PROBIOTICS FOR THE PREVENTION OF PEDIATRIC ANTIBIOTIC-ASSOCIATED DIARRHEA

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Cochrane Database of Systematic Reviews, Issue 03, 2012 (Status in this issue: NEW SEARCH FOR STUDIES AND CONTENT UPDATED (CONCLUSIONS CHANGED))

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DOI: 10.1002/14651858.CD004827.pub2


ABSTRACT

Background
Antibiotics alter the microbial balance within the gastrointestinal tract. Probiotics may prevent antibiotic-associated diarrhea (AAD) via restoration of the gut microflora. Antibiotics are prescribed frequently in children and AAD is common in this population.

Objective
The primary objectives were to assess the efficacy and safety of probiotics (any specified strain or dose) used for the prevention of AAD in children.

Criteria for considering studies for this review
MEDLINE, EMBASE, CENTRAL, CINAHL, AMED, and the Web of Science (inception to May 2010) were searched along with specialized registers including the Cochrane IBD/FBD review group, CISCOM (Centralized Information Service for Complementary Medicine), NHS Evidence, the International Bibliographic Information on Dietary Supplements as well as trial registries. Letters were sent to authors of included trials, nutra/pharmaceutical companies, and experts in the field requesting additional information on ongoing or unpublished trials. Conference proceedings, dissertation abstracts, and reference lists from included and relevant articles were also searched.

Selection criteria
Randomized, parallel, controlled trials in children (0 to 18 years) receiving antibiotics, that compare probiotics to placebo, active alternative prophylaxis, or no treatment and measure the incidence of diarrhea secondary to antibiotic use were considered for inclusion.

Data collection and analysis
Study selection, data extraction as well as methodological quality assessment using the risk of bias instrument was conducted independently and in duplicate by two authors. Dichotomous data (incidence of diarrhea, adverse events) were combined using a pooled relative risk and risk difference (adverse events), and continuous data (mean duration of diarrhea, mean daily stool frequency) as weighted mean differences, along with their corresponding 95% confidence intervals. For overall pooled results on the incidence of diarrhea, sensitivity analyses included available case versus extreme-plausible analyses and random- versus fixed-effect models. To explore possible explanations for heterogeneity, a priori subgroup analysis were conducted on probiotic strain, dose, definition of antibiotic-associated diarrhea, antibiotic agent as well as risk of bias.

Main results
Sixteen studies (3432 participants) met the inclusion criteria. Trials included treatment with either Bacillus spp., Bifidobacterium spp., Lactobacilli spp., Lactococcus spp., Leuconostoc cremoris, Saccharomyces spp., or Streptococcus spp., alone or in combination. Nine studies used a single strain probiotic agent, four combined two probiotic strains, one combined three probiotic strains, one product included ten probiotic agents, and one study included two probiotic arms that used three and two strains respectively. The risk of bias was determined to be high in 8 studies and low in 8 studies. Available case (patients who did not complete the studies were not included in the analysis) results from 15/16 trials reporting on the incidence of diarrhea show a large, precise benefit from probiotics compared to active, placebo or no treatment control. The incidence of AAD in the probiotic group was 9% compared to 18% in the control group (2874 participants; RR 0.52; 95% CI 0.38 to 0.72; I^2 = 56%). This benefit was not statistically significant in an extreme plausible (60% of children loss to follow-up in probiotic group and 20% loss to follow-up in the control group had diarrhea) intention to treat (ITT) sensitivity analysis. The incidence of AAD in the probiotic group was 16% compared to 18% in the control group (3392 participants; RR 0.81; 95% CI 0.63 to 1.04; I^2 = 59%). An a priori available case subgroup analysis exploring heterogeneity indicated that high dose (>5 billion CFUs/day) is more effective than low probiotic dose (< 5 billion CFUs/day), interaction P value = 0.010. For the high dose studies the incidence of AAD in the probiotic group was 8% compared to 22% in the control group (1474 participants; RR 0.40; 95% CI 0.29 to 0.55). For the low dose studies the...
incidence of AAD in the probiotic group was 8% compared to 11% in the control group (1382 participants; RR 0.80; 95% CI 0.53 to 1.21). An extreme plausible ITT subgroup analysis was marginally significant for high dose probiotics. For the high dose studies the incidence of AAD in the probiotic group was 17% compared to 22% in the control group (1776 participants; RR 0.72; 95% CI 0.53 to 0.99; I² = 58%). None of the 11 trials (n = 1583) that reported on adverse events documented any serious adverse events. Meta-analysis excluded all but an extremely small non-significant difference in adverse events between treatment and control (RD 0.00; 95% CI -0.01 to 0.02).

