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Meta-analyses

Effects of probiotics and synbiotics on diarrhea in undernourished children: Systematic review with meta-analysis

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SUMMARY

Background: Undernutrition predisposes children to a greater incidence and duration of diarrhea. No review and meta-analysis have yet been conducted to assess effectiveness of probiotics and synbiotics in undernourished children.

Aims: To assess the effectiveness of probiotics and synbiotics on diarrhea in undernourished children.

Methods: Randomized, double-blind, placebo-controlled trials evaluating the effects of probiotics and synbiotics on diarrhea in undernourished children were searched from 1990 to May 2020. Recommendations of the Cochrane Handbook and the Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) statement were followed.

Results: The systematic review identified 15 trials with 6986 patients. The meta-analysis revealed that treatment with probiotic or synbiotic reduced significantly both the duration of diarrhea [Weighted mean difference (WMD) = −1.05 day, 95% CI (−1.98, −0.11)] and the hospital stay duration [Standard mean difference (SMD) = −2.87 days, 95% CI (−5.33, −0.42)], especially in specific patient subsets. In both groups, similar rates of vomiting and nutritional recovery were observed. No probiotics or synbiotics-related adverse effects were reported. Subgroup analyses showed that probiotic and synbiotic treatment were more effective in reducing risk of diarrhea in outpatients [Risk ratio (RR) = 0.86, 95%CI (0.75−0.98)].

Conclusion: This meta-analysis supports the potential beneficial roles of probiotics and synbiotics on diarrhea in undernourished children.

1. Introduction

Globally, an estimated 200 million children younger than 5 years of age suffer from undernutrition, contributing to 45% of all child deaths every year, and 11% of the total global disability-adjusted-life-years worldwide [1,2]. Undernutrition leads to long term consequences in impairing the cognitive and physical development of children, and the economic productivity of individuals and societies [3].

Diarrhea continues to be a leading cause of death in children younger than 5 years of age in low- and middle-income countries, and a leading cause of morbidity worldwide. It leads to a high rate of hospitalization, and is associated with an economic burden inflicted by parents' absence from work [4]. Furthermore, diarrhea has long been linked to a reciprocal cycle of malnutrition and infection among vulnerable children in low- and middle-income countries [5]. This highlights the importance of developing safe, efficient and multifactorial intervention strategies to address malnutrition and enteric infections as major public health challenges [6].

The relationship between gut microbiota, diarrhea and undernutrition has attracted attention in recent years. Undernutrition predisposes children to a greater incidence and duration of diarrhea, and it can be triggered or worsened by significant

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diarrhea [7]. Diarrhea leads to reduced absorption of carbohydrates, protein, potassium, zinc and other nutrients, further contributing to undernutrition [8]. Breastfeeding, food and water, sanitation and hygiene are major protective factors against undernutrition and diarrhea [9]. Early depletion in gut *Bifidobacterium longum*, and later, the absence of the healthy mature anaerobic gut microbiota leads to deficient energy harvest, vitamin biosynthesis and immune protection, and is associated with diarrhea, malabsorption and systemic invasion by microbial pathogens. These two events represent the main steps in gut microbiota alteration, and are associated with undernutrition [9].

Germ-free animal studies have provided evidence of the mechanisms by which gut immaturity contributes to undernutrition through interaction with the diet. In a study involving 377 Malawian twin pairs in the first three years of live, Smith et al. [10] characterized the gut microbiome of twin pairs discordant for kwashiorkor during and after treatment with ready-to-use therapeutic food. A model of gnotobiotic mice was used to test the effectiveness of the same nutritional intervention, following transplantation of frozen fecal communities from three twin pairs, with one twin from each pair suffering from kwashiorkor, into germ-free mice. The animals were then fed the low-caloric density and nutrient-deficient diet similar to that of Malawian children. After 3 weeks, their diet was switched to the high energy, micronutrient rich ready-to-use therapeutic food for 2 weeks and then switched back to the initial Malawian diet. A significative difference in weight change between mice transplanted with healthy and kwashiorkor fecal communities was observed. Furthermore, a more dramatic change in body weight when switching between diets in kwashiorkor mice than in the healthy control mice was reported. This study suggested clinically important differences in the gut microbiota of children who develop kwashiorkor versus their healthy twin, which may determine the response to dietary change. Additionally, the study indicated that gnotobiotic mice, transplanted with the fecal communities of different groups, might be a valuable model in devising individualized dietary interventions.

Recently, Gehrig et al. [11] have integrated preclinical gnotobiotic animal models with human studies to understand the contributions of perturbed gut microbiota development to childhood undernutrition and to identify new microbiota-directed therapeutic approaches. They identified a set of proteins that distinguish the plasma proteomes of healthy children from those with severe acute malnutrition. This finding allowed them to develop microbiota-directed complementary foods that shifted the plasma proteome of children with moderate acute malnutrition toward that of healthy individuals, including proteins involved in linear growth, bone development, neurodevelopment, and immune function.

The past decade has seen a new era in medical science with increased use of probiotics and synbiotics. Probiotics are “live microorganisms which, when administered in adequate amounts, confer a health benefit on the host” [12]. Prebiotics have been consensually defined as a substrate that is selectively utilized by host microorganisms conferring a health benefit [13], whereas synbiotics are a mixture of probiotics and prebiotics [14]. Probiotic microorganisms act via a variety of means, including modulation of immune function, production of organic acids and antimicrobial compounds, interaction with resident microbiota, interfacing with the host, improving gut barrier integrity and enzyme formation. Prebiotics effects include defense against pathogens, immune modulation, mineral absorption, bowel function, metabolic effects and satiety [15].

In well-nourished children, meta-analyses have shown that probiotics and synbiotics may reduce the risk of diarrhea, as well as the duration of diarrhea and hospitalization [16–18]. Furthermore, other studies have reported that probiotics and synbiotics may also enhance child growth by preventing infections and micronutrient deficiencies, as they have been shown to improve the absorption of certain nutrients (calcium, zinc and vitamin B12) [19,20] and reduce the risk of anemia [21].

Although the last few years have seen data emerge from many probiotics and synbiotics human studies, a search of the Cochrane Library, PubMed, and Scopus did not reveal any review and meta-analysis in undernourished population. This review aims to assess the effectiveness of probiotics and synbiotics (any specified strain or dose), compared with placebo, in the management of diarrhea in undernourished children.

2. Materials and methods

This systematic review was conducted according to the Cochrane Handbook for systematic reviews of intervention guidelines, the Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) statement, and the Cochrane statistical methods guidelines [22,23]. It did not involve any animal, cell or human experimental research.

