143–51. Available from: http://www.ncbi.nlm.nih.gov/pubmed/28008787

52. Kerac M, Bunn J, Seal A, Thindwa M, Tomkins A, Sadler K, et al. Probiotics and prebiotics for severe acute malnutrition (PRONUT study): a double-blind efficacy randomised controlled trial in Malawi. Lancet [Internet]. 2009 Jul [cited 2016 Nov 15];374(9684):136–44. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0140673609608849

53. Klu YAK, Williams JH, Phillips RD, Chen J. Survival of Lactobacillus rhamnosus GG as Influenced by Storage Conditions and Product Matrixes. J Food Sci [Internet]. 2012 Dec [cited 2017 May 13];77(12):M659–63. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23106385

54. Vandenplas Y, De Hert S, Probiotical study group. Cost/benefit of synbiotics in acute infectious gastroenteritis: spend to save. Benef Microbes [Internet]. 2012 Sep [cited 2017 May 11];3(3):189–94. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22835702

55. Gutierrez-Castrellon P, Lopez-Velazquez G, Diaz-Garcia L, Jimenez-Gutierrez C, Mancilla-Ramirez J, Estevez-Jimenez J, et al. Diarrhea in Preschool Children and Lactobacillus reuteri: A Randomized Controlled Trial. Pediatrics [Internet]. 2014 [cited 2017 May 13];133(4). Available from: http://pediatrics.aappublications.org/content/133/4/e904.short

56. Marseille E, Larson B, Kazi DS, Kahn JG, Rosen S. Policy & practice Thresholds for the cost–effectiveness of interventions: alternative approaches. Bull World Heal Organ [Internet]. 2015 [cited 2017 Apr 17];93:118–24. Available from: http://dx.doi.org/10.2471/BLT.14.138206

57. Binns C, Lee A, Harding H, Gracey M, Barclay D. The CUPDAY Study: prebiotic-probiotic milk product in 1–3-year-old children attending childcare centres. Acta Paediatr [Internet]. 2007 Nov [cited 2017 May 1];96(11):1646–50. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17937689

58. Nichter M, Acuin CS, Vargas MA. Introducing Zinc in a Diarrhoeal Disease Control Programme Guide to conducting formative research [Internet]. Geneva; 2008 [cited 2017 May 1]. Available from: http://www.who.int/child-adolescent-health/

59. FAO & WHO. Towards country-specific smart commitments for action on nutrition [Internet]. 2016 [cited 2017 Apr 18]. Available from: http://www.who.int/nutrition/decade-of-action/smart_commitments.pdf?ua=1

60. Madhi SA, Cunliffe NA, Steele D, Witte D, Kirsten M, Louw C, et al. Effect of Human Rotavirus Vaccine on Severe Diarrhea in African Infants. N Engl J Med [Internet]. 2010 Jan 28 [cited 2017 May 20];362(4):289–98. Available from: http://www.nejm.org/doi/abs/10.1056/NEJMoa0904797

61. World Health Organization. Diarrhoea-related hospitalizations in children before and after implementation of monovalent rotavirus vaccination in Mexico. Bull World Health Organ [Internet]. 2015 [cited 2017 May 16]; Available from: http://www.who.int/bulletin/volumes/92/2/13-125286/en/

62. Hossain M, Choudhury N, Adib Binte Abdullah K, Mondal P, Jackson AA, Walson J, et al. Evidence-based approaches to childhood stunting in low and middle income countries: a systematic review. Arch Dis Child [Internet]. 2017 May 3 [cited 2017 May 17];archdischild-2016-311050. Available from: http://adc.bmj.com/lookup/doi/10.1136/archdischild-2016-311050

63. Chase C, Ngure F. Multisectoral Approaches to Improving Nutrition: Water, Sanitation, and Hygiene [Internet]. 2016 [cited 2017 May 17]. Available from: https://www.wsp.org/sites/wsp.org/files/publications/Multisectoral00itation00and0hygiene.pdf

64. International Food Policy Research Institute. Global Nutrition Report: From promise to Impact: Ending Malnutrition by 2030 [Internet]. Washington, DC.; 2016 [cited 2017 May 24]. Available from: https://data.unicef.org/wp-content/uploads/2016/06/130565-1.pdf