Authors’ conclusions
Despite heterogeneity in probiotic strain, dose, and duration, as well as in study quality, the overall evidence suggests a protective effect of probiotics in preventing AAD. Using 11 criteria to evaluate the credibility of the subgroup analysis on probiotic dose, the results indicate that the subgroup effect based on dose (≥5 billion CFU/day) was credible. Based on high-dose probiotics, the number needed to treat (NNT) to prevent one case of diarrhea is seven (NNT 7; 95% CI 6 to 10). However, a GRADE analysis indicated that the overall quality of the evidence for the primary endpoint (incidence of diarrhea) was low due to issues with risk of bias (due to high loss to follow-up) and imprecision (sparse data, 225 events). The benefit for high dose probiotics (Lactobacillus rhamnosus or Saccharomyces boulardii) needs to be confirmed by a large well-designed randomized trial. More refined trials are also needed that test strain specific probiotics and evaluate the efficacy (e.g. incidence and duration of diarrhea) and safety of probiotics with limited losses to follow-up. It is premature to draw conclusions about the efficacy and safety of other probiotic agents for pediatric AAD. Future trials would benefit from a standard and valid outcomes to measure AAD.

PLAIN LANGUAGE SUMMARY
Antibiotic-associated diarrhea (AAD) occurs when antibiotics disturb the natural balance of "good" and "bad" bacteria in the intestinal tract causing harmful bacteria to multiply beyond their normal numbers. The symptoms of AAD include frequent watery bowel movements and crampy abdominal pain. Probiotics are found in dietary supplements or yogurts and contain potentially beneficial bacteria or yeast. Probiotics may restore the natural balance of bacteria in the intestinal tract. Sixteen studies were reviewed and provide the best available evidence. The studies tested 3432 children (2 weeks to 17 years of age) who were receiving probiotics co-administered with antibiotics to prevent AAD. The participants received probiotics (Lactobacilli spp., Bifidobacterium spp., Streptococcus spp., or Saccharomyces boulardii alone or in combination), placebo (pills not including probiotics), other treatments thought to prevent AAD (i.e. diosmectite or infant formula) or no treatment. The studies were short-term, ranging in length from 10 days to 3 months. Analyses showed that probiotics may be effective for preventing AAD. Probiotics were generally well tolerated, and minor side effects occurred infrequently, with no significant difference between probiotic and control groups. Side effects reported in the studies include rash, nausea, gas, flatulence, vomiting, increased phlegm, chest pain, constipation, taste disturbance, and low appetite. The current data suggest that Lactobacillus rhamnosus and Saccharomyces boulardii at a high dosage of 5 to 40 billion CFU/day may prevent the onset of AAD, with no serious side effects documented in otherwise healthy children. This benefit for high dose probiotics needs to be confirmed by a large well designed randomized study. No conclusions about the effectiveness and safety of other probiotic agents for pediatric AAD can be drawn. More refined studies are also needed that evaluate strain specific probiotics and report both the effectiveness (e.g. incidence and duration of diarrhea) and safety of probiotics.

WHAT’S NEW
What's new
Last assessed as up-to-date: 24 May 2010.

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BACKGROUND
ANTIBIOTIC-ASSOCIATED DIARRHEA
More than 400 species of bacteria inhabit the human gut, and a balance of these micro-organisms is important for normal gastrointestinal function (Madsen 2001). Antibiotic treatment may disturb the colonization resistance of gastrointestinal flora, resulting in a range of symptoms, most notably, diarrhea. In particular, antibiotics such as aminopenicillins, cephalosporins and clindamycin that act on anaerobes are most commonly associated with diarrhea (Wistrom 2001; Owens 2008; McFarland 2008). In addition to frequent watery bowel movements, urgency and crampy abdominal pain, antibiotic-associated diarrhea (AAD) is associated with altered intestinal microflora, mucosal integrity and vitamin/mineral metabolism (Saavedra 1999). If severe, AAD may lead to electrolyte disturbances, volume depletion, pseudomembranous colitis, toxic megacolon and possibly death (Berrington 2004; Arvola 1999). Reports in the general population indicate that the incidence of AAD ranges from 5 to 62%, occurring at any point from the initiation of therapy to two months after the end of treatment (LaRosa 2003; Wistrom 2001; McFarland 1998; McFarland 2008). The incidence of diarrhea in children receiving broad spectrum antibiotics has been reported in the range of 11 to 40% (Turck 2003; Elstner 1983).
The overgrowth of many enteropathogens has been associated with antibiotic-induced diarrhea. Clostridium difficile (C. difficile) overgrowth is the bacterial agent most associated with AAD (McFarland 2008; McFarland 1998; Bartlett 1978). C. difficile diarrhea is associated with the most serious adverse events, and occurs most often in older, immunocompromised, hospitalized adults, but also occurs in children (Gogate 2005).

