

Probiotics for treating persistent diarrhoea in children (Review)

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[Intervention Review]

Probiotics for treating persistent diarrhoea in children

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ABSTRACT

Background

Persistent diarrhoea (diarrhoea lasting more than 14 days) accounts for one third of all diarrhoea related deaths in developing countries in some studies. Probiotics may help treatment.

Objectives

To evaluate probiotics for treating persistent diarrhoea in children.

Search strategy

In August 2010, we searched the Cochrane Infectious Diseases Group Specialized Register, CENTRAL, MEDLINE, EMBASE, and LILACS. We also contacted authors of included trials and organizations working in the field, and checked reference lists.

Selection criteria

Randomized controlled trials comparing a specified probiotic agent with placebo or no probiotic in children with persistent diarrhoea.

Data collection and analysis

Two review authors assessed the eligibility, risk of bias, extracted and analysed data. Differences were resolved by discussion. Statistical analysis were performed using the fixed-effect model and the results were expressed as mean difference (MD) for continuous outcomes with 95% confidence intervals (CI).

Main results

Four trials were included, with a total number of 464 participants; one trial had a low risk of bias. Meta-analysis showed that probiotics reduced the duration of persistent diarrhoea (mean difference 4.02 days, 95% CI 4.61 to 3.43 days, n=324, 2 trials). Stool frequency was reduced with probiotics in two trials. One trial reported a shorter hospital stay, which was significant, but numbers were small. No adverse events were reported.

Authors' conclusions

There is limited evidence suggesting probiotics may be effective in treating persistent diarrhoea in children.

PLAIN LANGUAGE SUMMARY

Probiotics for persistent diarrhoea in children

Persistent diarrhoea is defined as a diarrhoeal episode that starts acutely but then lasts for 14 days or more, and it is an important cause of morbidity and mortality in children under five years old in developing countries throughout the world. The cause of persistent diarrhoea is not completely understood but is likely to be complex; this in turn makes management of the condition difficult. Probiotics are bacteria and yeasts that are similar to the normal bacteria found in a healthy gut. These so called friendly bacteria have been used in several studies to treat acute infectious diarrhoea with encouraging results. This review found four trials involving children with persistent diarrhoea. Two studies with a combined total of 324, showed that probiotics shorten the duration of diarrhoea and reduce the stool frequency on day 5. One study (235 children) suggested that probiotics reduce the hospital stay. Three out of four trials reported that no adverse events occurred. However, this review is limited by few trials with small number of participants, and therefore may not represent a reliable estimate of probiotics' effect.

SUMMARY OF FINDINGS FOR THE MAIN COMPARISON *[Explanation]*

Probiotic compared to placebo for treating children with persistent diarrhoea						
Patient or population: Children with persistent diarrhoea Settings: Intervention: Probiotic Comparison: Placebo						
Outcomes	Illustrative comparative risks* (95% CI)		Relative effect (95% CI)	No of Participants (studies)	Quality of the evidence (GRADE)	Comments
	Assumed risk	Corresponding risk				
	Placebo	Probiotic				
Duration of diarrhoea days	The mean duration of diarrhoea ranged across control groups from 8.5-9.2 days	The mean Duration of diarrhoea in the intervention groups was 4.02 lower (4.61 to 3.43 lower)		324 (2 studies ⁵)	⊕⊕⊕○ moderate 1,2,3,4	
Stool frequency on day 5	See comment	See comment	Not estimable	327 (2 studies ⁵)	⊕⊕○○ low 1,3,6,7	Both studies showed a benefit with probiotics, however the size of the benefit was very different in the two trials so the data were not pooled
Hospital stay days	The mean hospital stay in the control groups was 15.5 days	The mean Hospital stay in the intervention groups was 8.2 lower (8.6 to 7.8 lower)		235 (1 study ¹²)	⊕⊕⊕○ moderate 8,9,10,11	
Death from any cause - not measured	See comment	See comment	Not estimable	-	See comment	Not estimable
Weight-for-age z score - not measured	See comment	See comment	Not estimable	-	See comment	Not estimable

*The basis for the **assumed risk** (e.g. the median control group risk across studies) is provided in footnotes. The **corresponding risk** (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).

CI: Confidence interval;

GRADE Working Group grades of evidence

High quality: Further research is very unlikely to change our confidence in the estimate of effect.

Moderate quality: Further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate.

Low quality: Further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate.

Very low quality: We are very uncertain about the estimate.

¹ No serious study limitations: Basu 2007 adequately concealed allocation and blinded both participants and study staff to be considered at low risk of bias. As this was the larger study contributing 86% of the data to the meta-analysis we did not downgrade for study limitations. Gaon 2003 did not adequately describe the study methodology.

² No serious inconsistency: There was no statistical heterogeneity. $I^2 = 0\%$

³ Serious indirectness: Only two studies, one from India and one from Argentina have assessed this comparison. Before the result can be confidently generalised to all situations further studies may be necessary. Basu 2007 included paediatric patients (mean age 4.2 yrs) who had had diarrhoea for 14 consecutive days, a stool pH <5.5 and reducing substances $<1\%$. 90% of participants had weight for age $<80\%$ of expected. The intervention was ORS plus lactobacillus vs ORS alone. Gaon 2003 included children age 6-24 months, and excluded breastfed infants, cow's milk allergies, and children with $<60\%$ of the 50th percentile for weight, or $>10\%$ dehydration. The intervention was cow's milk plus lactobacillus or *S. boulardii* vs cow's milk alone.

