

➤ Efficacy of probiotics in prevention of acute diarrhoea: a meta-analysis of masked, randomised, placebo-controlled trials

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Lancet Infect Dis 2006; 6:
374–82

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To evaluate the evidence for the use of probiotics in the prevention of acute diarrhoea, we did a meta-analysis of the available data from 34 masked, randomised, placebo-controlled trials. Only one trial was community based and carried out in a developing country. Most of the remaining 33 studies were carried out in a developed country in a health-care setting. Evaluating the evidence by types of acute diarrhoea suggests that probiotics significantly reduced antibiotic-associated diarrhoea by 52% (95% CI 35–65%), reduced the risk of travellers' diarrhoea by 8% (–6 to 21%), and that of acute diarrhoea of diverse causes by 34% (8–53%). Probiotics reduced the associated risk of acute diarrhoea among children by 57% (35–71%), and by 26% (7–49%) among adults. The protective effect did not vary significantly among the probiotic strains *Saccharomyces boulardii*, *Lactobacillus rhamnosus* GG, *Lactobacillus acidophilus*, *Lactobacillus bulgaricus*, and other strains used alone or in combinations of two or more strains. Although there is some suggestion that probiotics may be efficacious in preventing acute diarrhoea, there is a lack of data from community-based trials and from developing countries evaluating the effect on acute diarrhoea unrelated to antibiotic usage. The effect on acute diarrhoea is dependent on the age of the host and genera of strain used.

Introduction

Each year 4 billion diarrhoeal episodes occur worldwide, accounting for 4% of all deaths and 5% of days lost to disability. At an individual level, acute diarrhoea causes impairment in intestinal absorption of both micronutrients and macronutrients, malnutrition, and growth faltering.^{1,2} Prevention of acute diarrhoea is an important public-health challenge. A ubiquitous, simple, safe, and cost-effective intervention to prevent acute diarrhoea and its adverse health effects would have considerable public-health implications.

Hand washing is known to reduce the risk of acute diarrhoea. However, attempts to improve hand washing rates are limited by inadequate evidence of its cost-effectiveness, lack of evidence for its effectiveness, and the inevitable complexity of modifying human behaviours.^{3,4} Improving sanitary conditions, drinking water, and food preservation and handling methods can also prevent acute diarrhoea. However, it is unlikely that substantial improvement in these areas will be achieved in most developing countries in the near future.^{5,6} More recently, vaccines have been proposed as potential candidates to prevent acute diarrhoea, but an effective and affordable vaccine to prevent diarrhoea at the population level is not yet available.⁷

There is growing evidence that zinc⁸ and probiotics, either singly or in a combination, can effectively prevent diarrhoea. Probiotics are either monocultures or mixed cultures of live organisms that, applied to animals or human beings, beneficially affect the host by improving the properties of indigenous microflora, hampering the growth of diarrhoeal pathogens, and boosting cellular and humoral immunity.⁹ Although there is evidence for the therapeutic benefits of probiotics in viral or antibiotic-associated diarrhoea among children,^{10,11} evidence for the

role of probiotics in preventing acute diarrhoea is not clear. Two previously published meta-analyses included trials mostly carried out in developed countries and provide data on the efficacy of *Saccharomyces boulardii* and *Lactobacillus acidophilus* in preventing only antibiotic-associated diarrhoea.^{12,13} Two recently published reviews mainly focused on trials done in high-income countries in hospital settings, and were restricted to infants and children only.^{2,14}

We did a comprehensive analysis of data from all the currently available trials to evaluate the evidence for the efficacy of probiotics in preventing acute diarrhoea, and evaluated the efficacy of probiotics by strain, age groups, causes of acute diarrhoea, and varied formulations.

Methods

Search strategy and selection criteria

We searched the PubMed, Medline, Embase, and Cochrane Controlled Trials Registry (CENTRAL) databases using the keywords “probiotic”, “diarrhea”, “acute diarrhea”, “antibiotic associated diarrhea”, “traveler’s diarrhea”, “bacterial diarrhea”, “nosocomial diarrhea”, “diarrhea prophylaxis”, “*S boulardii*”, “*L rhamnosus* GG”, “*L acidophilus*”, “*L bulgaricus*”, “*Bifidobacterium*”, “*Lactobacilli*”, and “*Sacchromyces*” in different combinations. Publications in both English and French published up to February 2006 were included. We sought the references of the published review articles, and personally contacted researchers known to be working in this field to ensure the comprehensiveness of the search. Pre-defined inclusion criteria included only randomised, masked, placebo-controlled trials in which the experimental arm and the control arm differed only by the provision of a probiotic and in which the risk of acute diarrhoea in each arm was presented.

Data extraction and quality assessment of trials

Standardised, detailed forms for extraction of data from the selected trials (webappendix 1) were developed. The descriptive details, setting, strain of probiotic used, formulation and dosage of probiotic, duration and method of follow-up, and p values were extracted. The methodological details of each trial were also extracted so that we could score the trials for overall design.