The definition of AAD varies across trials. Although the World Health Organization (WHO) defines diarrhea as three or more loose or liquid stools per 24 hours, the definition in pediatric trials ranges from one to three abnormally loose stools per 24 to 48 hours (Johnston 2010). Additionally, stool frequency is more difficult to quantify in diaper-aged children with diarrhea and may vary substantially between infants and older children.

PROBIOTICS

Probiotics refer to so-called "friendly" non-pathogenic bacterial or yeast microbiota intended to benefit the host via altering the microflora by implantation or colonization (Schrezenmeir 2001). The rationale behind probiotic administration is based on re-inoculation and normalization of unbalanced indigenous microflora using specific probiotic strains. Probiotics have been administered both prophylactically and therapeutically in an attempt to modify the mucosal, epithelial, intestinal and systemic immune activity in ways that may benefit human health. Probiotics are reported to improve microbial balance in the intestinal tract and display both antibacterial and immune regulatory effects in humans (Gibson 1998; Goldin 1998). Probiotics commonly administered in randomized controlled trials of AAD are: Lactobacillus acidophilus, Lactobacillus bulgaricus, Lactobacillus casei, Lactobacillus rhamnosus, Bifidobacteria bifidum, Bifidobacteria longum, Streptococcus thermophilus, Saccharomyces boulardii and Clostridium butyicum.

PREVIOUS REVIEWS ON PROBIOTICS AND AAD

Four meta-analyses have addressed the use of probiotics, alone or in combination, for the prevention of AAD in the general population. The results favoured probiotic co-administration with antibiotics (RR 0.43; 95% CI 0.31 to 0.58; McFarland 2006; RR 0.48; 95% CI 0.35 to 0.65; Sazawal 2006; RR 0.40; 95% CI 0.28 to 0.57; Cremonini 2002 and OR 0.37; 95% CI 0.26 to 0.53; D’Souza 2002). Additionally, meta-analyses addressing the use of a single probiotic agent to prevent AAD examining Saccharomyces boulardii (S. boulardii) and Lactobacillus have also favored probiotic treatment (RR 0.35, 95% CI 0.19 to 0.67; Kale-Pradhan 2010; RR 0.47, 95% CI: 0.35 to 0.63; McFarland 2010; and RR 0.43; 95% CI: 0.23 to 0.78; Sazawal 2005). Three meta-analyses of randomized trials to evaluate the efficacy of probiotics for preventing antibiotic-induced diarrhea in children have also suggested benefit (RR 0.43; 95% CI 0.25 to 0.75; Johnston 2006; RR 0.44; 95% CI 0.25 to 0.77; Sazawal 2006; and RR 0.43, 95% CI 0.25 to 0.75; Johnston 2007), the latter of which this review aims to update.

SAFETY OF PROBIOTICS

According to the best available evidence, the safety of diverse probiotic interventions does not appear to be a concern in healthy individuals (Whelan 2010; Johnston 2007; Hammerman 2006; Borriello 2003). Infections (e.g. bacteremia, endocarditis, septicaemia, pneumonia, deep abdominal abscesses) resulting from probiotic use have been reported in neonates, severely debilitated and immuno-compromised individuals (Land 2005; Salminen 2004; Mackay 1999; Piarroux 1999; Raatto 1999; McFarland 1998; Salminen 1998; Saxelin 1996; Hata 1998; Sussman 1986), and studies do not indicate that safety is different in these populations. Nevertheless, prospective studies have demonstrated the safety of probiotics in immuno-compromised adults and children with HIV and preterm neonates, with no adverse effects secondary to probiotics reported (Bin-Nun 2005; Lin 2005; Salminen 2004; Cunningham-Rundles 2000).