⁴ No serious imprecision: The 95% CI around the pooled effect is narrow. Even the lower limit suggests a clinically important reduction in the duration of diarrhoea by 3 days.

⁵ Gaon 2003: A hospital-based study of 93 children in Argentina, and Basu 2007: A hospital-based study of 125 children in India

⁶ Serious inconsistency: There is substantial heterogeneity between the two trials: I^2 test for heterogeneity 80%. Both trials showed a benefit with probiotics, however the size of this effect was much larger in Basu 2007. The heterogeneity may be due to differences in the trial methodology, the characteristics of the study population or the nature of the different interventions. Due to the limited number of studies we were unable to further investigate this.

⁷ No serious imprecision: Not downgraded for imprecision. Data not pooled due to the substantial heterogeneity.

⁸ No serious limitations: Basu 2007 adequately concealed allocation and blinded both participants and study staff to be considered at low risk of bias.

⁹ No serious inconsistency: Not applicable as only one trial

¹⁰ Serious indirectness: Only one study has reported this outcome. Before the result can be confidently generalised to all situations further studies may be necessary.

¹¹ No serious imprecision: Both limits of the 95% CI suggest a clinically important reduction in hospital stay in the participants given the intervention.

¹² Basu 2007

BACKGROUND

The World Health Organization (WHO) defines persistent diarrhoea as an illness of proven or presumed infectious aetiology that lasts 14 days or more (Anonymous 1988). The definition excludes causes of chronic diarrhoea that may appear as persistent diarrhoea; for example, celiac disease, food-related enteropathies, and congenital enteropathies. Persistent diarrhoea accounts for 3% to 20% of all diarrhoeal episodes in children aged less than five years (IWGPD 1996). It is also directly responsible for between 36% and 54% of all diarrhoea-related deaths according to two large, community-based studies (Schorling 1990; Fauveau 1992). Thus, the main consequences of persistent diarrhoea are morbidity (with an increased risk of hospital admission), death, and malnutrition.

The cause of persistent diarrhoea is not known and the pathogenic mechanisms are not well understood; most of the viruses, parasites, and bacterial pathogens that cause acute diarrhoea have also been associated with persistent diarrhoea (Ochoa 2004). The management of persistent diarrhoea is complex because the etiology and pathogenesis are complex. It includes adequate dietary management, micronutrient supplementation, adequate rehydration, and antimicrobials (Ochoa 2004). In developing countries, where persistent diarrhoea is a problem, it is recognized that frequent recurrence of acute diarrhoeal episodes (less than 14 days' duration) result in nutritional compromise, which is in turn the most important epidemiological risk factor for persistent diarrhoea (Bhandari 1989). Other risk factors for persistent diarrhoea include lack of breastfeeding and immune deficiencies (Bhutta 2004).

Probiotics are defined as living organisms that when administered in adequate amounts confer a health benefit on the host (Pineiro 2007). They are used widely for various indications because of their widespread acceptance and general lack of adverse effects. Probiotics most commonly used include *Bifidobacterium* and two genera of lactic acid bacteria, namely, *Lactobacillus* and *Streptococcus*. Acute infectious diarrhoea is the most investigated field in the area of probiotic use in children; five recent systematic reviews have described the role of probiotics in acute infectious diarrhoea (Szajewska 2001; Huang 2002; Van Niel 2002; Allen 2003; McFarland 2006). Each review demonstrated that probiotics had a good safety profile, significantly reduced the duration of diarrhoea by 13.4 to 30.5 hours (range), reduced stool frequency, and reduced the duration of hospital stays. However, the effects of probiotics in acute diarrhoea are not generalizable to persistent diarrhoea, since most trials of probiotics in acute diarrhoea have been done in otherwise healthy, well nourished children from developed countries.

The rationale for using probiotics to treat infectious diarrhoea is based on the assumption that they modify the composition of the intestinal microflora and act against enteric pathogens. It has recently been reported that probiotics have multiple properties that attenuate inflammation in cases of inflammatory bowel disease. The main mechanisms of action include: induction of regulatory

T cells that suppress inflammation-inducing effector cells, maintenance of the gastrointestinal barrier function, and the interference with the ability of pathogens to colonize and infect the mucosa (Boirivant 2007). Probably two or more of these mechanisms operate simultaneously, and these mechanisms may also be beneficial in persistent diarrhoea. Moreover, the beneficial effects of probiotics in acute diarrhoea in children seem to be strain-dependent, dose-dependent (greater for doses of $> 10^{10}$ colony forming units), significant in people with viral gastroenteritis, and more evident when treatment with probiotics is initiated early in the course of disease (Szajewska 2005).

OBJECTIVES

To evaluate the efficacy and adverse effects of probiotics for the treatment of persistent diarrhoea in children.

METHODS

Criteria for considering studies for this review

Types of studies

Randomized controlled trials.