65. Lang D, MAL-ED Network Investigators M-EN. Opportunities to assess factors contributing to the development of the intestinal microbiota in infants living in developing countries. Microb Ecol Health Dis [Internet]. 2015 [cited 2017 Feb 18];26:28316. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26031686

66. Petri WA, Naylor C, Haque R, Haque R. Environmental enteropathy and malnutrition: do we know enough to intervene? BMC Med [Internet]. 2014 Oct 14 [cited 2017 May 17];12:187. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25604120

67. Hill R. How to GRADE the quality of the evidence [Internet]. Cochrane Consumers and Communication Group. Version 3.0. 2016 [cited 2017 Jun 1]. Available from: http://cccrg.cochrane.org/sites/cccrg.cochrane.org/files/public/uploads/how_to_grade_revising_1_december_2016.pdf

VIII. Annex

Annex 1. Excluded studies

Agustina, R. et al., 2012. Randomized Trial of Probiotics and Calcium on Diarrhea and Respiratory Tract Infections in Indonesian Children. *Pediatrics*, 129(5), pp.e1155–e1164. Available at: http://www.ncbi.nlm.nih.gov/pubmed/22492764

Ahmad, K. et al., 2013. Probiotics for the treatment of pediatric helicobacter pylori infection: a randomized double blind clinical trial. *Iranian journal of pediatrics*, 23(1), pp.79–84. Available at: http://www.ncbi.nlm.nih.gov/pubmed/23446685

Billoo, A.G. et al., 2006. Role of a probiotic (Saccharomyces boulardii) in management and prevention of diarrhoea. *World journal of gastroenterology*, 12(28), pp.4557–60. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16874872

Binns, C. et al., 2007. The CUPDAY Study: prebiotic-probiotic milk product in 1–3-year-old children attending childcare centres. *Acta Paediatrica*, 96(11), pp.1646–1650. Available at: http://www.ncbi.nlm.nih.gov/pubmed/17937689

Canani, R.B. et al., 2007. Probiotics for treatment of acute diarrhoea in children: randomised clinical trial of five different preparations. *BMJ*, 335(7615), pp.340–340. Available at: http://www.ncbi.nlm.nih.gov/pubmed/17690340

Chen, Y.-C. et al., 2012. Probiotic Supplement Use among Young Children in Taiwan: A Prospective Cohort Study H. R. Baradaran, ed. *PLoS ONE*, 7(9), p.e43885. Available at: http://www.ncbi.nlm.nih.gov/pubmed/22984450

Cobo Sanz, J.M., Mateos, J.A. & Muñoz Conejo, A., [Effect of Lactobacillus casei on the incidence of infectious conditions in children]. *Nutricion hospitalaria*, 21(4), pp.547–51. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16913215

Corrêa, N.B.O. et al., A randomized formula controlled trial of Bifidobacterium lactis and Streptococcus thermophilus for prevention of antibiotic-associated diarrhea in infants. *Journal of clinical gastroenterology*, 39(5), pp.385–9. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15815206

Corrêa, N.B.O. et al., 2011. Treatment of Acute Diarrhea With Saccharomyces Boulardii in Infants. *Journal of Pediatric Gastroenterology and Nutrition*, 53(5), p.1. Available at: http://www.ncbi.nlm.nih.gov/pubmed/21734600

Dinleyici, E.C. et al., 2009. Clinical efficacy of Saccharomyces boulardii and metronidazole compared to metronidazole alone in children with acute bloody diarrhea caused by amebiasis: a prospective, randomized, open label study. *The American journal of tropical medicine and hygiene*, 80(6), pp.953–5. Available at: http://www.ncbi.nlm.nih.gov/pubmed/19478257

Dinleyici, E.C. et al., 2015. Lactobacillus reuteri DSM 17938 shortens acute infectious diarrhea in a pediatric outpatient setting. *Jornal de Pediatria*, 91(4), pp.392–396. Available at: http://www.ncbi.nlm.nih.gov/pubmed/25986615

Dinleyici, E.C. et al., 2015. *Saccharomyces boulardii* CNCM I-745 reduces the duration of diarrhoea, length of emergency care and hospital stay in children with acute diarrhoea. *Beneficial Microbes*, 6(4), pp.415–421. Available at: http://www.ncbi.nlm.nih.gov/pubmed/25653151