Two recent systematic reviews have addressed the safety of Saccharomyces boulardii (S. boulardii) and other probiotics (McFarland 2010; Whelan 2010). The first, a systematic review of randomized controlled trials (RCTs), reports on a wide diversity of adult patients randomized to S. boulardii as part of a clinical trial (traveller’s diarrhea, n = 1596; AAD, n = 958; acute diarrhea, n = 156; enteral tube feeding, n = 103; IBD, n = 66; IBS, n = 16, HIV-related diarrhea, n = 18 and giardia infections, n = 50). These studies provide safety data for a total of 2963 adult patients. The only adverse reactions associated with S. boulardii were thirst (n = 5 patients) and constipation (n = 8 patients) in a trial of patients with C. difficile infections (McFarland 1998). No case of S. boulardii fungemia has been reported in these diverse patient populations (McFarland 2010).

A systematic review of case reports, randomized and non-randomized trials of probiotic safety in patients receiving nutritional support included 53 trials involving 4131 patients receiving probiotics. Most trials demonstrated either no effect or a positive effect on outcomes related to safety (e.g. infections, mortality). Three trials reported increased complications, which were largely noninfectious in nature and specific to patients with pancreatitis or undergoing transplant (Whelan 2010). The systematic review also reported 20 case reports of adverse events in 32 patients, 27 of which were infections due to S. boulardii (strain unspecified) or Lactobacillus rhamnosus GG (n = 5). Of the 32 patients having been administered S. boulardii with subsequent infections (i.e. fungemia, bacteremia), 11 of these were in children (either preterm neonates, severely debilitated or immuno-compromised children with underlying risk factors including central venous catheter use and disorders associated with bacterial/fungal translocation). Each of the children recovered after S boulardii or Lactobacillus GG was discontinued, removing the central venous catheter (n = 7) and an antibiotic or anti-fungal was administered (n = 11). The authors of the study reported that these case reports likely reflect the wide use of S boulardii and Lactobacillus GG in clinical settings, rather than their increased virulence (Whelan 2010).

AIMS OF TREATMENT

The aim of treatment with probiotics is to prevent or ameliorate diarrhea (i.e., shorten duration and/or severity of diarrhea).

OBJECTIVES

PRIMARY
1) To systematically assess whether probiotics (any specified strain or dose) co-administered with antibiotics (any agent) reduce the incidence of antibiotic-associated diarrhea in children.

2) To systematically assess adverse events of probiotics when co-administered with antibiotics in children.

SECONDARY
1) To systematically assess which probiotic strain(s) and dose(s) yield the most beneficial results in reducing the incidence of diarrhea.

2) To systematically assess whether probiotics (any specified strain or dose) co-administered with antibiotics (any agent) reduce the duration of diarrhea.

3) To systematically assess whether probiotics (any specified strain or dose) co-administered with antibiotics (any agent) reduce the daily stool frequency.

METHODS OF THE REVIEW

CRITERIA FOR CONSIDERING STUDIES FOR THIS REVIEW

Types of studies
All randomized controlled trials irrespective of language or publication status, in which a specified probiotic agent has been compared to placebo, active, or no treatment control were considered for inclusion.

Types of participants
Children (0 to 18 years of age), male or female of any ethnic group being administered antibiotic therapy for any reason were considered for inclusion.

Types of intervention
Intervention group: specific, identified probiotic in any form (e.g. capsule, sachet, yogurt). Trials investigating non-specific probiotic or yogurt agents (e.g. products that do not label the probiotic strain and dose) were not considered. Trials combining probiotics with prebiotics were included if the prebiotic dose was less than 2.5 grams, as this was judged to be of limited impact to alter the gut milieu (Davis 2010; Gibson 2004; Roberfroid 1998). Control group: placebo, active, or no treatment control. All studies comparing probiotics to conventional care (i.e. diosmectite, loperamide) or probiotics plus conventional care versus conventional care plus placebo or no treatment were considered for the review.