Types of participants

Children (0 to 18 years of age) with persistent diarrhoea (duration ≥ 14 days) that is proven (pathogens isolated from stools) or presumed to be caused by an infectious agent.

Types of interventions

Intervention

Specific, identified probiotic.

Trials investigating yogurt or other fermented foods in which a specific probiotic agent is not identified are not eligible.

Control

Placebo or no treatment.

Intervention and control arms to be otherwise treated identically in relation to other treatments and drugs.

Types of outcome measures

Primary

1. Duration of diarrhoea.

Secondary

1. Stool frequency.
2. Stool volume.
3. Weight-for-age z score.
4. Hospital stay.
5. Death from any cause.

Adverse events

1. Serious (leads to death, hospitalisation, or disability, is life-threatening, or requires intervention to prevent permanent impairment).
2. Requiring discontinuation of treatment.
3. Other.

Search methods for identification of studies

We identified all relevant trials regardless of language or publication status (published, unpublished, in press, and in progress).

Databases

We searched the following databases using the search terms and strategy as described in [Table 1](#): Cochrane Infectious Disease Group Specialized Register; Cochrane Central Register of Controlled Trials (CENTRAL), published in *The Cochrane Library*; MEDLINE; EMBASE; and LILACS. We also searched the *metaRegister of Controlled Trials (mRCT)* using 'diarrhoea', 'probiotic*', 'lactobacill*' and 'bifidobacter*' as search terms.

Table 1. Detailed search strategies

Search set	CIDG SR ^a	CENTRAL	MEDLINE ^b	EMBASE ^b	LILACS ^b
1	diarrhea	diarrhea	diarrhea	diarrhea	diarrhea
2	diarrhoea	diarrhoea	diarrhoea	diarrhoea	diarrhoea
3	1 or 2	1 or 2	1 or 2	1 or 2	1 or 2
4	probiotic*	lactobacill*	lactobacill*	lactobacill\$	probiotic\$
5	lactobacill*	lactococc*	lactococc*	lactococc\$	lactobacill\$
6	Bifidobacter*	Bifidobacter*	Bifidobacter*	Bifidobacter\$	Bifidobacter\$
7	4 or 5 or 6	Enterococc*	Enterococc*	Enterococc\$	4 or 5 or 6
8	3 and 7	Streptococc*	Streptococc*	Streptococc\$	3 and 7
9	child*	Saccharomyces	Saccharomyces	Saccharomyces	child\$
10	infant*	4-9/OR	4-9/OR	4-9/OR	Infant\$
11	pediatr*	3 and 10	3 and 10	3 and 10	pediatr\$
12	9 or 10 or 11	child*	child*	child\$	9 or 10 or 11
13	8 and 12	Infant*	Infant*	Infant\$	8 and 12
14	-	pediatr*	pediatr*	pediatr\$	-
15	-	12 or 13 or 14	12 or 13 or 14	12 or 13 or 14	-
16	-	11 and 15	11 and 15	11 and 15	-

^aCochrane Infectious Diseases Group Specialized Register.

^bSearch terms used in combination with the search strategy for retrieving trials developed by The Cochrane Collaboration ([Lefebvre 2008](#)); upper case: MeSH or EMTREE heading; lower case: free text term.

Organizations

To help identify unpublished and ongoing trials, we contacted researchers at organizations including the International Scientific Association for Probiotics and Prebiotics.

Reference lists

We checked the reference lists of all studies identified by the above methods.

Data collection and analysis

Selection of studies

Two authors (CABM and NYCP) independently screened the search results using article titles and abstracts (where available). The full text of the selected articles was retrieved and scrutinized to ensure that multiple publications from the same trial were included only once. The same authors (CABM and NYCP) then independently selected articles for inclusion according to a standardized form to assess the eligibility of trials. Disagreements were resolved through discussion with a third author (GBA). The trial authors were contacted for clarification if it was unclear whether a trial is eligible for inclusion. Excluded trials along with the reason for exclusion are listed in the section, '[Characteristics of excluded studies](#)'.

Data extraction and management

Two authors (GBA and RARG) independently extracted the data using standard forms. Any differences were resolved through discussion with a third author (CABM). Attempts were made to obtain any missing data from the trial authors. We aimed to extract the following data: hazard ratios and standard deviations for duration of diarrhoea if trials reported them, otherwise we extracted mean and standard deviations; the number of stools and the number of person days; the mean stool output from the start of the intervention; the mean weight for age z score; the mean duration of hospital stay and its standard deviations; and the number of deaths in each group. The authors carried out an intention-to-treat analysis, and extracted the number of participants randomized and analysed in each group for all outcomes.

Assessment of risk of bias in included studies

Two authors (GBA and NYCP) independently assessed the risk of bias of each trial using The Cochrane Collaboration's risk of bias tool ([Higgins 2008](#)). We followed the guidance to make judgements on the risk of bias in six domains: sequence generation; allocation concealment; blinding (of participants, personnel, and outcome assessors); incomplete outcome data; selective outcome

reporting; and other sources of bias. We categorized these judgements as 'yes' (low risk of bias), 'no' (high risk of bias), or 'unclear'. Where our judgment was unclear we attempted to contact the trial authors for clarification.