We assessed the quality of each trial using previously published and validated checklists.¹⁵⁻¹⁷ The trials were primarily assessed for randomisation, masking, and dropouts from the trial.

Three authors (GH, PM, and SD) evaluated articles for eligibility and quality and abstracted the data independently. Any disagreement among authors in scoring or data abstraction was resolved by discussion and review of the publication(s).

Statistical analyses

All statistical analyses were done using Stata version 8 (Stata Corporation, College Station, TX, USA). Incidence of acute diarrhoea was identified as the outcome of interest, and estimated from the proportion of patients who had acute diarrhoea during follow-up, since this was the only variable consistently available.

The DerSimonian-Laird random effects model¹⁸ was used to calculate pooled risk ratios, 95% CIs, and weights. χ^2 tests were done to determine statistical heterogeneity across the trials. The proportion of total statistical heterogeneity not explained by chance was estimated using the I^2 statistic. I^2 (calculated as $I^2 = 100\% \times (Q - df) / Q$; where Q is Cochran's heterogeneity statistic and df is the degrees of freedom) lies between 0% and 100%; hence, values less than 0% or more than 100% were set as 0% and 100%, respectively.^{19,20} Heterogeneity was explored by doing subgroup and stratified analyses including the effects of age, setting of the trial, type of diarrhoea, probiotic strain(s) used, formulation of probiotics administered, influence of setting, and quality score of trials.

Publication bias was assessed by funnel plot asymmetry,^{21,22} by means of plotting the standard error against the precision for each trial. Assuming that trials with either no statistical significance or possible reverse effect were not published, we used the "Fail-safe N" method²³ to estimate the number of such studies that would be needed to change the overall conclusion of our meta-analysis. This method estimates the number of unpublished studies with non-significant p values needed to overturn the results obtained using published studies. We assumed a p value of more than 0.05 for studies with statistically non-significant 95% CIs that did not report the p values. Further, to provide an estimate of effect size after correcting for potential publication bias, we used the "trim and fill" method.^{24,25} This method first trims the asymmetric trials on the right side of funnel plot, leaving a symmetric remainder to estimate the true centre of funnel. The trimmed trials are then added and their

assumed counterparts (mirror images) are filled, thus enabling adjusted overall confidence intervals to be calculated. Influence analysis (pooled effect size estimation after systematically dropping trials one at a time) was done to estimate the robustness of the findings and evaluate if the results were heavily influenced by any particular trial. To express our results in clinical terms, we estimated the number needed to treat (NNT) to prevent a case of acute diarrhoea among children or adults. We estimated the absolute difference in the proportion of acute diarrhoea in the intervention group and the control group, and inverted it.²⁶

Results

Search results

Our original search yielded 690 publications. 28 of these trials fulfilled the eligibility criteria for inclusion in our analysis (figure 1 and table 1). Webappendix 2 contains a list of the 25 studies that were excluded following detailed evaluation. Five of the included trials^{27,34,37,46,50} presented two or more independently analysable results that were distinctly different from each other; hence, we considered them as individual trials. Therefore a total of 34 trials were used for analysis.

Trial descriptions

The 34 trials comprised 4844 patients. One trial was triple masked,⁴⁶ one was single masked,⁵¹ and the rest

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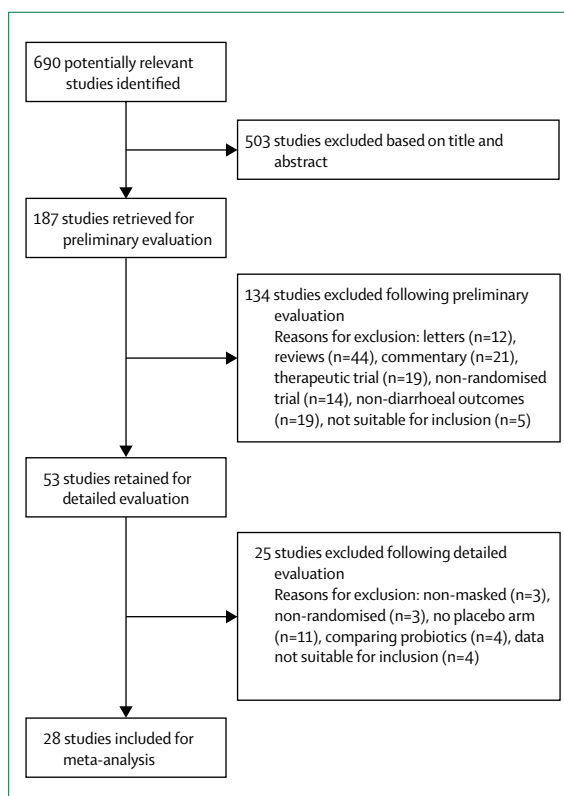