Types of outcome measures

PRIMARY OUTCOMES
1. Incidence of diarrhea using the primary investigators' definition (i.e. frequency, consistency of bowel movements)
2. Number and type of adverse events (e.g. bacteremia, meningitis)

SECONDARY OUTCOMES
1. Mean duration of diarrhea
2. Mean stool frequency

SEARCH METHODS FOR IDENTIFICATION OF STUDIES

Search methods for identification of studies
In May 2010, a comprehensive search of the following relevant databases irrespective of publication status or language was conducted: The Cochrane Central Register of Controlled Trials (CENTRAL) on the Cochrane Library (2010 Issue 2), the trial registers of the Cochrane IBD/FBD Review Group, the Cochrane Complementary Medicine Field's Register of Controlled Trials, MEDLINE (1966 to 2010), EMBASE (1980 to 2010), CINAHL (1982 to 2010), AMED (1985 to 2010), Web of Science (1945 to 2010).

HANDSEARCHES
Bibliographies of randomised controlled trials and review articles were checked for additional studies not identified by the electronic searches.

ADDITIONAL SEARCHES
We searched the International Bibliographic Information on Dietary Supplements (IBIDS) as well as ongoing trials through ClinicalTrials.gov and Current Controlled Trial Register, which houses the NHS Controlled Trials Register, the National Institute of Health Register, the National Research Register, and the International Standard Randomized Controlled Trial Number Register.

The MEDLINE search strategy was as follows:

1. exp PROBIOTICS/tu or probiotic$.tw. or "l acidophilus".tw. or "l casei".tw. or bifidobacter$.mp. or "b infantis".tw. or "b bifidum".tw. or "b longum".tw. or saccharomyce$.mp. or "s boulardii".tw. or clostridium butyricum.tw. or clostridium difficile.mp. or "streptococcus thermophilus".tw. or enterococcus faecium.mp. or...
DATA COLLECTION AND ANALYSIS

Data collection and analysis

The screening, selection, data extraction and risk of bias assessment were done independently and in duplicate by two investigators.

STUDY SELECTION
Search results were screened using titles of papers, and when available, abstracts. The full-text of the selected articles was retrieved and assessed for inclusion according to pre-specified selection criteria. Inter-rater reliability was measured using kappa statistics and disagreement was resolved by discussion.

DATA EXTRACTION
Using a standardized data extraction form we extracted the following data: author, year of publication, language, study setting, funding source, definition and diagnostic criteria for diarrhea, inclusion and exclusion criteria for participants, patient characteristics (age, gender, diagnosis, socioeconomic status), number of patients allocated to each group, presence/absence of intention to treat analysis (whether patients for whom data were available were analyzed as randomized), participants lost to follow-up (LTFU), if so, reasons for LTFU described and information about methods of imputation, measures of compliance, specified antibiotic, specified probiotic, duration, dosage and schedule of antibiotic, duration, dosage and schedule of probiotic, and outcome measures (incidence of diarrhea, number of adverse events, mean duration of diarrhea, mean stool consistency, and mean stool frequency).

QUALITY ASSESSMENTS
Quality components for each included RCT were assessed for selection, detection, performance, reporting and loss to follow-up bias. Each of the included studies was evaluated using the risk of bias instrument to assess sequence generation, allocation concealment, blinding, incomplete outcome data, selective outcome reporting, and other sources of bias (Hartling 2009).

We also employed the GRADE system for rating overall quality of evidence for each of the outcomes. In particular, randomized trials begin as high quality evidence, but may be rated down by one or more of five categories of limitations: (1) risk of bias, (2) consistency, (3) directness, (4) imprecision, and (5) reporting bias. The quality of evidence for each main outcome can be determined after considering each of these elements, and categorized as either (we are very confident that the true effect lies close to that of the estimate of the effect); moderate (we are moderately confident in the effect estimate: the true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different); low (our confidence in the effect estimate is limited: the true effect may be substantially different from the estimate of the effect); very low (we have very little confidence in the effect estimate: the true effect is likely to be substantially different from the estimate of the effect) (Guyatt 2008).