Assessment of reporting biases

We assessed publication bias using the funnel plot if there were about 10 or more trials included in a meta-analysis.

Data synthesis

We analysed the data using [Review Manager 5](#). Results were combined unless diversity (clinical and methodological heterogeneity) or statistical heterogeneity (non-overlapping confidence intervals) made this unreasonable. We pooled dichotomous data using the risk ratio and calculated the number needed to treat when appropriate. If continuous data were summarized by arithmetic means and standard deviation data, then we combined them using the mean differences; where continuous data were summarized using geometric means, we combined them on the log scale using the generic inverse variance method and reported them on the natural scale. The hazard ratio was combined on the log scale using the generic inverse variance method for time to event data. We presented all results with 95% confidence intervals.

The intention-to-treat principle was applied; however, if there was a discrepancy in the number randomized and the number analysed in each group, we calculated the percentage loss to follow up in each group. We assessed pooled data using available case analysis rather than intention-to-treat analysis with imputation. We used a fixed-effect model unless there was statistically significant heterogeneity between trials, in which case used a random-effects model.

Subgroup analysis and investigation of heterogeneity

Heterogeneity amongst trials was investigated by looking at whether a graphical plot of the confidence intervals for the results of each study overlapped, using a standard Chi^2 test with significance set at $P < 0.10$, and using the I^2 test statistic ($> 50\%$ will be considered as substantial heterogeneity). Subgroup analyses were subdivided by: identified diarrhoeal pathogens, trial low- to middle-income/high-income setting, probiotic strain and dosage of probiotic.

Sensitivity analysis

We performed sensitivity analysis in order to explore whether effect size was different in adequately concealed trials compared with the rest.

RESULTS

Description of studies

See: [Characteristics of included studies](#); [Characteristics of excluded studies](#); [Characteristics of studies awaiting classification](#).

See: [Characteristics of included studies](#); [Characteristics of excluded studies](#); [Characteristics of studies awaiting classification](#).

Our search (in August 2010) identified 198 potentially relevant studies. Independent review (CABM, NYCP) of titles and/or abstracts identified 23 potentially relevant studies for review (21 full-text and 2 abstracts). An additional study was found through the reference list of included studies, and one through a different search undertaken for another related publication. Of these studies, two were duplicated publications of prior reports, and one was a preliminary report of a potentially relevant study. Finally, four trials met inclusion criteria and were included in the review. In one trial, which referred to chronic diarrhoea, participants actually met the criteria for persistent diarrhoea (Castañeda 1995). One unpublished trial is awaiting further evaluation. The International Scientific Association for Probiotics and Prebiotics was contacted in October 2008 but no additional references were found.

Risk of bias in included studies

See: [Characteristics of included studies](#).

Generation of allocation sequence

Three trials used adequate methods to generate the allocation sequence. The method used in the other trial was unclear (Gaón 2003).

Allocation concealment

Allocation concealment was mixed. One study demonstrated adequate allocation concealment (Basu 2007), two studies were unclear as to whether allocation concealment was performed (Castañeda 1995; Gaón 2003), and in the remaining study allocation concealment was not performed.

Blinding

Blinding of the participants, providers, and assessors was only done in one trial (Basu 2007). Two trials described double blinding in broad terms, which made it impossible to know exactly who was blinded (Castañeda 1995; Gaón 2003). The remaining study was open-labelled.

Follow up and exclusions

Reported outcome data was considered satisfactory with a low risk of bias in three studies. One trial (Touhami 1992) were considered to be at high risk of bias due to number of exclusions (>10%).

Selective reporting

Reporting appeared to include all important outcomes in one study (Basu 2007). In two studies (Touhami 1992; Castañeda 1995) it was unclear whether important persistent diarrhoea outcomes had not occurred or had not been reported. In Gaón 2003, the treatment failure outcome was not reported.

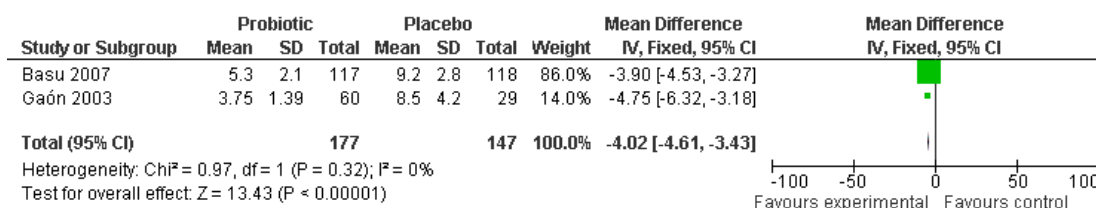
Other potential sources of bias

One trial was apparently free of other problems that could put it at a risk of bias (Basu 2007). It was unclear whether the remaining trials were free from potential sources of bias. Small number of patients were recruited in the three intervention groups in Gaón 2003, there was insufficient data to assess the baseline balance in Castañeda 1995, and the incorrect enrolment of 14 children could have introduced baseline imbalance in Touhami 1992.

Effects of interventions

See: [Summary of findings for the main comparison Probiotic compared to placebo for treating children with persistent diarrhoea](#). The primary outcome was reported in two studies (Gaón 2003; Basu 2007), and after combining the results of the trials, diarrhoea duration was reduced with probiotics by -4.02 days (MD, 95% CI -4.61 to -3.43; 324 participants, two trials, Figure 1).