Dinleyici, E.C. et al., 2013. The effect of a multispecies synbiotic mixture on the duration of diarrhea and length of hospital stay in children with acute diarrhea in Turkey: Single blinded randomized study. *European Journal of Pediatrics*, 172(4), pp.459–464. Available at: http://www.ncbi.nlm.nih.gov/pubmed/23239048

Dinleyici, E.C., PROBAGE Study Group, Y. & Vandenplas, Y., 2014. Lactobacillus reuteri DSM 17938 effectively reduces the duration of acute diarrhoea in hospitalised children. *Acta paediatrica*, 103(7), pp.e300-5. Available at: http://doi.wiley.com/10.1111/apa.12617

Duggan, C. et al., 2003. Oligofructose-supplemented infant cereal: 2 randomized, blinded, community-based trials in Peruvian infants. *The American journal of clinical nutrition*, 77(4), pp.937–42. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12663295

Erdeve, O. et al., 2005. Saccharomyces boulardii and antibiotic-associated diarrhoea in children. *Alimentary Pharmacology and Therapeutics*, 21(12), pp.1508–1509. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15948821

Erdeve, O., Tiras, U. & Dallar, Y., 2004. The probiotic effect of Saccharomyces boulardii in a pediatric age group. *Journal of tropical pediatrics*, 50(4), pp.234–6. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15357564

Erdoğan, Ö. et al., 2012. The Comparition of the Efficacy of Two Different Probiotics in Rotavirus Gastroenteritis in Children. *Journal of Tropical Medicine*, 2012, pp.1–5. Available at: http://www.ncbi.nlm.nih.gov/pubmed/22778754

Fox, M.J. et al., 2015. Can probiotic yogurt prevent diarrhoea in children on antibiotics? A double-blind, randomised, placebo-controlled study. *BMJ Open*, 5(1), pp.e006474–e006474. Available at: http://www.ncbi.nlm.nih.gov/pubmed/25588782 [Accessed February 22, 2017].

Francavilla, R. et al., 2012. Randomised clinical trial: Lactobacillus reuteri DSM 17938 vs. placebo in children with acute diarrhoea - a double-blind study. *Alimentary Pharmacology & Therapeutics*, 36(4), pp.363–369. Available at: http://www.ncbi.nlm.nih.gov/pubmed/22680836

Gaón, D. et al., 2003. Effect of Lactobacillus strains and Saccharomyces boulardii on persistent diarrhea in children. *Medicina*, 63(4), pp.293–8. Available at: http://www.ncbi.nlm.nih.gov/pubmed/14518142

Guandalini, S. et al., 2000. Lactobacillus GG administered in oral rehydration solution to children with acute diarrhea: a multicenter European trial. *Journal of pediatric gastroenterology and nutrition*, 30(1), pp.54–60. Available at: http://www.ncbi.nlm.nih.gov/pubmed/10630440

Hatakka, K. et al., 2001. Effect of long term consumption of probiotic milk on infections in children attending day care centres: double blind, randomised trial. *BMJ (Clinical research ed.)*, 322(7298), p.1327. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11387176

Henker, J. et al., 2007. The probiotic Escherichia coli strain Nissle 1917 (EcN) stops acute diarrhoea in infants and toddlers. *European Journal of Pediatrics*, 166(4), pp.311–318. Available at: http://www.ncbi.nlm.nih.gov/pubmed/17287932

Hojsak, I. et al., 2010. Lactobacillus GG in the prevention of gastrointestinal and respiratory tract infections in children who attend day care centers: A randomized, double-blind, placebo-controlled trial. *Clinical Nutrition*, 29(3), pp.312–316. Available at: http://www.ncbi.nlm.nih.gov/pubmed/19896252

Htwe, K. et al., 2008. Effect of Saccharomyces boulardii in the treatment of acute watery diarrhea in Myanmar children: a randomized controlled study. *The American journal of tropical medicine and hygiene*, 78(2), pp.214–6. Available at: http://www.ncbi.nlm.nih.gov/pubmed/18256417

Huang, Y.-F. et al., 2014. Three-Combination Probiotics Therapy in Children With Salmonella and Rotavirus Gastroenteritis. *Journal of Clinical Gastroenterology*, 48(1), pp.37–42. Available at: http://www.ncbi.nlm.nih.gov/pubmed/23632352