STATISTICAL ANALYSIS
Results were combined unless diversity (clinical and/or statistical heterogeneity) suggested combination was unreasonable. Dichotomous data are presented as relative risks, and continuous data as weighted mean differences, along with their corresponding 95% confidence intervals. Using control event risks from the included trials, the number needed to treat (NNT) or the number needed to harm (NNH) was calculated for statistically significant dichotomous outcomes. Adverse events were summarized using risk difference since these events were rare. Random-effects models were used and fixed-effect models were considered in sensitivity analyses. Heterogeneity was investigated using the I^2 statistic (Higgins 2003). Meta-regression or the Chi^2 test for heterogeneity - depending on the number of trials included - were used to address a priori hypotheses explaining heterogeneity. To explore possible explanations for heterogeneity, subgroup analyses were subdivided by: probiotic strain(s) (when two or more trials administered the same strains), antibiotics that are specific to anaerobes (most associated with diarrhea adverse events), diagnostic criteria for diarrhea (e.g. ≥ 3 watery/liquid stools per day for 2 consecutive days versus ≥ 3 watery/liquid stools per day), dosage of probiotic (≥ 5 billion colony forming units of live bacteria/yeast, < 5 billion colony forming units of live bacteria/yeast) and quality criterion (e.g. risk of bias instrument). To assess the potential influence of missing responses (e.g. children lost to follow up) sensitivity analyses were applied for the primary outcome, incidence of diarrhea. Although many approaches exist for evaluating the sensitivity of results to missing responses (Akl 2009; Hollis 1999), we elected to make assumptions about the missing data which were extreme but still plausible (i.e. 60% of children lost to follow-up in probiotic group and 20% loss to follow-up in the control group had diarrhea). To evaluate the potential for publication bias, funnel plots, the rank correlation test (Begg 1994) and weighted regression (Egger 1997) were applied to the main efficacy outcome, incidence of diarrhea. If publication bias was apparent, adjustment of the pooled estimates was considered using the trim and fill method (Duval 2001). More than one method to evaluate publication bias was considered since the relative merits of the methods are not

http://cochrane.bvsalud.org/cochrane/show.php?db=reviews&mf=n=&id=CD004827&lang=en&blang=en&lib=COC#
well established.

**METHODOLOGICAL QUALITY**

**RESULTS**

**Results**

**Description of studies**

See: Characteristics of included studies; Characteristics of excluded studies.

A previous literature search conducted in August 2006 identified 10 relevant studies for inclusion (7 English, 2 Italian, 1 French) and is described in detail elsewhere (Johnston 2007). An updated search was conducted in May 2010 (search dates: January 2005 to May 25, 2010). A total of 416 studies were identified from the primary electronic databases (Medline 144, EMBASE 92, CENTRAL 41, CINAHL 41, Web of Science 98, AMED 0). Of these 173 were identified as duplicates, leaving 243 abstracts and/or titles identified as original publications. A grey literature search (ClinicalTrials.gov, NHS Evidence, IBIDS) identified 1 additional relevant study for full-text review. Independent review (BCJ, JZG) of these titles and/or abstracts identified 14 potentially relevant studies for full-text review. Two authors (JZG, XS) independently assessed these studies and identified six that met the inclusion criteria. The inter-rater proportion of overall agreement on inclusion and exclusion was 93% with a kappa coefficient of 0.85 (95% CI 0.57 to 1.00). Reasons for exclusion were as follows: five studies did not include children (Kim 2008; Koning 2008; Wenus 2008; Beausoleil 2007; Can 2006); one study used a gastro-intestinal symptoms rating scale that, while inclusive of stool frequency and consistency, did not report data specific to those outcomes (Lionetti 2006); one study involved Saccharomyces boulardii for pediatric infectious diarrhea (i.e., amebiasis-associated diarrhea) (Savas-Erdeve 2009); and one study did not administer probiotics concurrently with antibiotics (Honeycutt 2007). A detailed summary of all included and excluded studies can be found in the "Characteristics of Included Studies" and "Characteristics of Excluded Studies" tables.

**Design**

All included studies were prospective, randomized, controlled trials (placebo, active or no treatment control arm).