2.1. Search strategy and selection criteria

The Cochrane Central Register of Controlled Trials (CENTRAL, the Cochrane Library), MEDLINE, and EMBASE databases were searched, with no language restriction nor geographical limits. The search strategy was conducted with Medical Subject Headings (MeSH) and search text word terms outlined in Table 1. Furthermore, reference lists of all included studies and review articles identified by the search were also checked to identify other relevant studies.

2.2. Eligibility and exclusion criteria

Only randomized controlled trials (RCTs) were eligible for inclusion. Participants were undernourished children. Childhood undernutrition encompassed various clinical forms, including stunting (low height-for-age < −2 Standard deviation (SD)), wasting (low weight-for-age <-2 SD) and underweight (low weight-for-height <-2 SD). Patients in the experimental groups received probiotics or synbiotics at any type, dose and duration. Subjects in the control group received placebo or no additional intervention. The primary outcome measures were the incidence of diarrhea; the duration of diarrhea, the duration of hospitalization; and the number of episodes of diarrhea. The secondary outcome measures were the duration of vomiting; the adverse effects of probiotics/synbiotics; and the nutritional recovery.

Exclusion criteria were: (i) studies in well-nourished children; (ii) studies no related to study outcomes; (iii) studies related to neonates or adult persons; (v) studies in animals; and (vi) no RCTs.

Table 1
Search terms used in search strategy.

1)	Malnutrition OR malnourished OR undernutrition OR underweight
2)	Severe acute malnutrition OR kwashiorkor OR marasmus OR stunting OR wasting
3)	Protein-energy malnutrition OR child nutrition disorders OR infant nutrition disorders
4)	OR 1-3
5)	Randomized controlled trial OR randomized OR randomly OR trial
6)	Controlled clinical trial OR clinical trial as topic OR placebo
7)	OR 5-6
8)	Probiotics OR probiotics and prebiotics OR Synbiotics OR fermented milk products
9)	4 AND 7 AND 8
10)	Limit 9 to human

2.3. Selection of studies and data extraction

Two review authors (RMK and FIN) independently screened all studies by title and abstract. Studies fulfilling the inclusion criteria have been selected. Disagreements was resolved by discussion between the 2 authors. Two investigators (GAN and JBK) independently extracted the data from each included study. The following data were extracted: author, year of publication, country, age group, patient source, type of undernutrition, sample size, experimental and control treatments, dose, and duration of intervention. Data regarding main outcome indicators included the following: incidence of diarrhea, duration of diarrhea, duration of hospitalization, and number of episodes of diarrhea per child year. Secondary outcome data included duration of vomiting, adverse effects of probiotics and synbiotics, and nutritional recovery. If the study data were unclear, we contacted the trial authors to obtain clarification.

2.4. Risk of bias assessment

Risk of bias was assessed independently by 2 authors (GAN and JBK), and disagreements were resolved by discussion between the 2 authors, and, when necessary, by consulting a third review author (RMK). The Revised Cochrane risk-of-bias tool for randomized trials (RoB2) was used. The risk of bias parameters included the type of randomization method (selection bias), allocation concealment (selection bias), blinding of participants and personnel (performance bias), blinding of outcome assessment (detection bias) and incomplete outcome data (attrition bias). Each trial was graded as 'yes', 'probably yes', no', 'probably no', or 'no information' with respect to the abovementioned aspects, representing a high risk of bias, a low risk of bias, or some concerns bias, respectively [24].

2.5. Rating the quality of evidence

The GRADE (Grading of Recommendations, Assessment Development and Evaluations) approach was used to assess the quality of the supporting evidence for main outcomes. GRADE pro software (https://gdt.grade pro.org) [25] was used to import the data from Review Manager 5.3 (RevMan 5.3) to create "summary of findings" tables. These tables provided outcome-specific information concerning the overall quality of evidence derived from the included studies, the magnitude of the effects of the interventions examined and the sum of the available data on the outcomes that were considered. The quality of the evidence was categorized as high, moderate, low or very low based on consideration of the risk of bias, the directness of evidence, consistency and precision of the estimates.

2.6. Statistical analysis

Statistical analyses were performed using RevMan 5.3 software. Regardless of heterogeneity between the pooled studies, we

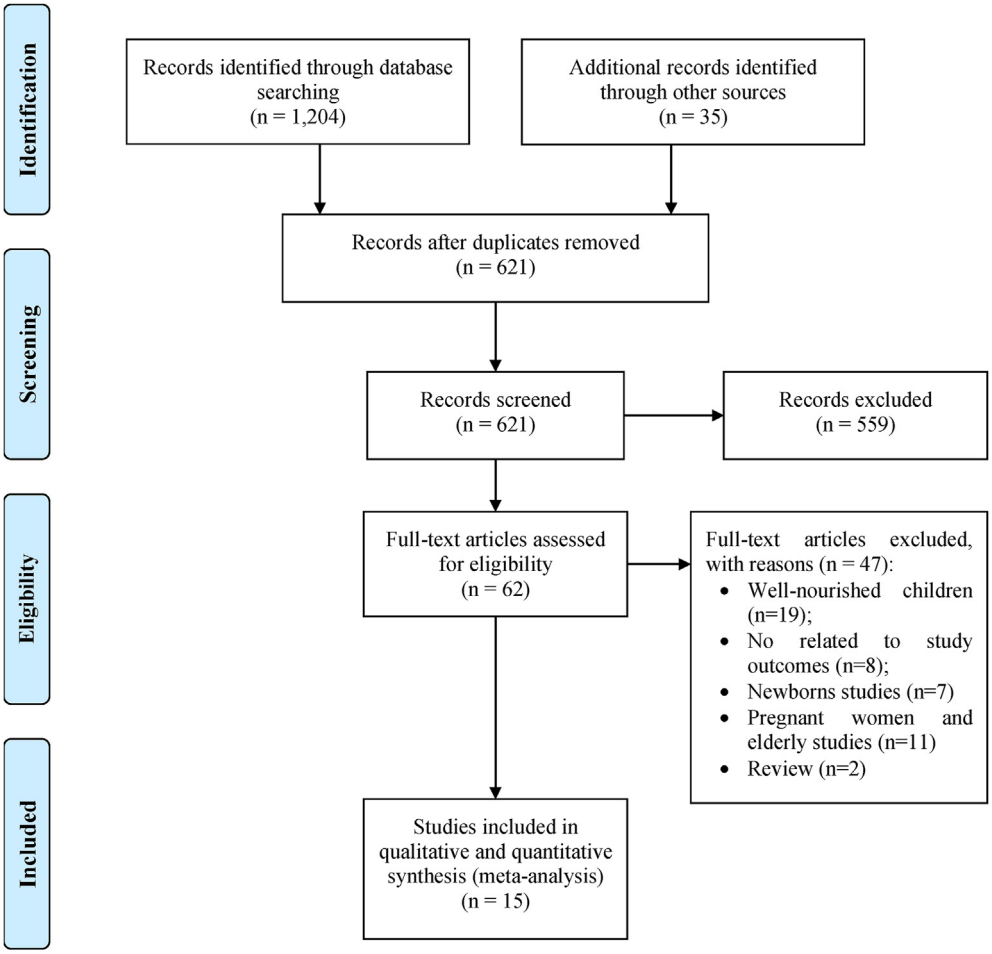


Fig. 1. PRISMA flow diagram for selection of studies.