Figure 1: Trial flow for masked, randomised, placebo-controlled trials

were double masked. We categorised acute diarrhoea as antibiotic-associated diarrhoea (n=19) and travellers' diarrhoea (n=6). All non-antibiotic-associated diarrhoea and non-travellers' diarrhoea was categorised as other

Reference	Quality score*	Setting	Diarrhoea	Sample size (treatment group; placebo group)	Age group	Follow-up duration	Follow-up visitation	Probiotic(s) (formulation)	Dosage
Pozo-Olano et al ²⁷ (a)	4	Community	Travellers'	31 (17; 14)	Adults	8 days	Passive	<i>Lactobacillus acidophilus</i> plus <i>Lactobacillus bulgaricus</i> (granules)	30 × 10 ⁷ to 60 × 10 ⁷ counts of lactobacilli per tablet Four tablets at each mealtime for about 8 days
Pozo-Olano et al ²⁷ (b)	4	Community	Travellers'	51 (27; 24)	Adults	28 days	Passive	<i>L acidophilus</i> plus <i>L bulgaricus</i> (granules)	30 × 10 ⁷ to 60 × 10 ⁷ counts of lactobacilli per tablet Four tablets at each mealtime for about 8 days
Gotz et al ²⁸	4	Health care	Antibiotic associated	79 (36; 43)	Adults	Hospital stay	Active	<i>L acidophilus</i> plus <i>L bulgaricus</i> (granules)	One packet of Lactinex four times a day for first 5 days of ampicillin therapy
Clements et al ²⁹	3	Health care	<i>Escherichia coli</i> diarrhoea	48 (23; 25)	Adults	Up to 5 days	Active	<i>L acidophilus</i> plus <i>L bulgaricus</i> (granules)	2 × 10 ⁸ viable <i>L acidophilus</i> plus <i>L bulgaricus</i> per dose
Wunderlich et al ³⁰	4	Health care	Antibiotic associated	45 (23; 22)	Adults	7 days	Active	<i>Enterococcus</i> SF68 (capsule)	7.5 × 10 ⁶ lyophilised bacteria twice daily
Surawicz et al ³¹	5	Health care	Antibiotic associated	180 (116; 64)	Adults	17.3 ± 8.6 days	Active	<i>Saccharomyces boulardii</i> (capsule)	1 g per day
Tankanow et al ³²	4	Health care	Antibiotic associated	38 (15; 23)	Children	10 days	Passive	<i>L acidophilus</i> plus <i>L bulgaricus</i> (granules)	5.1 × 10 ⁸ lactobacilli per 1 g of packet; administered four times a day for 10 days
Oksanen et al ³³	4	Community	Travellers'	756 (373; 383)	10–80 years	1–2 weeks	Active	<i>Lactobacillus rhamnosus</i> GG (powder)	2 × 10 ⁸ bacteria per day for 1–2 weeks
Orrhage et al ³⁴ (a)	4	Health care	Antibiotic associated	20 (10; 10)	Adults	21 days	Active	<i>Bifidobacterium longum</i> BB 536 plus <i>L acidophilus</i> NCFB 1748 (fermented milk product)	5 × 10 ⁷ to 2 × 10 ⁸ cfu/mL of fermented milk of <i>B longum</i> BB 536 plus 2 × 10 ⁸ to 3 × 10 ⁸ cfu/mL of fermented milk of <i>L acidophilus</i> NCFB 1748
Orrhage et al ³⁴ (b)	4	Health care	Antibiotic associated	20 (10; 10)	Adults	21 days	Active	<i>B longum</i> BB 536 (fermented milk product)	5 × 10 ⁷ to 2 × 10 ⁸ cfu/mL of <i>B longum</i> BB 536
Saavedra et al ³⁵	5	Health care	Acute	55 (29; 26)	Children	14 days	Active	<i>Bifidobacterium bifidum</i> plus <i>Streptococcus thermophilus</i> (powder)	1.9 × 10 ⁸ cfu/g of <i>B bifidum</i> plus 0.14 × 10 ⁸ cfu/g of <i>S thermophilus</i>
McFarland et al ³⁶	5	Health care	Antibiotic associated	193 (97; 96)	Adults	7 weeks	Active	<i>S boulardii</i> (capsule)	3 × 10 ¹⁰ cfu given for a maximum of 4 weeks
Katellaris et al ³⁷ (a)	4	Community	Travellers'	181 (80; 101)	Adults	3 weeks	Passive	<i>Lactobacillus fermentum</i> strain KLD (capsule)	10 ¹¹ cfu daily for 3 weeks or until diarrhoea occurred
Katellaris et al ³⁷ (b)	4	Community	Travellers'	202 (101; 101)	Adults	3 weeks	Passive	<i>L acidophilus</i> (capsule)	10 ¹¹ cfu daily for 3 weeks or until diarrhoea occurred
Hilton et al ³⁸	4	Health care	Travellers'	245 (126; 119)	Adults	2743 travel days	Active	<i>L rhamnosus</i> GG (capsule)	20 × 10 ⁸ cfu per day for 1–3 weeks
Lewis et al ³⁹	5	Health care	Antibiotic associated	69 (33; 36)	Adults	7 days	Active	<i>S boulardii</i> (capsule)	113 g twice daily until administration of antibiotics
Arvola et al ⁴⁰	4	Health care	Antibiotic associated	119 (61; 58)	Children	2 weeks	Passive	<i>L rhamnosus</i> GG (capsule)	2 × 10 ¹⁰ cfu twice daily during antimicrobial therapy.
Vanderhoof et al ⁴¹	5	Health care	Antibiotic associated	188 (93; 95)	Children	10 days	Active	<i>L rhamnosus</i> GG (capsule)	Body weight less than 12 kg: 1 × 10 ¹⁰ to 2 × 10 ¹⁰ cfu once daily; Body weight over 12 kg: 1 × 10 ¹⁰ to 2 × 10 ¹⁰ cfu twice daily, with antimicrobial treatment
Oberhelman et al ⁴²	5	Community	General	204 (99; 105)	Children	31 270 child days	Active	<i>L rhamnosus</i> GG (liquid cherry gelatin)	3.7 × 10 ¹⁰ organisms once daily for 6 days a week for 15 months
Thomas et al ⁴³	5	Health care	Antibiotic associated	267 (133; 134)	Adults	21 days	Active	<i>L rhamnosus</i> GG (capsule)	20 × 10 ⁸ cfu per day for 14 days
Szajewska et al ⁴⁴	5	Health care	Nosocomial	81 (45; 36)	Children	3 days	Active	<i>L rhamnosus</i> GG (powder)	6 × 10 ⁹ cfu twice daily
Armuzzi et al ⁴⁵	4	Health care	Antibiotic associated	60 (30; 30)	Adults	7 days	Passive	<i>L rhamnosus</i> GG (powder)	6 × 10 ⁹ viable bacteria twice daily for 7 days