**Patient population**

For the purposes of this systematic review LT FU can be understood as incomplete ascertainment of the primary outcome for some participants in an RCT. Patients for whom data were not available for the primary outcome were classified as LT FU. After accounting for LT FU the 16 studies included a total of 2941 patients (1516 treatment, 1425 controls). Patients were diagnosed with upper and lower respiratory tract, ear, gastrointestinal, dermatological, or other infections (Merenstein 2009; Szajewska 2009; Ruszczynski 2008; Szymanski 2008; Kotowska 2005; LaRosa 2003; Arvola 1999; Benhamou 1999; Vanderhoof 1999; Contardi 1991; Tankanow 1990; Destura unpublished), and meningitis or septicemia (Jirapinyo 2002). In one study the type of infection that necessitated antibiotic therapy was not reported (Conway 2007). The health care setting was reported in 14 studies and consisted of: private primary care practices (Merenstein 2009; Conway 2007; Benhamou 1999; Vanderhoof 1999; Contardi 1991; Tankanow 1990), hospitalized inpatients (Szajewska 2009; Correa 2005; Jirapinyo 2002), an outpatient university teaching hospital (Kotowska 2005; Arvola 1999), and both inpatient and outpatient hospital populations (Destura unpublished). In addition to inpatient and outpatient hospital populations, Ruszczynski 2008 also enrolled from a private practice, and Szymanski 2008 also enrolled from outpatient clinics. Children enrolled were from families of diverse socioeconomic status, including developed and developing countries such as Brazil (Correa 2005), the Philippines (Destura unpublished), Poland (Szajewska 2009; Ruszczynski 2008; Szymanski 2008), Thailand (Jirapinyo 2002) and Turkey (Erdeve 2004). Children ranged from 1 month to 18 years of age. Eleven studies provided information regarding the participants' mean age: 4.5 years (Arvola 1999), 2.4 years (Benhamou 1999), 1.8 years (Correa 2005), treatment 4.1 years and control 4 years (Destura unpublished), 4.8 years (Kotowska 2005), 6.6 years (LaRosa 2003), 2.9 years treatment and 3.2 years control (Merenstein 2009), treatment 4.6 years and control 4.5 years (Ruszczynski 2008), 12.3 years treatment and 11.9 years control (Szajewska 2009), 2.5 years (Tankanow 1990) and 4 years (Vanderhoof 1999). Two studies provided only the age range of enrolled participants: 8 months to 3 years (Contardi 1991) and 1 month to 3 years (Jirapinyo 2002). One study provided median age with a range: 7 years (range 1 to 15) (Szymanski 2008). Eleven studies included both males and females (838 males and 821 females), and five studies did not state sufficient information regarding gender (Conway 2007; Erdeve 2004; Jirapinyo 2002; Arvola 1999; Benhamou 1999).

**Interventions**

Overall the trials provided between 3 and 30 days of antibiotic therapy. Most trials provided oral antibiotics. Three trials administered intravenous antibiotics to some patients (e.g., cefuroxime): 60/246 (24.3%) (Kotowska 2005); 87/240 (36.3%) (Ruszczynski 2008); 6/78 (7.7%) (Szymanski 2008). Ruszczynski 2008 also provided intravenous (IV) antibiotics followed by oral antibiotics (17/240; 7.1%) and intramuscular (IM) antibiotics (2/240; 0.8%). In three trials it was unclear what antibiotics and/or route was used (Merenstein 2009; Conway 2007; Destura unpublished). Two studies provided oral amoxicillin alone (Contardi 1991; Tankanow 1990) using a standard pediatric dosage range (20 to 50 mg/kg/day), whereas the remaining trials provided a mixture of oral antibiotic agents including: bactericidal cephalosporins (e.g., cefotaxime, ceproflox), bacteriostatic macrolides (e.g., clarithromycin, erythromycin), and the bactericidal beta-lactams/penicillins. In particular, nine studies described the antibiotic classes administered. Four studies administered a host of cephalosporins (n = 341) and beta-lactams/penicillins (n = 931) (Correa 2005; Kotowska 2005; Benhamou 1999; Destura unpublished), one study provided cephalosporins (n = 49), beta-lactams/penicillins in the form of amoxicillin-clavulanate (n = 36) and macrolides in the form of erythromycin (n = 34) (LaRosa 2003), and one study provided beta-
lactams/penicillins in the form of sulbactam-ampicillin (n = 234) and macrolides in the form of azithromycin (n = 232) (Erdeve 2004). One study provided all participants (n = 83) with three concurrent pharmaceuticals: amoxicillin, clarithromycin, and omeprazole (Szajewska 2009). Szymanski 2008 provided cephalosporins (n = 20); beta-lactams/penicillins in the forms of penicillin, amoxicillin, or amoxicillin+clavulanate (n = 39); macrolides (n = 18); and aminoglycosides (n = 1). Ruszczynski 2008 provided cephalosporins (n = 89); beta-lactams/penicillins in the forms of penicillin, amoxicillin, or amoxicillin+clavulanate (n = 134); macrolides (n = 15); and clindamycin (n = 2).