Investigating Group for Prevention of AAD in Children with Pneumonia by Clostridium Butyricum and Bifidobacterium, 2012. [Multicenter, randomized, controlled clinical trial on preventing antibiotic-associated diarrhea in children with pneumonia using the live Clostridium butyricum and Bifidobacterium combined Powder]. *Zhonghua er ke za zhi = Chinese journal of pediatrics*, 50(10), pp.732–6. Available at: http://www.ncbi.nlm.nih.gov/pubmed/23302558

Kerac, M. et al., 2009. Probiotics and prebiotics for severe acute malnutrition (PRONUT study): a double-blind efficacy randomised controlled trial in Malawi. *The Lancet*, 374(9684), pp.136–144. Available at: http://linkinghub.elsevier.com/retrieve/pii/S0140673609608849

Khanna, V. et al., 2005. Efficacy of tyndalized Lactobacillus acidophilus in acute diarrhea. *Indian journal of pediatrics*, 72(11), pp.935–8. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16391447

Kotowska, M., Albrecht, P. & Szajewska, H., 2005. Saccharomyces boulardii in the prevention of antibiotic-associated diarrhoea in children: a randomized double-blind placebo-controlled trial. *Alimentary Pharmacology and Therapeutics*, 21(5), pp.583–590. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15740542

Krammer, H. et al., 2006. Probiotische Arzneimitteltherapie mit *E. coli* Stamm Nissle 1917 (EcN): Ergebnisse einer prospektiven Datenerhebung mit 3807 Patienten. *Zeitschrift für Gastroenterologie*, 44(8), pp.651–656. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16902895

Lee, D.K. et al., 2015. Probiotic bacteria, B. longum and L. acidophilus inhibit infection by rotavirus in vitro and decrease the duration of diarrhea in pediatric patients. *Clinics and Research in Hepatology and Gastroenterology*, 39(2), pp.237–244. Available at: http://www.ncbi.nlm.nih.gov/pubmed/25459995

Le Luyer, B., Makhoul, G. & Duhamel, J.-F., 2010. Étude multicentrique, contrôlée en double insu d'une formule adaptée enrichie en Saccharomyces boulardii dans le traitement des diarrhées aiguës du nourrisson. *Archives de Pédiatrie*, 17(5), pp.459–465. Available at: http://www.ncbi.nlm.nih.gov/pubmed/20236813

Mao, M. et al., 2008. Effect of a lactose-free milk formula supplemented with bifidobacteria and streptococci on the recovery from acute diarrhoea. *Asia Pacific journal of clinical nutrition*, 17(1), pp.30–4. Available at: http://www.ncbi.nlm.nih.gov/pubmed/18364323

Merenstein, D.J., Foster, J. & D'Amico, F., 2009. A Randomized Clinical Trial Measuring the Influence of Kefir on Antibiotic-Associated Diarrhea. *Archives of Pediatrics & Adolescent Medicine*, 163(8), pp.750–4. Available at: http://www.ncbi.nlm.nih.gov/pubmed/19652108

Nixon, A.F. et al., 2012. The Effect of Lactobacillus GG on Acute Diarrheal Illness in the Pediatric Emergency Department. *Pediatric Emergency Care*, 28(10), pp.1048–1051. Available at: http://www.ncbi.nlm.nih.gov/pubmed/23023475

Olek, A. et al., 2017. Efficacy and Safety of Lactobacillus plantarum DSM 9843 (LP299V) in the Prevention of Antibiotic-Associated Gastrointestinal Symptoms in Children—Randomized, Double-Blind, Placebo-Controlled Study. *The Journal of Pediatrics*. Available at: http://www.ncbi.nlm.nih.gov/pubmed/28438377

Ritchie, B.K. et al., 2010. Efficacy of Lactobacillus GG in Aboriginal Children With Acute Diarrhoeal Disease: A Randomised Clinical Trial. *Journal of Pediatric Gastroenterology and Nutrition*, 50(6), pp.619–624. Available at: http://www.ncbi.nlm.nih.gov/pubmed/20400916