Table 2
Characteristics of the included studies.

Study	Country	Age range (month)	Patient source	Type of undernutrition	n (exp/control)	Intervention	Control	Dosage (CFU/day)	Duration
Aflatoonian et al., 2020 [36]	Iran	24–59	Outpatients	Underweight	69 (37/32)	Synbiotic Probiotics: *Lactobacillus acidophilus, Lactobacillus rhamnosus, Lactobacillus bulgaricus, Lactobacillus casei, Bifidobacterium infantis, Bifidobacterium breve*, and *Streptococcus thermophilus* Prebiotic: Fructo-oligosaccharide	Placebo sachet daily which similar taste, smell, and appearance with synbiotic sachet	1.0×10^9	30 days
Kara et al., 2019 [30]	Turkey	6–59	Outpatients	Underweight, Stunting and wasting	100 (50/50)	*Lactobacillus rhamnosus* GG	No treatment	1.0×10^9	3 months
Grenov et al., 2017a [29]	Uganda	6–59	Inpatients	Wasting	400 (200/200)	*Bifidobacterium animalis* subsp *lactis* and *Lactobacillus rhamnosus*	One sachet daily containing 1g of maltodextrin without probiotic	1.0×10^{10}	8–12 weeks
Grenov et al., 2017b [29]	Uganda	6–59	Outpatients	Wasting	327 (164/163)	*Bifidobacterium animalis* subsp *lactis* and *Lactobacillus rhamnosus*	One sachet daily containing 1g of maltodextrin without probiotic	1.0×10^{10}	8–12 weeks
Sur et al., 2011 [31]	India	12–59	Outpatients	Underweight	3585 (1802/1783)	*Lactobacillus casei*	The same nutrient drink daily without probiotic	6.5×10^9	12 weeks
Sawazal et al., 2010 [37]	India	12–36	Outpatients	Wasting ad stunting	422 (205/217)	Synbiotic Probiotic: *Bifidobacterium lactis* HN019 Prebiotic: Oligosaccharide	The same milk without Synbiotic	1.9×10^7	1 year
Basu et al., 2009a [28]	India	Unclear	Inpatients	Underweight	371 (186/185)	*Lactobacillus rhamnosus* GG	ORS without probiotics	10^{12} CFU	Minimum period of 7 days
Basu et al., 2009b [28]	India	Unclear	Inpatients	Underweight	373 (188/185)	*Lactobacillus rhamnosus* GG	ORS without probiotics	10^{10} CFU	Minimum period of 7 days
Kerac et al., 2009 [38]	Malawi	5–168	Inpatients and outpatients	Wasting	795 (399/396)	Synbiotic Probiotics: *Pediococcus pentosaceus, Leuconostoc mesenteroides, Lactobacillus paracasei* ssp *paracasei* and *Lactobacillus plantarum* Prebiotic: oat bran, inulin, pectin, and resistant starch	RUTF without Synbiotic	1×10^{11}	10 weeks
Basu et al., 2007 [32]	India	>24	Inpatients	Underweight	235 (117/118)	*Lactobacillus rhamnosus* GG	Readymade disposable poly packs of ORS without *Lactobacillus rhamnosus* GG	6×10^7	Minimum 7 days or till diarrhea has stopped
Agarwal 2002a [27]	India	6–59	Inpatients and outpatients	Underweight	102 (52/50)	*Lactococcus lactis, Lactococcus lactis cremoris*, and *Leuconostac mesenteroides cremoris*	Ultra-heat-treated yoghurt preparation	1×10^8	3 months
Agarwal 2002b [27]	India	6–59	Inpatients and outpatients	Underweight	98 (48/50)	*Lactobacillus casei, Lactobacillus bulgaricus* and *Streptococcus thermophilus*	Ultra-heat-treated yoghurt preparation	1×10^8	3 months
Saran 2002 [33]	India	24–59	Outpatients	Stunting	100 (50/50)	*Lactobacillus acidophilus*	an isocaloric supplement (two biscuits each)	1×10^8	6 months
Oberhelman et al., 1999 [34]	Peru	6–24	Outpatients	Underweight	204 (99/105)	*Lactobacillus rhamnosus* GG	Identical-appearing placebo capsule administered in identical fashion.	3.7×10^{10}	15 months
Raza et al., 1995 [35]	Pakistan	1–24	Inpatients	Underweight	40 (21/19)	*Lactobacillus rhamnosus* GG	Microcrystalline cellulose with identical appearance mixed in 10 ml of ORS twice a day	1×10^{10}	2 days

CFU: Colony-forming units; exp: exposed; ORS: Oral Rehydration Salts; RUTF: ready-to-use therapeutic food.

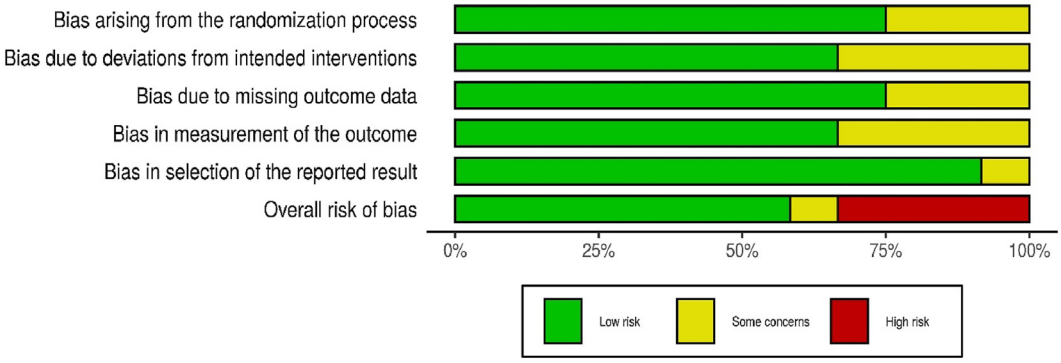


Fig. 2. Bias risk assessment of the RCTs on the effects of probiotics and synbiotics in undernourished children with diarrhea.

used a random-effects model to synthesize all data. We sought missing data from the trial authors. Outcome measures were analyzed on an Intention-to-treat population. Continuous outcome variables were assessed with Mean Differences (MDs) and 95% confidence intervals (CIs), and dichotomous outcomes were evaluated with aggregated risk ratios (RRs) and 95% CIs. We calculated the rate ratio of episode rates (episodes per child year) of diarrhea between two groups and the standard error of rate ratio according to the Cochrane Handbook for Systematic Reviews of Interventions [23]. We used the generic inverse variance data to pool these studies.