Figure 1. Forest plot of comparison: I Probiotic versus placebo, outcome: I.I duration of diarrhoea.



For the secondary efficacy endpoint of stool frequency on day 5, data were available from two trials (Gaón 2003; Basu 2007). The data from the three-arm study by Gaón 2003 showed that on day 5, the stool frequency decreased to 2.0 ± 2.0 in the saccharomyces group and 1.5 ± 0.9 in the lactobacilli group, respectively, in comparison with placebo (5.2 ± 3.0), ($P < 0.001$). The data from the study by Basu 2007 showed a significant difference in stool frequency on day 5 during treatment between probiotic and placebo groups (5.2 ± 2.1 versus 10.2 ± 3.2).

For the secondary efficacy endpoint of stool volume, data from one trial (Touhami 1992) suggested no difference in total stool volume between probiotic group and placebo group (1130 ± 250 versus 830 ± 180 , $P = 0.89$, authors' calculation). For the secondary endpoint of hospital stay, one trial (Basu 2007) found a reduction in hospital stay (7.3 ± 1.6 versus 15.5 ± 1.5 , $P < 0.05$). No studies reported weight-for-age Z score or death from any cause. Three out of four trials reported that no adverse events occurred.

Despite we planned an intention-to-treat analysis, it was not feasible, so we performed a complete-case analysis.

DISCUSSION

Summary of main results

See: Summary of findings table 1.

Despite the comprehensive search strategy used, only four relevant trials were identified. There were variability between studies in probiotic tested, treatment regimens, and definitions of outcomes measures; and the small number of studies limited the ability to perform a subgroup analysis, especially with regards to the probiotic strains and identified diarrhoeal pathogens.

Concerning the primary outcome, two trials (Gaón 2003; Basu 2007) examined whether probiotics reduced the duration of diarrhoea. The results of the pooled analysis were significant in favour of the intervention group. Because there were only two trials and the number of participants in the studies were small, it is hard to draw a definitive conclusion about the effects of probiotics on the duration of persistent diarrhoea. One problem with the analysis presented in these trials was that the primary outcome was not treated as a time-to-event outcome.

Concerning secondary outcomes, there was evidence from two studies (Gaón 2003; Basu 2007) that probiotic treatment leads to a reduction in stool frequency on day 5. One study (Touhami 1992) reported that stool volume showed no significant difference between the two groups, and other study (Basu 2007) showed a significant reduction in hospital stay. No conclusion regarding probiotic's impact on weight-for-age Z score or death can be drawn from this review as trials included were not designed to look at these outcomes.

One trial did not report adverse events (Touhami 1992), and three trials reported that no events occurred.

Completeness and applicability of evidence

All trials were conducted in hospitals and involved children up to the age of six years from middle-income countries. Studies including children from low-income countries, where persistent diarrhoea is more common and interventions most likely to be beneficial, were not found. Moreover, the number of studies and the number of participants included in review analysis were small, and review outcomes were poorly reported.

Although two of the included trials (Gaón 2003; Basu 2007) showed a significant reduction of diarrhoea duration and stool frequency, the available data does not support the use of probiotics as a standard treatment for persistent diarrhoea.

One trial, Castañeda 1995, classified participants as having chronic diarrhoea; however, they met criteria for persistent diarrhoea (which was confirmed by the author). In addition, the endpoints of this trial were not the same as the intended outcome measures of the review.

Quality of the evidence

All four included trials were randomized, controlled studies. However, only one trial (Basu 2007) was at a low risk of bias because it had adequate concealment of randomisation, good follow up, and blinded outcome assessment. Two trials (Castañeda 1995; Gaón 2003) were at a unclear risk of bias, since they had an unclear risk of bias for one or more key domains, specifically for allocation concealment and blinding. The remaining trial (Touhami 1992) was at a high risk of bias with no allocation concealment, no blinding, and excessive loss to follow-up (more than 10%). Hence, the identified evidence did not permit a robust conclusion to be reached on the efficacy of probiotics for treating persistent diarrhoea.

Potential biases in the review process

The literature search was conducted by the Cochrane Infectious Diseases Group Information Specialist, making it unlikely that any relevant trial have been missed; however, it is possible that we have overlooked small unpublished trials.

Although we tried to collect all relevant data, the possibility of missing data remained. We have contacted the authors for data but have not yet received a reply or have not obtained useful information.

Another limitation of this review is the fact that the meta-analysis for duration of diarrhoea was performed with only two trials, especially when one of them is a small three-arm trial. The two intervention groups of the three-arm trial were combined into one before performing the meta-analysis.

AUTHORS' CONCLUSIONS

Implications for practice

The current data are promising, but inconclusive. The use of probiotics appear to hold promise as adjunctive therapy for persistent diarrhoea, but there is insufficient evidence to recommend their use at this time.

Implications for research

Methodologically well-designed and sufficiently powered trials are needed to determine whether probiotics as a part of the therapy will alter the clinical outcome in children with persistent diarrhoea.