La Rosa, M. et al., 2003. [Prevention of antibiotic-associated diarrhea with Lactobacillus sporogens and fructo-oligosaccharides in children. A multicentric double-blind vs placebo study]. *Minerva pediatrica*, 55(5), pp.447–52. Available at: http://www.ncbi.nlm.nih.gov/pubmed/14608267

Rosenfeldt, V. et al., 2002. Effect of probiotic Lactobacillus strains in young children hospitalized with acute diarrhea. *The Pediatric infectious disease journal*, 21(5), pp.411–6. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12150178

Ruszczyński, M., Radzikowski, A. & Szajewska, H., 2008. Clinical trial: effectiveness of Lactobacillus rhamnosus (strains E/N, Oxy and Pen) in the prevention of antibiotic-associated diarrhoea in children. *Alimentary Pharmacology & Therapeutics*, 28(1), pp.154–161. Available at: http://www.ncbi.nlm.nih.gov/pubmed/18410562

Salazar-Lindo, E. et al., 2004. Lactobacillus caseistrain GG in the treatment of infants with acute watery diarrhea: A randomized, double-blind, placebo controlled clinical trial [ISRCTN67363048]. *BMC Pediatrics*, 4(1), p.18. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15345099

Savaş-Erdeve, S., Gökay, S. & Dallar, Y., Efficacy and safety of Saccharomyces boulardii in amebiasis-associated diarrhea in children. *The Turkish journal of pediatrics*, 51(3), pp.220–4. Available at: http://www.ncbi.nlm.nih.gov/pubmed/19817264

Schrezenmeir, J. et al., 2004. Benefits of Oral Supplementation With and Without Synbiotics in Young Children With Acute Bacterial Infections. *Clinical Pediatrics*, 43(3), pp.239–249. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15094948

Seki, H. et al., 2003. Prevention of antibiotic-associated diarrhea in children by Clostridium butyricum MIYAIRI. *Pediatrics international : official journal of the Japan Pediatric Society*, 45(1), pp.86–90. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12654076

Shamir, R. et al., 2005. Evaluation of a diet containing probiotics and zinc for the treatment of mild diarrheal illness in children younger than one year of age. *Journal of the American College of Nutrition*, 24(5), pp.370–5. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16192262

Shan, L.-S. et al., 2013. Prevention and treatment of diarrhoea with *Saccharomyces boulardii* in children with acute lower respiratory tract infections. *Beneficial Microbes*, 4(4), pp.329–334. Available at: http://www.ncbi.nlm.nih.gov/pubmed/24311316

Sharif, M.R. et al., 2016. The Effect of a Yeast Probiotic on Acute Diarrhea in Children. *Probiotics and Antimicrobial Proteins*, 8(4), pp.211–214. Available at: http://www.ncbi.nlm.nih.gov/pubmed/27530282

Simakachorn, N. et al., 2011. Tolerance, Safety, and Effect on the Faecal Microbiota of an Enteral Formula Supplemented With Pre- and Probiotics in Critically Ill Children. *Journal of Pediatric Gastroenterology and Nutrition*, 53(2), pp.174–181. Available at: http://www.ncbi.nlm.nih.gov/pubmed/21788759

Szajewska, H. et al., 2001. Efficacy of Lactobacillus GG in prevention of nosocomial diarrhea in infants. *The Journal of Pediatrics*, 138(3), pp.361–365. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11241043

Szymański, H. et al., 2008. Bifidobacterium longum&lt PL03, Lactobacillus rhamnosus KL53A, and Lactobacillus plantarum PL02 in the Prevention of Antibiotic-Associated Diarrhea in Children: A Randomized Controlled Pilot Trial. *Digestion*, 78(1), pp.13–17. Available at: http://www.ncbi.nlm.nih.gov/pubmed/18701826

Szymanski, H. et al., 2006. Treatment of acute infectious diarrhoea in infants and children with a mixture of three Lactobacillus rhamnosus strains - a randomized, double-blind, placebo-controlled trial. *Alimentary Pharmacology and Therapeutics*, 23(2), pp.247–253. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16393304

Thibault, H., Aubert-Jacquin, C. & Goulet, O., 2004. Effects of long-term consumption of a fermented infant formula (with Bifidobacterium breve c50 and Streptococcus thermophilus 065) on acute diarrhea in healthy infants. *Journal of pediatric gastroenterology and nutrition*, 39(2), pp.147–52. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15269618