Statistical heterogeneity was evaluated with the Chi^2 test with significance being set at P value < 0.1. The degree of heterogeneity among the studies was measured by the I^2 statistic. An I^2 statistic <40% was considered to be a low level of heterogeneity, 41%–60% a moderate level, 61%–90% a substantial heterogeneity, and >90% a considerable heterogeneity [26]. We did not assess the presence of publication bias in this review, due that funnel plots are difficult to detect with small numbers of studies (i.e. less than 10) in systematic reviews.

Subgroup analyses were conducted to evaluate the influence of patient source, type of undernutrition, combination of probiotics, dose, and duration of intervention on the main overall outcome indicators. We did not perform a sensitivity analysis since only a few studies were included in each subgroup.

3. Results

3.1. Study selection

A total of 1239 studies were included after an initial search, of which 1181 studies did not fulfil the inclusion criteria as determined by screening the titles and abstracts. Sixty-two potential trials were selected for full-text assessment, and 47 studies were excluded. A total of 15 RCTs were included in the meta-analysis. The detailed selection process is presented in Fig. 1.

3.2. Characteristics of the included studies

A total of 6986 participants (3618 in the experimental group and 3368 in the control group) in 15 RCTs were identified in the literature. Two experimental arms in the study of Agarwal et al. [27] were listed separately to exhibit different strains of probiotics, which were marked as Agarwal 2002a and Agarwal 2002b. Similarly, two experimental arms in the study of Basu et al. [28] were listed separately to exhibit different doses of probiotics, which were marked as Basu 2009a and Basu 2009b. In the study of Grenov et al. [29], two experimental arms were also listed separately to exhibit different management and follow-up, which were marked as Grenov 2017a and Grenov 2017b. Eleven trials were conducted in Asia, three in Africa and one in South America. Patients were followed up as outpatients (7 RCTs), inpatients (5 RCTs) and both (3 RCTs). Twelve studies [27–35] presented the results of probiotic interventions, whereas synbiotic interventions were reported in 3 trials [36–38]. Six trials used a mixture of probiotics including 2 to 7 strains [27,29,36,38], while 8 studies used a single probiotic strain [28,30–35,37]. *Lactobacillus, Bifidobacterium, Streptococcus, Lactococcus, Pediococcus* and *Leucostonac* were the 6 genera of probiotics used in the trials. *Lactobacillus* was the most commonly genus of probiotics used (13 RCTs), including the *Lactobacillus rhamnosus* GG strain in 9 RCTs. The daily dose of probiotics in these trials ranged from 1.9×10^7 to 1×10^{12} colony-forming units (CFU). Other detailed characteristics of the included RCTs are presented in Table 2.

3.3. Risk of bias assessment

Most trials were at risk of bias for at least one of the domains (see Fig. 2). Only 72% of trials adequately generated their randomization sequence; Fifty-six percent adequately concealed allocation, and 61% blinded participants, study personnel, and outcome assessors. There were incomplete outcome data in almost 28% of trials. Overall, only five studies were considered to be at low risk of bias with regard to adequate randomization, allocation concealment, blinding, and follow-up.

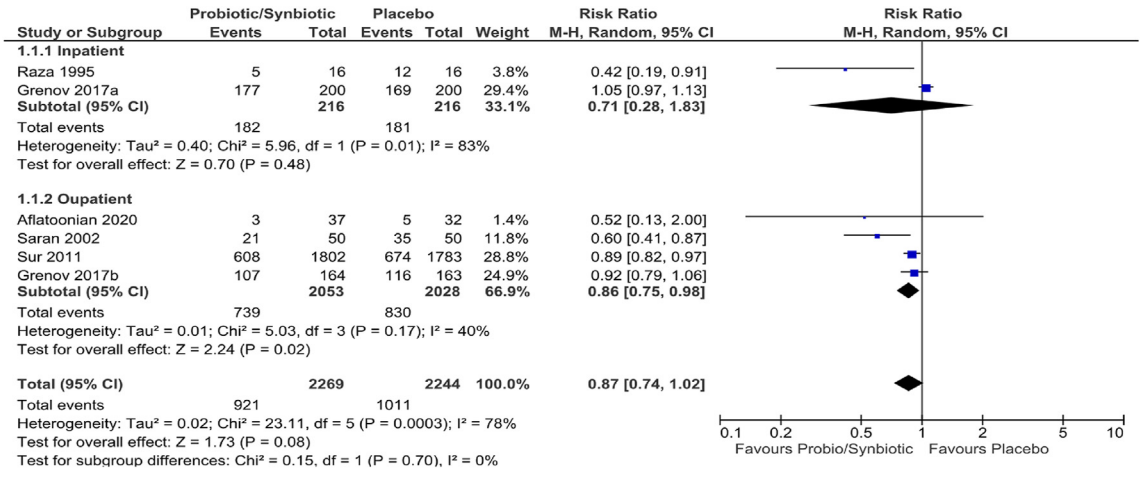
3.4. Main outcome indicators

3.4.1. Incidence of diarrhea

A total of 6 studies [29–31,33,36] with 1932 individuals reported the incidence of diarrhea. A pooled analysis of data from these studies revealed that, compared with placebo, probiotics and synbiotics did not reduce significantly the risk of diarrhea [RR 0.87, 95% CI (0.74, 1.02)]. There was a substantial heterogeneity among studies ($P < 0.1$, $I^2 = 78\%$) (Fig. 3A).

3.4.2. Duration of diarrhea

Nine trials [27–29,32–34] were included in the pooled analysis of the effects of probiotics and synbiotics on the duration of diarrhea, including 1094 undernourished children allocated to treatment groups, and 900 undernourished children allocated to control groups. The pooled results suggest that probiotic and synbiotic supplementation can reduce the duration of diarrhea in undernourished children with diarrhea [WMD -1.05 day, 95% CI