Trials need to use standardized definitions for persistent diarrhoea and resolution of illness, and the primary outcome for this review will ideally be represented in a time-to-event analysis. All future studies should use specific probiotic strains and doses in well-defined participants subgroups.

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* Indicates the major publication for the study

CHARACTERISTICS OF STUDIES

Characteristics of included studies [ordered by year of study]

Touhami 1992

Methods	Study design: RCT; Duration: 3 years, from January 1987 to February 1990.	
Participants	<p>Inclusion criteria: children aged 3 to 36 months with 3 or more liquid stools per day for > 13 days but < 29 days; and fed predominantly or exclusively milk formula at the time of onset of the diarrhoea.</p> <p>Exclusion criteria: exclusive breast feeding; gross blood in the stools; additional disease requiring antimicrobial treatment; severe clinical malnutrition (weight/height < 70% NCHS); failure to give informed consent.</p> <p>Number completing study: 36/40 (90%) in probiotic group (1 withdrawn by the parents, 4 developed urinary tract infection, 1 developed bronchiolitis); 35/38 (92.1%) in placebo group (3 developed urinary tract infection).</p>	
Interventions	<p>(1) Yogurt formula prepared by fermentation with <i>streptococcus thermophilus</i> and <i>lactobacillus bulgaricus</i> (4×10^8 CFU/Litre of yogurt).</p> <p>(2) Non-fermented milk formula.</p> <p>Intervention started after a 2-day observation period. The repartition of milk or yogurt varied with age as follow: between 3 and 6 months 120 mL/kg four times a day; 7 and 12 months 90 mL/kg three times a day; 13 and 18 months 60 mL/kg twice a day, during 5 days. ORS solution was offered according to WHO recommendations.</p>	
Outcomes	<p>(1) Treatment failure defined as a 5% loss of body weight in 24 hours, or if liquid stools were still present at the end of the 5-day period.</p> <p>(2) Total number of stools.</p> <p>(3) Stool volume.</p> <p>No comment regarding adverse events.</p> <p>Resolution of diarrhoea defined as the last liquid or semi-liquid stool evacuated before two formed stools in 12 hours, or no passage of stools for 12 hours before a formed stool.</p>	
Notes	<p>Study location: Algeria</p> <p>Stool analyses: Rotavirus 8, Campylobacters jejuni 3, EPEC 3, and Giardia Lamblia 2.</p> <p>14 children were enrolled in error.</p> <p>Source of funding: WHO and INSERM.</p>	
Risk of bias		
Item	Authors' judgement	Description
Adequate sequence generation?	Yes	Investigators describe use of random permutation of fixed length, separately for boys and girls.
Allocation concealment?	No	Information obtained from trial author.

Touhami 1992 (Continued)

Blinding? Duration of diarrhoea	No	"...non-blinded clinical trial that lasted for 5 days."
Blinding? Duration of vomiting	No	"...non-blinded clinical trial that lasted for 5 days."
Blinding? Stool frequency by day of treatment	No	"...non-blinded clinical trial that lasted for 5 days."
Blinding? Frequency of vomiting by day of treatment	No	"...non-blinded clinical trial that lasted for 5 days."
Blinding? Duration of hospital stay	No	"...non-blinded clinical trial that lasted for 5 days."
Incomplete outcome data addressed? All outcomes	No	6/40 missing outcome data from yogurt group; 3/38 missing outcome data from milk group. The proportion of missing outcomes could induce clinically relevant bias in intervention effect estimate.
Free of selective reporting?	Unclear	Insufficient information.
Free of other bias?	Unclear	The incorrect enrolment of 14 children can introduce baseline imbalance.

Castañeda 1995

Methods	Study design: RCT; Duration: no available.
Participants	Inclusion criteria: diarrhoea for 3 or 4 weeks; persistence of diarrhoea after antibiotic treatment. Exclusion criteria: not stated. Number completing study: 20/20 (100%) in probiotic group and 20/20 (100%) in placebo group.
Interventions	(1) <i>Saccharomyces boulardii</i> (5×10^9 /250 mg) diluted with water or juice, twice daily for 30 days. (2) Identical placebo, but without any active ingredient, at the same dose. Timing of start of administration not stated.
Outcomes	(1) Number of stools on a daily basis and assigned to categories of 1-3, 4-6, 7-10, and more than 10 per day. (2) Degree of reversal of histological changes (partial, subtotal, total atrophy). No adverse events observed.

Castañeda 1995 (Continued)

Notes	Study location: Cuba. Stool analyses (probiotic group/placebo group): Giardia lamblia 17/18, Shigella 2/2. Duration of diarrhoea not measured. Source of funding: no available.	
Risk of bias		
Item	Authors' judgement	Description
Adequate sequence generation?	Yes	"...randomly assigned to treatment with the active product or with placebo on the basis of a table of random numbers".
Allocation concealment?	Unclear	Insufficient information.
Blinding? Duration of diarrhoea	Unclear	Insufficient information to know who were blinded.
Blinding? Duration of vomiting	Unclear	Insufficient information to know who were blinded.
Blinding? Stool frequency by day of treatment	Unclear	Insufficient information to know who were blinded.
Blinding? Frequency of vomiting by day of treatment	Unclear	Insufficient information to know who were blinded.
Blinding? Duration of hospital stay	Unclear	Insufficient information to know who were blinded.
Incomplete outcome data addressed? All outcomes	Yes	No missing outcome data.
Free of selective reporting?	Unclear	Insufficient information.
Free of other bias?	Unclear	Insufficient information to assess the base-line balance.