Trois, L., Cardoso, E.M. & Miura, E., 2007. Use of Probiotics in HIV-infected Children: A Randomized Double-blind Controlled Study. *Journal of Tropical Pediatrics*, 54(1), pp.19–24. Available at: http://www.ncbi.nlm.nih.gov/pubmed/17878180

Urbańska, M. et al., 2016. Effectiveness of Lactobacillus reuteri DSM 17938 for the Prevention of Nosocomial Diarrhea in Children. *The Pediatric Infectious Disease Journal*, 35(2), pp.142–145. Available at: http://www.ncbi.nlm.nih.gov/pubmed/26551445

Villarruel, G. et al., 2007. Saccharomyces boulardii in acute childhood diarrhoea: a randomized, placebo-controlled study. *Acta Paediatrica*, 96(4), pp.538–541. Available at: http://www.ncbi.nlm.nih.gov/pubmed/17306006

Waligora-Dupriet, A.-J. et al., 2007. Effect of oligofructose supplementation on gut microflora and well-being in young children attending a day care centre. *International Journal of Food Microbiology*, 113(1), pp.108–113. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16996154

Zhao, H.-M. et al., 2014. [Clinical effect of triple therapy combined with Saccharomyces boulardii in the treatment of Helicobacter pylori infection in children]. *Zhongguo dang dai er ke za zhi = Chinese journal of contemporary pediatrics*, 16(3), pp.230–3. Available at: http://www.ncbi.nlm.nih.gov/pubmed/24661511

Annex 2. Conceptual proposal of an intervention to prevent diarrhea in children under 5 years old using probiotic supplementation and WASH improvement

TITTLE OF THE PROJECT

Probiotic supplementation and WASH improvement to prevent diarrhea in children under 5 years old

OVERVIEW

Project Duration	**3 years**
Intervention area	Low middle-income country to be determined
Project Objective	The overall objective of the project is to contribute to the reduction of mortality related to malnutrition among the children under 5 years old
Budget	Pending until setting is determined

1. Context

According to the World Health Organization, malnutrition contributes to nearly one-third of the infant mortality worldwide (1). Diarrhea is a leading cause of malnutrition in children under five years old and is the second cause of death in this age group. The situation is of particular concern in low-income countries where children experience on average three episodes of diarrhea every year in their first three years of life (2).

Evidence suggests the importance of implementing nutrition sensitive interventions since even when scaling up to 90% coverage of the proven nutrition specific interventions there is only a reduction of 35% in diarrhea-specific mortality and 20% in stunting (3).

As part of a nutrition-sensitive intervention, Water, Sanitation and Hygiene (WASH) programs could interrupt specific pathways for fecal-oral transmission while probiotics could contribute to support a healthy microbiota, and theoretically potentiate a reduction in the risk of diarrhea in children. This combination of strategies seems increasingly pertinent considering the rise in evidence regarding the involvement of environmental enteropathy (EE) in nutritional status. EE is characterized by a gut and systemic inflammation, alterations in gut barrier function, and absorptive capacity. Poor WASH practices have been associated with the prevalence of this condition (4) and probiotics have the potential to participate in EE modulation (5). Jointly they could tackle the underlying causes of malnutrition without waiting for the onset of symptoms especially considering that a child with EE might not present diarrhea.

The utilization of probiotics as a public health tool integrated response to benefit populations, which carry the highest burden of diarrhea could be key to interrupt the vicious diarrhea-malnutrition circle.

2. Outputs and Indicators

1. Prevent diarrhea episodes through probiotics supplementation
- Proportion of at-risk children receiving the intervention
- Product quality assurance by analysis of viable bacterial count
- Health outcome: mean diarrhea duration, children weight change, number of episodes, number of patients admitted to health facility, inpatient treatment duration, malnutrition rate
- Community perception: caregivers level of perception of the risk of diarrhea occurrence, benefits of treatment and acceptability of the administration method

2. Increased access to improved WASH practices
- Number of community members trained on management of water, sanitation and hygiene service
- Number of water points that are built or fixed
- Proportion of people with adequate domestic sanitation facilities
- Proportion of people provided with sustained access to safe water supply
- Number of households receiving a hygiene kit

3. Appropriate knowledge of WASH practices and probiotics use
- Number of community members trained on management of WASH services
- Number of hygiene awareness campaigns conducted
- Number of people receiving direct hygiene promotion and correct probiotics usage messages

3. Means of Verification

Output 1. Laboratory records of viable bacterial count of product. Health workers and hospital records.