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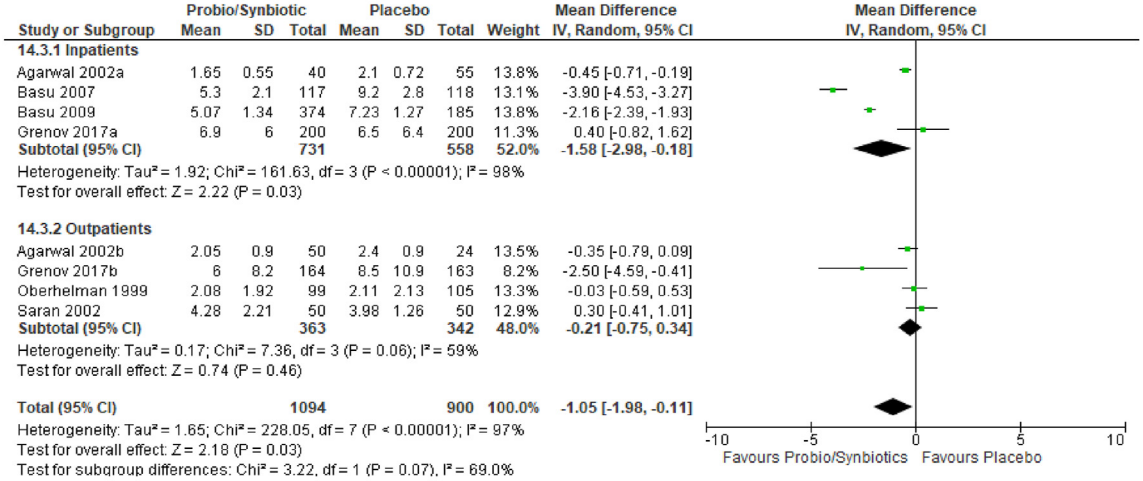

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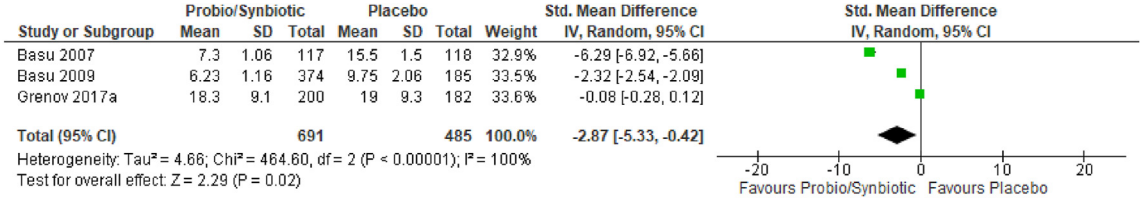

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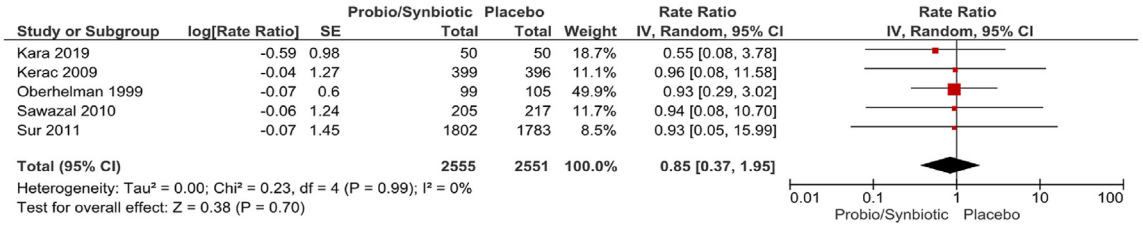
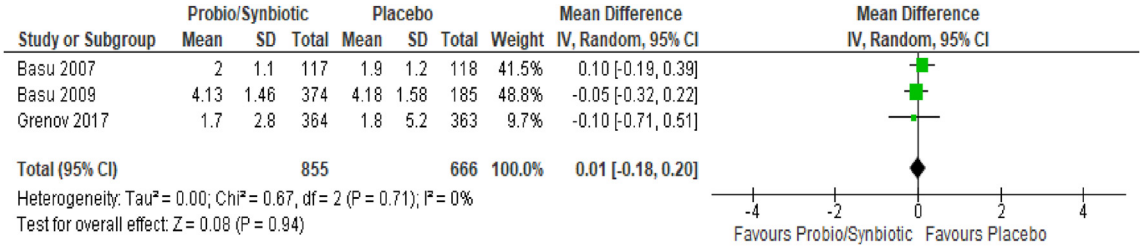


D

Fig. 3. A: Meta-analysis results for probiotics and synbiotics on incidence of diarrhea in undernourished children. B: Meta-analysis results for probiotics and synbiotics on duration of diarrhea in undernourished children. C: Meta-analysis results for probiotics and synbiotics on duration of hospitalization in undernourished children. D: Meta-analysis results for probiotics and synbiotics on number of episodes of diarrhea in undernourished children.



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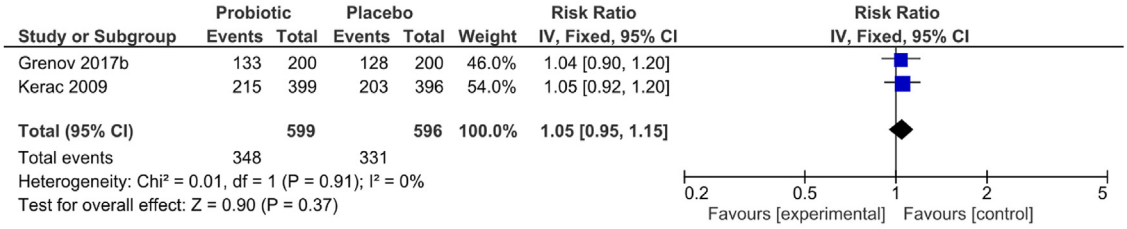


B

Fig. 4. A: Meta-analysis results for probiotics and synbiotics on duration of vomiting in undernourished children. B: Meta-analysis results for probiotics and synbiotics on nutritional recovery in undernourished children.

(−1.98, −0.11)]. There was a considerable heterogeneity among trials (I^2 = 96%, $P < 0.1$) (Fig. 3B).

3.4.3. Duration of hospitalization

Four studies [28,30,32] were included in the meta-analysis of the effects of probiotics and synbiotics on the duration of hospitalization, including 691 undernourished children assigned to treatment groups and 485 undernourished children assigned to control groups. The aggregated results suggest that probiotic and synbiotic supplementation can considerably reduce the duration of hospitalization in undernourished children with diarrhea [Standard Mean Difference (SMD) = −2.87 days, 95% CI (−5.33, −0.42)], and the heterogeneity among trials was considerable (I^2 = 98%, $P < 0.1$) (Fig. 3C).

3.4.4. Number of episodes of diarrhea

Five trials [29,31,34,37,38] reported the total number of episodes of diarrhea, including 2555 undernourished children in the probiotic and synbiotic group, and 2551 undernourished children in the placebo group. Pooled analyses showed that across these studies, the episode rates of diarrhea were not statistically significant between groups [rate ratio 0.85, 95%CI (0.37, 1.95)]. There was no statistically significant heterogeneity between studies (P = 0.99; I^2 = 0%) (Fig. 3D).