Gaón 2003

Methods	Study design: RCT; Duration: 2 years, from January 1996 to April 1998.
Participants	Inclusion criteria: a history of frequent loose stools (>3 per day) for the last consecutive 14 days or more; not being breast fed; no history of allergy to cow's milk; no history of treatment with antimicrobials or antidiarrhoeal agents within the preceding 7 days; ability to take oral food. Exclusion criteria: presence of concurrent systemic illness; a weight for age < 60% of the value for the 50 th percentile according to the tables of the NCHS; dehydration of more

Gaón 2003 (Continued)

	<p>than 10% of body weight (severe). Number completing study: 30/31 (96.8%) in <i>saccharomyces</i> group (1 developed urinary tract infection); 30/31 (96.8%) in <i>lactobacillus</i> group (1 developed urinary tract infection); and 29/31 (93.5%) in control group (2 developed vomiting).</p>	
Interventions	<p>(1) Pasteurized cow's milk containing <i>Saccharomyces boulardii</i> (10^{10} - 10^{12} CFU/g), 175 g twice a day for a 5 day period. (2) Pasteurized cow's milk containing <i>Lactobacillus casei</i> and <i>Lactobacillus acidophilus sp</i> (10^{10} - 10^{12} CFU/g), 175 g twice a day for a 5 day period. (3) Identical placebo (pasteurised cow milk). Intervention started after the initial correction of dehydration. After rehydration and until diarrhoea stopped, ORS solution was offered according to WHO recommendations.</p>	
Outcomes	<p>(1) Duration of diarrhoea. (2) Stool frequency by day of treatment. (3) Proportion of participants with diarrhoea at end of intervention (day 5). (4) Duration of symptoms. Resolution of diarrhoea defined as an unformed stool follow by two stools that retains its shape and does not stick to the container (formed stool). Results for rotavirus subgroup also presented. No adverse events observed.</p>	
Notes	<p>Study location: Argentina Stool analyses (saccharomyces group/ lactobacillus group/ placebo group): Rotavirus 9/8/7; E. coli, Salmonella, Shigella 4/3/4. Patients with malnutrition 6/30 (20%) in saccharomyces group, 7/30 (23.3%) in lactobacillus group, and 6/29 (20.7%) in placebo group. Stool frequency by day of treatment derived from graph. Source of funding: Sancor Cooperativas Unidas and CONICET.</p>	
Risk of bias		
Item	Authors' judgement	Description
Adequate sequence generation?	Unclear	Randomised, but no further details given.
Allocation concealment?	Unclear	The method of concealment is not describe in sufficient detail.
Blinding? Duration of diarrhoea	Unclear	Insufficient information to know who were blinded.
Blinding? Duration of vomiting	Unclear	Insufficient information to know who were blinded.
Blinding? Stool frequency by day of treatment	Unclear	Insufficient information to know who were blinded.

Gaón 2003 (Continued)

Blinding? Frequency of vomiting by day of treatment	Unclear	Insufficient information to know who were blinded.
Blinding? Duration of hospital stay	Unclear	Insufficient information to know who were blinded.
Incomplete outcome data addressed? All outcomes	Yes	2/62 missing outcome data from intervention group (both due to vomiting); 2/31 missing outcome data from control group (both due to urinary tract infection).
Free of selective reporting?	No	The pre-specified treatment failure outcome was not reported.
Free of other bias?	Unclear	Small number of patients were recruited in the three study groups.

Basu 2007

Methods	Study design: RCT; Duration: 2 years, from January 2003 to December 2004.
Participants	Inclusion criteria: history of diarrhoea persisting for 14 days or more without any remission in between; stool pH < 5.5; stool reducing substance > 1%. Exclusion criteria: presence of any systemic illness other than diarrhoea on admission; development of any systemic complication of diarrhoea during hospital stay; failure to give informed consent. Number completing study: 117/125 (93.6%) in probiotic group (1 developed septicaemia, 1 developed renal failure, 4 withdrew consent, 2 discharged on request) and 118/128 in control group (3 developed septicaemia, 3 withdrew consent, 4 discharged on request).
Interventions	(1) <i>Lactobacillus GG</i> powder containing 60 million cells was dissolved in 100 ml of ORS twice daily for a minimum period of 7 days or till diarrhoea stopped. (2) ORS twice daily for the same duration. Intervention started after the initial correction of dehydration. All participants with positive stool culture received antibiotics. All participants were given lactose-free diet; however, breast fed children continue breastfeeding.
Outcomes	(1) Duration of diarrhoea. (2) Duration of vomiting. (3) Stool frequency by day of treatment. (4) Frequency of vomiting by day of treatment. (5) Duration of hospital stay. No complication could be documented.
Notes	Study location: India Stool analyses (probiotic group/placebo group): <i>E. Coli</i> 12/10; <i>Shigella</i> spp 9/7; <i>C.</i>