Output 2. Training attendance records, work completion forms and water quality testing reports. Kit distribution reports.

Output 3. Education sessions attendance records. Awareness campaigns reports.

4. Activities

Output 1. Recruitment community health workers. Test viable bacterial count of product in different stages of the intervention at main storage and household level. Weekly visits to the houses and health facility. Distribution of the probiotic and usage guide. Individual interviews, and focus groups.

Output 2. Recruitment of a WASH team in the community. Instruction of local mechanics to maintain infrastructure and distribute the necessary tool kit. Test water quality water at distribution source and household. Distribution of hygiene kits and sensitization campaigns for their usage.

Output 3. Training of hygiene promotion leaders. Conduct tailored hygiene promotion campaigns and messaging. Creation of mother-to-mother groups to facilitate information exchange.

5. Methodology

Recruitment will be performed using hospital records, children under five years old will be eligible for the intervention. Supplementation of *L. rhamnosus GG* in a dosage of 5 x 10^{10} colony forming units will be provided once a day through the length of the study. The probiotic product will be delivered in sachets from a reliable source, from which the manufacturing process has been closely regulated and supply chain closely monitored. Distribution of product will be performed alongside the hygiene kit. Caregivers will be instructed on how to incorporate the sachet content into semi-solid foods and storage practices. Each child will receive weekly visits from the community health worker. In each visit diarrhea surveillance, anthropometric measures and evaluation of clinical outcomes will be assessed. Children who develop complication will be referred to the nearest health outpost for management by medical officers.

WASH improvements will be performed by a specialized team with support of local authorities and community groups. Most of the infrastructure activities will be completed in the first months of the project. Both interventions will be performed parallel to WASH practices and probiotics usage education and sensitization programs.

6. Beneficiaries

Direct beneficiaries: children under five years old
Indirect beneficiaries: the community and the national Health programs

7. Monitoring and Evaluation Approach

Field staff and supervisors are responsible for daily monitoring of activity process. Weekly reports will be performed by the WASH supervisor and the Nutrition specialist. Monthly interventions reports are assessed by the project coordinators. Financial reports are performed monthly and communicated to investors every six months. Independent auditors will assess the overall cost-effectiveness of the intervention.

8. Posible Partners

Local: Ministry of Health, NGOs, community groups, universities, hospitals, research centers
International: World Bank, UNICEF, OXFAM, ACF

9. Sustainability

Throughout the project implementation, the participating agencies will apply a community-empowerment approach for future sustainability. Community groups will be established to manage the physical infrastructure, services provision, and education programs in target areas. The maintenance of effective information exchange systems between the community, local organizations, public officials and international actors will help to promote the ownership of the project.

References

1. World Health Organization. Guideline: Updates on the Management of Severe Acute Malnutrition in Infants and Children [Internet]. World Health Organization. Geneva; 2013 [cited 2016 Nov 20]. Available from: www.who.int/about/licensing/copyright_form/en/index.

2. World Health Organization. Diarrhoeal disease: fact sheet [Internet]. WHO. World Health Organization; 2017 [cited 2017 May 7]. Available from: http://www.who.int/mediacentre/factsheets/fs330/en/

3. Bhutta ZA, Das JK, Rizvi A, Gaffey MF, Walker N, Horton S, et al. Evidence-based interventions for improvement of maternal and child nutrition: what can be done and at what cost? Lancet (London, England) [Internet]. 2013 Aug 3 [cited 2016 Dec 4];382(9890):452–77. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23746776

4. Galpin L, Manary MJ, Fleming K, Ou C-N, Ashorn P, Shulman RJ. Effect of Lactobacillus GG on intestinal integrity in Malawian children at risk of tropical enteropathy. Am J Clin Nutr [Internet]. 2005 Nov [cited 2016 Nov 20];82(5):1040–5. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16280436

5. Petri WA, Naylor C, Haque R, Haque R. Environmental enteropathy and malnutrition: do we know enough to intervene? BMC Med [Internet]. 2014 Oct 14 [cited 2017 May 17];12:187. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25604120