3.5. Secondary outcome indicators

3.5.1. Duration of vomiting

A total of 5 studies reported the duration of vomiting, including 855 individuals in the probiotic and synbiotic group, and 666 individuals in the placebo group. Compared with the placebo group, no statistically significant reduction in the duration of vomiting was noted with the probiotic and synbiotic group [WMD -0.01 day, 95% CI (−0.18, 0.15)]. There was no statistically significant heterogeneity between studies (P = 0.49, I^2 = 0%) (Fig. 4A).

3.5.2. Adverse effects

Data regarding probiotics and synbiotics-related adverse events were collected in twelve trials [27−32,35,37,38]. In eleven of them, adverse events were not observed. In one trial [35], authors reported one case of myoclonic jerks of the limbs among two children, one receiving *Lactobacillus casei* and one receiving placebo.

3.5.3. Nutritional recovery

Two trials [29,38] reported the nutritional recovery, including 599 undernourished children in the probiotic and synbiotic group and 596 undernourished children in the placebo group. Pooled analyses showed that there was no statistically significant difference between groups [RR 1.05, 95% CI (0.95, 1.95)]. There was no statistically significant heterogeneity between studies (P = 0.91, I^2 = 0%) (Fig. 4B).

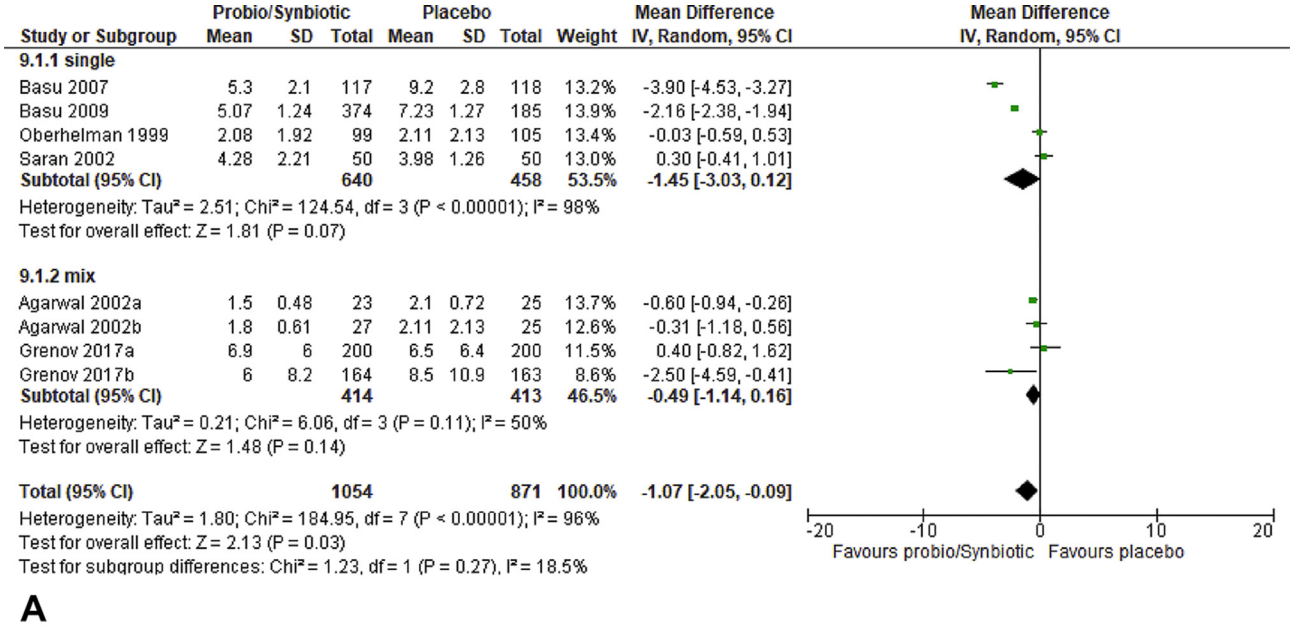
3.6. Subgroup analyses

Subgroup analyses were conducted based on sociodemographic and intervention features such as patient source, type of undernutrition, combination of probiotics, dose, and duration of intervention. Patient source explained the statistically heterogeneity concerning incidence of diarrhea. Indeed, the test of subgroup differences (outpatients versus inpatients) showed that there was no statically significant heterogeneity (P = 0.70, I^2 = 0%). Compared with placebo, probiotics and synbiotics reduced significantly the risk of diarrhea in outpatients [RR 0.86, 95%CI (0.75−0.98)] (Fig. 3A).

Regression analysis between the duration of diarrhea and probiotic type and dose revealed that different types and doses of probiotic/synbiotic contributed to the heterogeneity. The test of subgroup differences (single versus combination of probiotics) showed that there was no statically significant heterogeneity ($P = 0.27$, $I^2 = 18.5\%$) (Fig. 5A). Similarly, the test of subgroup differences (<10 billion CFU versus ≥ 10 billion CFU) showed that there was no statically significant heterogeneity ($P = 0.86$, $I^2 = 0\%$) (Fig. 5B).

4. Discussion

This systematic review identified 15 trials that randomized 6986 undernourished children with diarrhea in a control group with an experimental group treated with probiotic or synbiotic. The analysis revealed that treatment with probiotic or synbiotic reduced significantly both the duration of diarrhea and the hospital stay duration. In both groups, similar rates of vomiting and nutritional recovery were observed. No probiotics or synbiotics-related