Basu 2007 (Continued)

		difficile 6/8; E. histolytica 7/9; G. lamblia 5/8; mixed infections 6/3. Patients with PEM (body weight < 80% expected) 107/117 (91.5%) in the probiotic group and 105/118 (89%) in placebo group. Source of funding: no available.
Risk of bias		
Item	Authors' judgement	Description
Adequate sequence generation?	Yes	"Randomization was performed by computer generated random numbers"
Allocation concealment?	Yes	"...by opaque and sealed envelopes."
Blinding? Duration of diarrhoea	Yes	Blinding of participants and key study personnel ensured.
Blinding? Duration of vomiting	Yes	Blinding of participants and key study personnel ensured.
Blinding? Stool frequency by day of treatment	Yes	Blinding of participants and key study personnel ensured.
Blinding? Frequency of vomiting by day of treatment	Yes	Blinding of participants and key study personnel ensured.
Blinding? Duration of hospital stay	Yes	Blinding of participants and key study personnel ensured.
Incomplete outcome data addressed? All outcomes	Yes	8/125 missing outcome data from intervention group; 10/128 missing outcome data from control group. Reasons similar across groups.
Free of selective reporting?	Yes	Published report include all expected outcomes.
Free of other bias?	Yes	The study appears to be free of other sources of bias.

RCT: randomized controlled trial; NHCS: National Center for Health Statistics; CFU: colony-forming units; ORS: oral rehydration solution; WHO: World Health Organization; EPEC: enteropathogenic escherichia coli; PEM: protein-energy malnutrition; IN-SERM: National Institute of Health and Medical Research; CONICET: National Research Council of Argentine.

Characteristics of excluded studies *[ordered by study ID]*

Study	Reason for exclusion
Balli 1992	Included children with chronic non-specific diarrhoea only.
Boudraa 1990	Preliminary report of included trial.
Locascio 2002	No mention of randomization.
Roggero 1990	Included children with chronic non specific diarrhoea only.

Characteristics of studies awaiting assessment *[ordered by study ID]*

Chouraqui 1995

Methods	Study design: RCT
Participants	Infants with toddler diarrhoea who did not improved after one month of dietary modifications.
Interventions	(1) <i>Saccharomyces boulardii</i> 25 mg/kg/d for three months. (2) Placebo.
Outcomes	Clinical score based on stool characteristics, and gastrointestinal symptoms and signs.
Notes	Unpublished data.

DATA AND ANALYSES

Comparison 1. Probiotic versus placebo

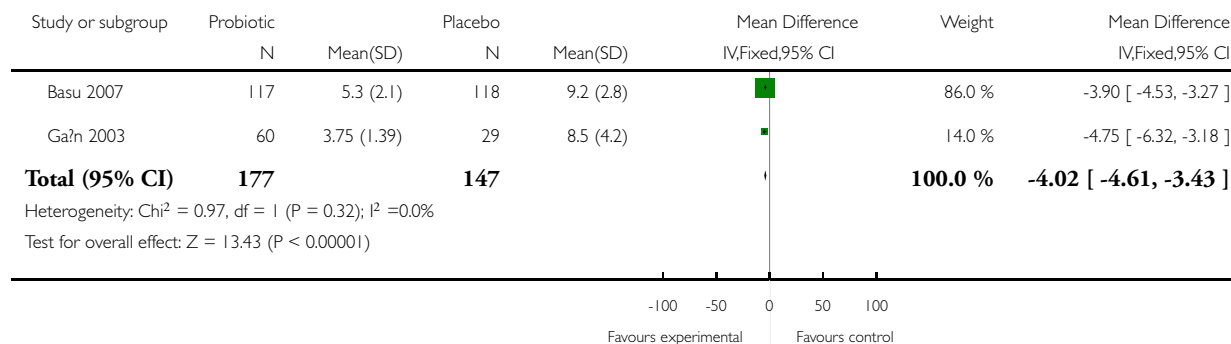
Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 duration of diarrhoea	2	324	Mean Difference (IV, Fixed, 95% CI)	-4.02 [-4.61, -3.43]

Analysis 1.1. Comparison 1 Probiotic versus placebo, Outcome 1 duration of diarrhoea.

Review: Probiotics for treating persistent diarrhoea in children

Comparison: 1 Probiotic versus placebo

Outcome: 1 duration of diarrhoea



HISTORY

Protocol first published: Issue 4, 2008

Review first published: Issue 11, 2010

CONTRIBUTIONS OF AUTHORS

G Bernaola Aponte conceived the idea for this systematic review, participated with the development of the methodological aspects of the protocol, and has co-ordinated its development. CA Bada Mancilla and NY Carreazo Pariasca have helped develop the search strategy. RA Rojas Galarza gave advice regarding persistent diarrhoea.

DECLARATIONS OF INTEREST

None known.

SOURCES OF SUPPORT

Internal sources

- No sources of support supplied

External sources

- Iberoamerican Cochrane Center, Spain.

DIFFERENCES BETWEEN PROTOCOL AND REVIEW

Secondary outcome:

“Stool frequency” replaced by “Mean stool frequency on day 5” because the former was not reported in trials.

Risk difference for adverse events was not employed since adverse events have not occurred or have been not reported.