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Cremonini et al ⁴⁵ (a)	4	Health care	Antibiotic associated	42 (21; 21)	Adults	4 weeks	Passive	<i>L rhamnosus</i> GG (powder)	Twice daily during <i>Helicobacter pylori</i> treatment week, and a week thereafter
Cremonini et al ⁴⁵ (b)	4	Health care	Antibiotic associated	42 (22; 21)	Adults	4 weeks	Passive	<i>S boulardii</i> (powder)	Twice daily during <i>H pylori</i> treatment week, and a week thereafter
Cremonini et al ⁴⁵ (c)	4	Health care	Antibiotic associated	42 (21; 21)	Adults	4 weeks	Passive	Combination containing bifidobacteria (powder)	Twice daily during <i>H pylori</i> treatment week, and a week thereafter
Jirapinyo et al ⁴⁷	4	Health care	Antibiotic associated	18 (8; 10)	Children	Hospital stay	Active	<i>L acidophilus</i> plus <i>Bifidobacterium infantis</i> (capsule)	One capsule three times a day for 7 days
Beniwal et al ⁴⁸	4	Health care	Antibiotic associated	202 (105; 97)	Adults	8 days	Active	<i>L acidophilus</i> plus <i>L bulgaricus</i> , <i>S thermophilus</i> (yogurt)	10 ⁶ cultures per g of yogurt (8 oz container) twice daily for 8 days
Plummer et al ⁴⁹	5	Health care	<i>Clostridium difficile</i> associated	138 (69; 69)	Adults	20 days	Active	<i>L acidophilus</i> plus <i>B bifidum</i> (capsule)	2 × 10 ¹⁰ cfu <i>L acidophilus</i> and <i>B bifidum</i> per capsule; each patient received one capsule per day for 20 days
Weizman et al ⁵⁰ (a)	5	Health care	Infectious	129 (71; 58)	Children	12 weeks	Active	<i>Bifidobacterium lactis</i> BB12 (cow milk powder)	1 × 10 ⁷ cfu/g of formula powder for 12 weeks
Weizman et al ⁵⁰ (b)	5	Health care	Infectious	123 (65; 58)	Children	12 weeks	Active	<i>Lactobacillus reuteri</i> (cow milk powder)	1 × 10 ⁷ cfu/g of formula powder for 12 weeks
Pereg et al ⁵¹	4	Community	Infectious	502 (254; 248)	Adults	8 weeks	Passive	<i>Lactobacillus casei</i> (yogurt)	10 ¹⁰ cfu per day per 100 mL yogurt for 48 days
Chouraqui et al ⁵²	4	Health care	Infectious	90 (46; 44)	Children	137.1 ± 60.1 days	Active	<i>B lactis</i> BB12 (acidified infant formula)	At least 10 ⁸ cfu/g per day
Kotowska et al ⁵³	5	Health care	Antibiotic associated	269 (132; 137)	Children	2 weeks	Passive	<i>S boulardii</i> (capsule)	250 mg twice daily for duration of antibiotic treatment
Correa et al ⁵⁴	5	Health care	Antibiotic associated	157 (80; 77)	Children	30 days	Active	<i>B lactis</i> plus <i>S thermophilus</i> (fortified infant formula)	10 ⁷ cfu/g of <i>B lactis</i> and 10 ⁶ of <i>S thermophilus</i> for 15 days

*Maximum score of 5. cfu=colony forming unit.