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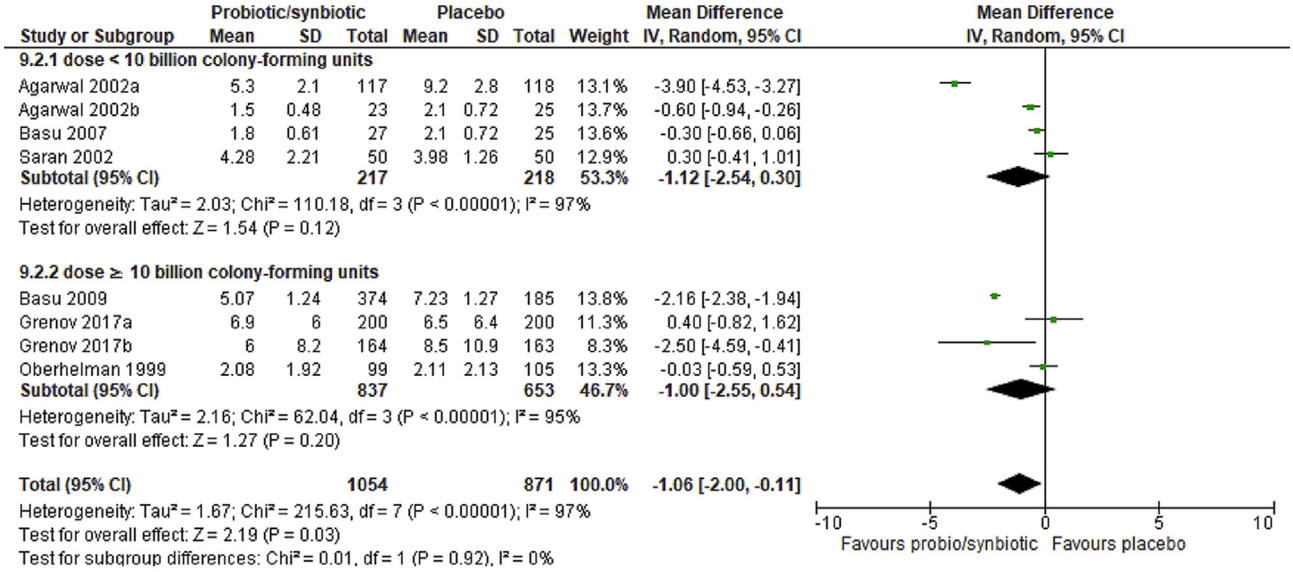
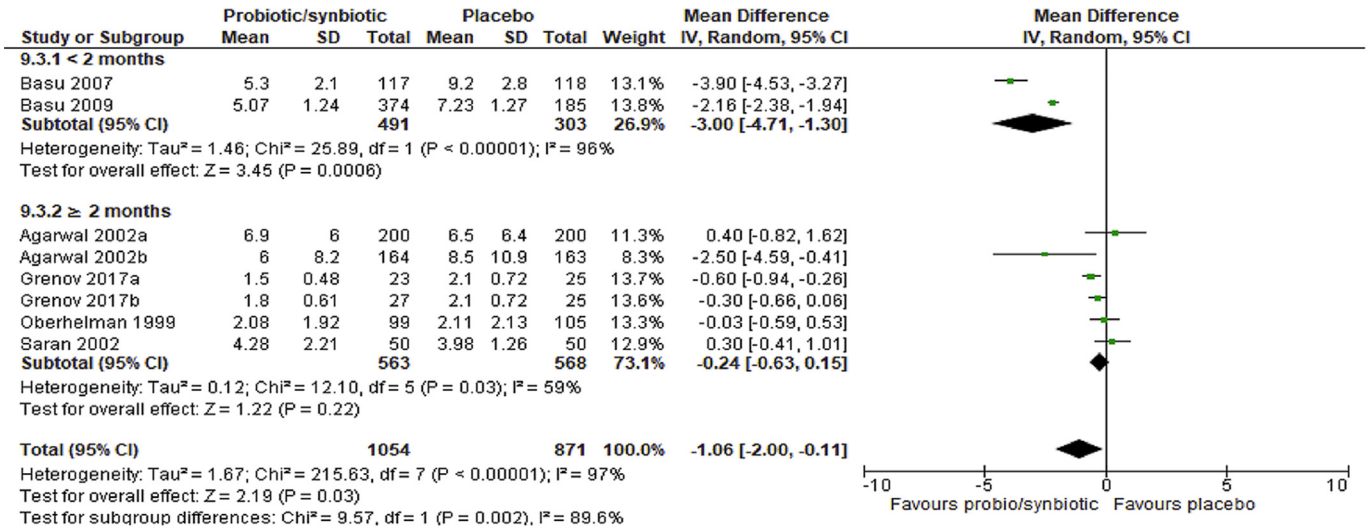
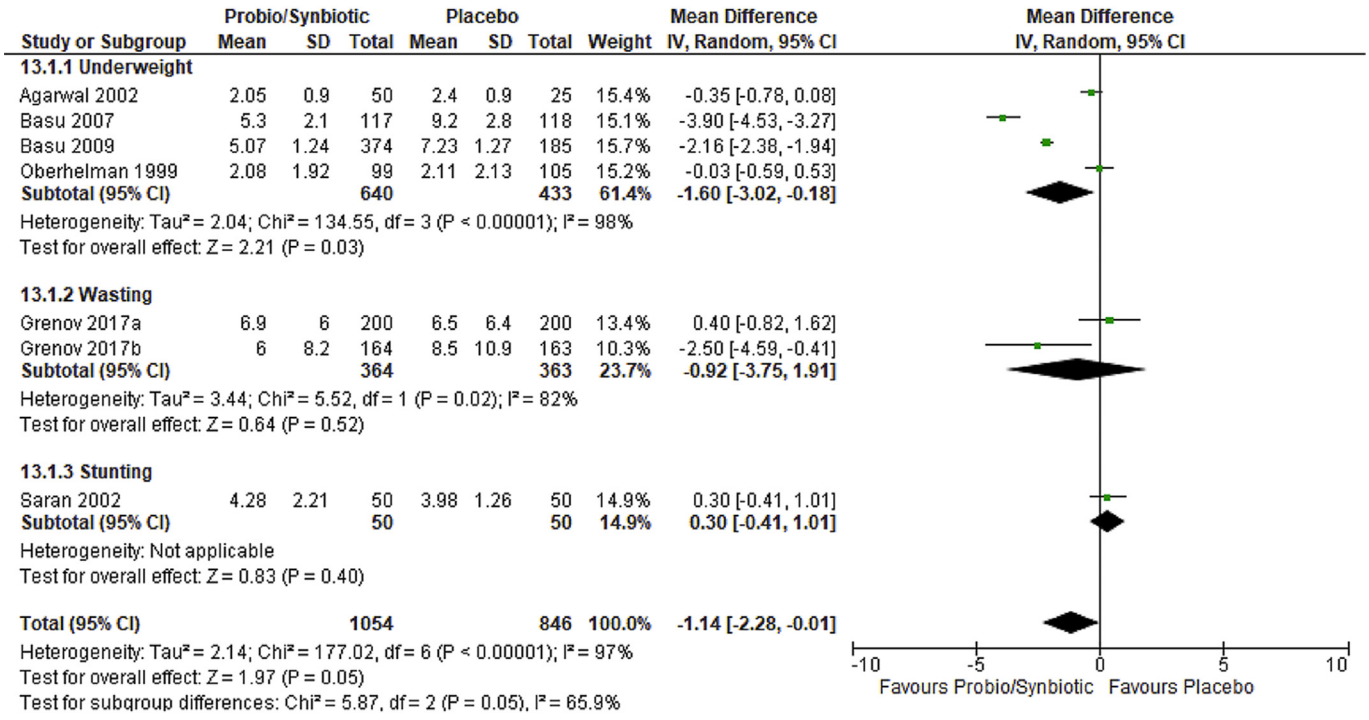


B

Fig. 5. A: Subgroup analysis regarding duration of diarrhea: single versus combination of probiotics. B: Subgroup analysis regarding duration of diarrhea: dose of probiotics 10^{10}. C: Subgroup analysis regarding duration of diarrhea: duration of intervention. D: Subgroup analysis regarding duration of diarrhea: types of undernutrition.



C



D

Fig. 5. (*continued*).

adverse effects were reported. Subgroup analyses showed that probiotic or synbiotic treatment was more effective in reducing risk of diarrhea in outpatients.

The beneficial effects of probiotics or synbiotics in reducing the duration of diarrhea and the hospital stay duration highlighted in this meta-analysis is consistent with studies' findings conducted in well-nourished children with acute diarrhea [16–18,39]. Undernutrition and diarrhea are major causes of morbidity and mortality among children worldwide. When they coincident, they place the child at increased risk of death and disability [40,41]. The situation is of particular concern in low-income countries where children under three years old experience on average three episodes of diarrhea every year. Each episode deprives the child of the nutrition necessary for growth [42]. In resource-poor settings, the high diarrheal disease burden is related to increased transmission of disease pathogens through ingestion of fecally contaminated water or food [40]. Children living in deprived areas with poor sanitation, inadequate hygiene, and unsafe drinking water have greater exposure to enteric pathogens and an increased risk of morbidity and severity of diarrheal illnesses [43–45].

Diarrhea is a common complication in children hospitalized with undernutrition. Mortality for children who had diarrhea at any

Table 3
Rating the quality of evidence.

Certainty assessment							№ of patients		Effect		Certainty	Importance
№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	probiotics or synbiotics	Placebo	Relative (95% CI)	Absolute (95% CI)		
Incidence of diarrhea												
6	randomized trials	serious[a]	serious[b]	not serious	not serious	none	921/2269 (40.6%)	1011/2244 (45.1%)	RR 0.87 (0.74–1.02)	59 fewer per 1 000 (from 117 fewer to 9 more)	⊕⊕◯◯ LOW	IMPORTANT
Duration of diarrhea												
9	randomized trials	serious[c]	serious[d]	not serious	not serious	none	1104	1106	–	MD 1.03 lower (1.74 lower to 0.32 lower)	⊕⊕◯◯ LOW	IMPORTANT
Duration hospitalization												
4	randomized trials	serious[e]	serious[f]	not serious	not serious	none	691	688	–	MD 4.05 lower (7.47 lower to 0.62 lower)	⊕⊕◯◯ LOW	IMPORTANT
Episodes of diarrhea												
5	randomized trials	serious[g]	not serious	not serious	not serious	none	2555	2551	Rate ratio 0.85 (0.37–1.95)	per 1000 patient(s) per years	⊕⊕⊕◯ MODERATE	IMPORTANT

CI: Confidence interval; RR: Risk ratio; MD: Mean difference; a, c, e, and g: Downgraded one level for limitation in study design due to a high risk of attrition bias; b, d and f: Downgraded one level for considerable heterogeneity.

time during their admission is significantly higher than the mortality without diarrhea as reported in studies' findings conducted in Kenya [46]. Severely undernourished children often experience long hospital stay duration ranged 2–4 weeks [47]. Both community- and nosocomially-acquired diarrhea are major challenges to the successful management of children hospitalized with severe undernutrition, as reported elsewhere [48,49].

Management of diarrhea and dehydration continue to be controversial issues in the treatment of malnutrition. No specific WHO guidelines exist to provide guidance on the treatment of diarrhea in children with undernutrition. The implementation of a program including both Water, Sanitation and Hygiene (WASH) improvement and probiotic supplementation as a public health strategy in low and middle-income countries could be an attractive approach. It would prevent the diarrhea occurrence, and reduce the diarrhea duration and the hospital stay duration [50]. WASH programs (including hand washing with soap, improved sanitation, and water supply) 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. Hence, the combination of probiotics with WASH could be potentially beneficial in disrupting the vicious diarrhea-malnutrition circle and its consequences, particularly in high disease endemic areas.

In the subgroup analyses, no significant differences between combination or single-strain probiotics were observed in terms of incidence of diarrhea, duration of diarrhea and hospitalization. Chapman et al. showed the beneficial effects of mixtures of probiotics on diarrhea, respiratory tract infections and gut microbiota modulation [51]. However, Grandy et al. revealed no differences in the duration of diarrhea when they compared the use of a single probiotic *(Saccharomyces boulardii)* against a group receiving a combination of them [52]. Similarly, Grenov et al. reported no effect of mixtures of probiotics (*Bifidobacterium animalis subsplactis* and *L. rhamnosus* GG) on diarrhea in children with severe acute malnutrition [29], while Dubey et al. observed a reduction in the duration of diarrhea, stool frequency, and overall better recovery rates by incorporation of eight different strains [53]. The effect of combination versus single strain probiotics remain inconclusive. Negative effects related to competition among different probiotic strains may occur, and more trials are needed to evaluate how to combine probiotics so that they can act synergistically.

In poor settings, the cost of probiotics/synbiotics may limit their accessibility. In Uganda, dairy farmers and small-scale entrepreneurs were taught to convert their milk into a probiotic yoghurt using an innovative bacterial starter culture and basic equipment [54]. This self-support provides better cost-effectiveness. The implementation of such projects in poor settings could be one of the keys in the control of malnutrition and diarrhea.

The study presents several strengths. To our knowledge, this meta-analysis is the first to investigate probiotics or synbiotics effects for the treatment of diarrhea in undernourished children. We included 15 RCTs involving 6986 undernourished children and we performed a detailed subgroup analysis to explore the sources of heterogeneity between trials. Finally, we used the GRADE approach to assess the quality of evidence (Table 3).

There are several limitations of our study. First, the findings were limited by the available studies. The overall quality of studies was low, largely due to missing information regarding randomization procedures and missing outcome data, all of which affect internal validity. Second, the categories of undernourished individuals were not differentiated in trials. Indeed, the effectiveness of probiotics or synbiotics can be dramatically altered by the cause of undernutrition (lack of intake or lack of absorbance). Third, heterogeneity between individual trials was observed, related to

the different phenotypes of undernutrition, dose, duration and choice of strain of probiotics, and severe/moderate undernutrition phenotype.

5. Conclusion

Current evidence supports the potential role of probiotics and synbiotics in the management of diarrhea in undernourished children. A probiotic/synbiotic intervention capable of reducing durations of diarrhea and hospitalization in undernourished children, even with modest efficacy, would have an enormous impact in resource-poor settings. Its broad implementation should be envisaged in order to improve the prognosis of undernourished children.

Author contributions

RMK and DVDL conceptualized the study. RMK and FIN selected and screened studies. GAN and JBK extracted data from included trials. RMK was responsible of data analysis. RMK wrote the manuscript. DVDL and LBB critically revised the manuscript. All authors read and approved the final manuscript.

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Conflict of interest

The authors declare no conflict of interest.

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