Table 1: Details of included trials

acute diarrhoea (n=9). The age range was 6 months to 71 years. 12 trials were in children (≤18 years), and 21 trials in adults (>18 years). Individuals aged 10–80 years were included in one trial.³³ Major strains of probiotics used were: *S boulardii* (n=5), *L rhamnosus* GG (n=10), and *L acidophilus* plus *L bulgaricus* (n=7). 25 of the trials administered the probiotics either as a capsule, tablets, powder, or granules. Nine trials administered it by pre-mixing with a food vehicle such as flavoured gelatin,⁴² fermented milk,³⁴ or yogurt.⁴⁸ A substantial proportion of the reported trials were carried out in health-care facilities (either hospitals or clinics); very few (n=4) were undertaken in community settings (table 1). In all but two trials, the episodes of diarrhoea were reported as a proportion of patients developing diarrhoea; two trials^{42,53} reported episodes of acute diarrhoea as the numerator and person-time as the denominator. There were no methodological problems identified in the studies that could potentially have invalidated/biased the results. The median quality assessment score was 4 out of a maximum of 5.

Overall effect of probiotics

28 trials had protective point estimates; ten of them attained statistical significance (webfigure 1). Six trials had statistically non-significant non-protective point

estimates (figure 2). The pooled estimate of efficacy of probiotics in prevention of diarrhoea was a reduction of 35% (95% CI 22–44%; p<0.001), with substantial heterogeneity (χ^2 p<0.001; I²=63%, 95% CI 52–75%). The results of the overall and subgroup analyses are summarised in table 2 and figure 2.

Effect by types of acute diarrhoea

Of 19 trials with data on antibiotic-associated diarrhoea, 18 had positive point estimates; six of these attained statistical significance, with an overall reduction of 52% (95% CI 35–65%; p<0.001; webfigure 2). Of six trials in travellers' diarrhoea, three had positive point estimates, although none of these results attained statistical significance. An overall reduction of 8% (95% CI –6 to 21%; p=0.235) with statistically non-significant heterogeneity (χ^2 p=0.455) was observed. Of nine trials with data on other types of acute diarrhoea, seven had positive point estimates, and four achieved statistical significance, with an overall reduction of 34% (95% CI 8–53%; p=0.013). Between strata I² was 89%, whereas adjusted within strata I² was 56% (95% CI 46–69%).

Effects of age

All 12 trials with data on children showed protective point estimates for reducing acute diarrhoea; seven

See Online for webfigures 1 and 2

	Number of trials	Effect size		Heterogeneity test
		Risk ratio	95% CI	I ² (95% CI)
Overall effect	34	0.65	0.55-0.78*†	63% (52-75)
Types of diarrhoea				
Antibiotic-associated diarrhoea	19	0.48	0.35-0.65*†	53% (40-68)
Travellers' diarrhoea	6	0.92	0.80-1.06	0% (0-1)‡
Others	9	0.66	0.47-0.92*†	72% (51-100)
Age group				
Children	12	0.43	0.29-0.65*†	80% (61-100)
Adult	21	0.74	0.59-0.94*	42% (32-54)
Probiotic strain				
<i>Saccharomyces boulardii</i>	5	0.48	0.24-0.96*	58% (35-95)
<i>Lactobacillus rhamnosus</i> GG	10	0.72	0.57-0.93*†	66% (48-93)
<i>Lactobacillus acidophilus</i> plus <i>Lactobacillus bulgaricus</i>	7	0.70	0.36-1.33*	78% (54-100)
Other single strain	6	0.83	0.62-1.09*	17% (12-26)
Other combination of strains	6	0.48	0.34-0.67	0% (0-5)
Formulation				
Capsule, tablet, powder, or granules (C/T/P/G)	25	0.66	0.52-0.83*†	59% (48-74)
Pre-mixed with a food vehicle (PFV)	9	0.57	0.41-0.81*	72% (51-100)
Formulation and age group				
C/T/P/G in children	7	0.35	0.18-0.68*	73% (50-100)
PFV in children	5	0.54	0.31-0.94*	80% (52-100)
C/T/P/G in adults	17	0.80	0.61-1.04*	44% (33-58)
PFV in adults	4	0.62	0.45-0.86	0% (0-2)
Assigned quality score				
≤4	21	0.74	0.61-0.91*	40% (31-52)
>4	13	0.52	0.36-0.74*	78% (60-100)

* χ^2 test of heterogeneity: p<0.05. †Publication bias: p<0.05. ‡Upper limit of 95% confidence interval rounded off to 1.

Table 2: Results of the overall and subgroup analyses

trials attained statistical significance. In children, the overall reduction was 57% (95% CI 35–71%; p<0.001; webfigure 3). Of 21 trials with data on adults, 15 showed a protective point estimate and three attained statistical significance, with an overall reduction of 26% (95% CI 7–41%; p=0.011; webfigure 3). One trial was not included in this analysis since it included patients aged 10–80 years. However, I² was still 65% (95% CI 54–78%) after adjusting for age.

Effect by probiotic strain

Among the five trials with data on effects of *S. boulardii*, four had positive point estimates and two attained statistical significance with an overall reduction of 52% (95% CI 4–76%; p=0.037). Of the ten trials using *L. rhamnosus* GG, all but one trial had a positive point estimate and three attained statistical significance, with an overall reduction of 28% (95% CI 7–43%; p=0.011). *L. acidophilus* plus *L. bulgaricus* were used in seven trials. The point estimates for four trials were positive and two of them attained statistical significance. An overall reduction of 30% for the *L. acidophilus* plus *L. bulgaricus* trials was not statistically significant (p=0.275). All six trials that used combinations of two or more probiotics resulted in protective point estimates; three attained

statistical significance with an overall pooled reduction of 52% (95% CI 33–66%; p<0.001; webfigure 4). Within strain I², corrected for heterogeneity between strains, was 59% (95% CI 48–72%).

Effect by mode of delivery of probiotic

Among 25 trials administering probiotics as capsules, tablets, granules, or powder, 19 had protective point estimates and six of them attained statistical significance (webfigure 5). The overall pooled reduction was 34% (95% CI 17–48%; p<0.001). All nine trials administering probiotic premixed with a food vehicle had a protective point estimate; three attained statistical significance, with an overall reduction of 43% (95% CI 19–60%; p=0.005). The effect by mode of delivery was not influenced by patient age. Stratification by mode of delivery and age did not explain the observed heterogeneity; within strata I² corrected for between strata was 65% (95% CI 53–78%).

Effect by assigned quality scores

The protective effect of probiotics did not differ significantly between the studies with quality scores of four or less and studies with quality scores of more than four.

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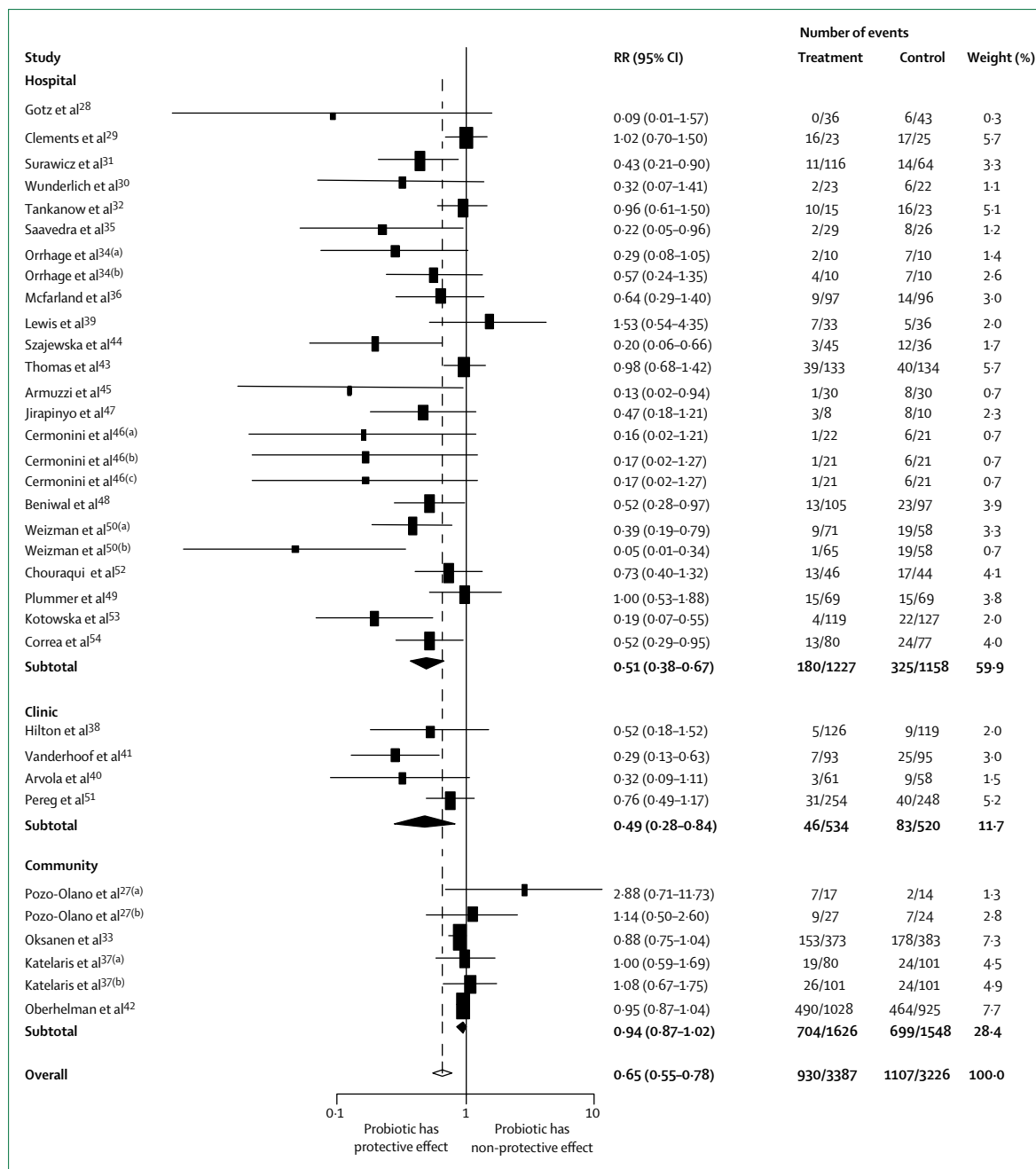


Figure 2: Effects of probiotics on diarrhoeal morbidity

The rectangles represent the risk ratios of the study and the size of the rectangle represents the weight given to each study in the meta-analysis. The black diamonds represent the overall risk ratios for the hospital-based studies, clinic-based studies, and community-based studies. The clear diamond and vertical broken line represent the overall risk ratio. The solid vertical line is the null value.

Publication bias, influence analysis, and NNT analysis

We found evidence of publication bias by the Egger (weighted regression) and Begg (rank correlation) methods (webfigure 6 and webfigure 7).^{21,22} By the fail-safe N method, we estimated that a total of 330 unpublished trials with non-significant p values were required to overturn the current results. Using the trim and fill

method (figure 3), correcting for publication bias yielded an overall reduction of 33% (95% CI 21–44%), which is not very different from the uncorrected overall reduction of 35% (95% CI 22–45%).

Although the results of this meta-analysis were heavily influenced by Oberhelman and colleagues' trial,⁴² influence analysis revealed that excluding this trial did

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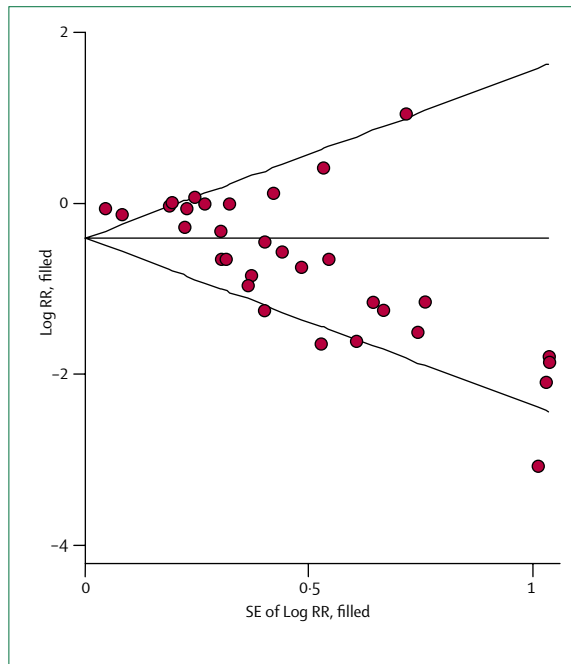


Figure 3: Trim and fill plot for publication bias

not have a significant effect on the direction and magnitude of preventive effects of probiotics.

NNT analyses by age group revealed that for every six children given probiotics, one child could be prevented from having acute diarrhoea. One adult in every four could be prevented from having acute diarrhoea by administration of probiotics.

Discussion

To our knowledge, this is the only comprehensive meta-analysis that examines the preventive role of probiotics by different age groups, setting, cause of acute diarrhoea, probiotic strains, and commonly used probiotic formulation(s). Our results highlight the fact that very few trials have been carried out in the community setting. There has been only one community-based trial to evaluate the efficacy of probiotics in preventing acute diarrhoea among young children in developing countries, where a large proportion of diarrhoeal burden exists. The abstracted data indicate that most of the trials are of adequate quality, since most of them are less than two decades old. However, short follow-up and not estimating person-time analysis (which is the most appropriate type of analysis in a recurrent disease such as diarrhoea) are limitations. There was a large variation in the dosage of probiotics, frequency of administration, and formulations used. Further variation was seen with regard to the timing of administration relative to a number of factors, including travel,^{33,38} concurrent treatment with antimicrobial therapy,^{30,55} administration within 72 hours of initiation of antimicrobial therapy to prevent antibiotic-associated diarrhoea,^{31,33,43,49} or

administration together with eradication therapy for *Helicobacter pylori*.^{35,46} These factors need to be considered when interpreting and extrapolating overall results.

Despite individual variation in methods and intervention among the included studies, the overall analysis suggests that probiotics are efficacious in preventing acute diarrhoea. Although variable, the magnitude of this effect shows a reduction of at least 21%. These results concur with those reported in other meta-analyses^{2,14} with a subset of studies.

Pooled analysis of zinc supplementation trials suggested an 18% (95% CI 7–28%) efficacy of zinc supplementation in preventing acute diarrhoea.^{8,36} Thus probiotics appear to have a marginally higher impact; however, zinc supplementation trials have been predominantly done in developing countries and with longer follow-up. A high proportion of the probiotic trials are hospital-based studies, done among children suffering from antibiotic-associated diarrhoea in developed countries. Given that both probiotics and zinc supplementation can have an important role in the prevention of acute diarrhoea, it will be worthwhile to explore if administering both treatments together would have a synergistic effect.

The effect size of probiotic treatment does vary by type of diarrhoea. The effect size was highest and more conclusive for antibiotic-associated diarrhoea, with results from 18 trials. The effect size was lowest for travellers' diarrhoea, and with data from only six studies, evidence for this effect seems inconclusive. A moderately significant impact was observed for effect on non-antibiotic-associated and non-travellers' diarrhoea.

This analysis also compared the efficacy of probiotics in preventing acute diarrhoea among children with that in adults. Although a statistically significant protection was observed in both children and adults, the effect size was significantly higher among children when compared with adults. These differences may be due to differences between colonisation and gut flora in children and adults, or the higher prevalence of antibiotic-associated and infectious diarrhoea among children. This analysis did not have the ability to evaluate the effect of probiotics in preventing diarrhoea among breast-fed infants aged 6–12 months⁴² and nosocomial infection-related diarrhoea in children. This question remains unresolved.⁵⁷

For a microorganism to be classified as a probiotic it has to be of human origin, exhibit non-pathogenic properties, be viable in delivery vehicles, be stable in acid and bile, adhere to target epithelial tissue, persist within the gastrointestinal tract, produce antimicrobial substances, modulate the immune system, and influence metabolic activities.⁵⁸ The variety of microorganisms that meet these requirements may or may not have similar impacts on specific health outcomes. Data from this analysis does not indicate any meaningful differences in impact between different strains, even

Search strategy and selection criteria

Details of the search strategy and selection criteria can be found in the Methods section.

after considering the confidence intervals of the effect size. Non-significant results with wide confidence intervals were only observed in studies using *L acidophilus* plus *L bulgaricus*, despite similar point estimates, suggestive of greater variability among studies. All other subgroups of probiotics evaluated showed a statistically significant preventive effect; studies using a combination of strains showed marginally better effects. Unfortunately, we were unable to evaluate variability in efficacy of probiotics by the dosage of probiotic or inoculum size of pathogen.

There is some evidence of publication bias in the reported trials. However, it is highly unlikely that this bias has substantially affected the magnitude and direction of impact of probiotics, nor would it change or invalidate the findings of these analyses.

There is evidence of heterogeneity in impact beyond chance in the studies included in this meta-analysis. Stratification of data based on the trial characteristics that could potentially have contributed to this heterogeneity yielded within strata I^2 estimates (corrected for between strata heterogeneity) of more than 50% in all analyses. Thus, it seems that there is either an inherent variation in impact or an unmeasured factor relating to impact. Given an I^2 estimate of 0% in travellers' diarrhoea, which is usually caused by a narrow range of bacterial agents, probiotics may have a variable effect in diarrhoea of different aetiologies. Since none of the studies have evaluated aetiology it is not possible to ascertain this effect. However, variation occurs more in the magnitude of the effect rather than the direction of effect, and since the aetiology of diarrhoea is not investigated in most cases in clinical or public-health practice, incorporating this uncertainty in estimates using a random effects model may be justified.

In conclusion, the available data from the medical literature provides sufficient evidence for the role of probiotics in the prevention of acute diarrhoea. Although higher in children, the effect of probiotics is also observed in adults. The effect on antibiotic-associated diarrhoea is most pronounced, but is also observed in non-antibiotic-associated diarrhoea and non-travellers' diarrhoea. There does not seem to be significant variation in impact by genera of strains. However, there is insufficient evidence for extrapolation of these results for global recommendations, since trials among children in community settings in the developing countries are lacking. Such trials need to be carried out before further conclusions can be drawn.

Conflicts of interest

We declare that we have no conflicts of interest.

Acknowledgments

Support from the Johns Hopkins Family Health and Child Survival Cooperative Agreement and funds from United States Agency for International Development is acknowledged (GH, SS, UD).

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