Trials included treatment with either Bacteri spp., Bifidobacterium spp., Lactobacillus spp., Lactococcus spp., Leuconostoc cremoris, Saccharomyces spp., or Streptococcus spp. The strain(s) and daily dosage of the probiotic interventions included: Lactobacillus GG, 1 billion colony forming units (CFU) bacteria/day (Szajewska 2009); Lactobacillus GG, 20 to 40 billion CFU bacteria/day (Arvola 1999); Lactobacillus GG and inulin (a prebiotic), 10 to 20 billion CFU bacteria/day equaling 100 mg and 225 mg of the prebiotic inulin/day (the only study to use a weight-based approach) (Vanderhoof 1999); Saccharomyces boulardii, 4.5 billion yeast/day (Benhamou 1999); Lactobacillus acidophilus and Bifidobacterium bifidus, 3 billion CFU bacteria/day (Contardi 1991); Bifidobacterium lactis and Streptococcus thermophilus, 825 million CFU bacteria/day (Correa 2005); Bacillus clausii, 4 billion CFU bacteria/day (Destura unpublished); Saccharomyces boulardii, 5 billion CFU yeast/day (Erdeve 2004); Lactobacillus acidophilus and Bifidobacterium infantis, dose not reported (Jirapinyo 2002); Saccharomyces boulardii, 10 billion CFU of yeast/day (Kotowska 2005); Lactobacillus sporogenes and fructo-oligosaccaride (a prebiotic); 5.5 billion CFU bacteria/day and 250 mg prebiotic/day (LaRosa 2003); Lactococcus lactis, L. plantarum, L. rhamnosus, L. casei, L. lactis subspecies diacetylactis, Leuconostoc cremoris, Bifidobacterium longum, B. breve, Lactobacillus acidophilus, and Saccharomyces florentinus, at least half of a 150 ml drink containing 7 to 10 billion CFU bacteria and yeast/day (Merenstein 2009); Lactobacillus rhamnosus, 40 billion CFU bacteria/day (Ruszczynski 2008); Bifidobacterium longum PL03, Lactobacillus rhamnosus KL53A, and Lactobacillus plantarum PL02, 200 billion CFU bacteria/day (Szymanski 2008); Lactobacillus acidophilus and Lactobacillus bulgaricus, 2 billion CFU bacteria/day (Tankanow 1990); and finally Streptococcus thermophilus, Lactobacillus acidophilus, and Bifidobacteria anamalis subsp. lactis or Streptococcus thermophilus and Lactobacillus delbrueckii subsp. bulgaris, 1 billion CFU bacteria/day (Conway 2007).

Comparison
In 10 studies, the probiotic(s) intervention was compared to a placebo control group, two trials compared probiotics to conventional care including formula and diosmectite (Correa 2005; Benhamou 1999), two trials compared probiotics to no treatment (Erdeve 2004; Destura unpublished), one trial compared a live probiotic drink to a heat-killed probiotics drink (Merenstein 2009), and one trial used three arms: 'bioyogurt,' commercial yogurt, and no yogurt (Conway 2007). In order to avoid unit of analysis errors, for the purposes of this review we grouped the two yogurt arms of the latter trial together. In one placebo-controlled trial, contact with authors revealed that the placebo contained an inert amount of inulin (325 mg) - a prebiotic used as capsule filler (Vanderhoof 1999). Five additional placebo-controlled trials provided information on the choice of comparison stating that the placebos contained maltodextrine, non-fat milk and saccharose, saccharose, lactose, 'sugar,' and 'lactose' respectively (Szajewska 2009; Ruszczynski 2008; Kotowska 2005; Jirapinyo 2002; Tankanow 1990). For the two trials involving active controls with conventional care, one trial administered diosmectite (an anti-diarrheal gastrointestinal protectant drug) (Benhamou 1999) and the second administered formula containing vitamins, minerals and protein (Correa 2005).

Outcomes
Fifteen studies (n = 2882) provided data on the incidence of diarrhea, 11 studies (n = 1583) reported on adverse events, five studies (n = 897) reported mean duration of diarrhea, and four studies (n = 425) reported mean stool frequency. The criteria for defining diarrhea varied among the studies and ranged from clinical determination of diarrhea incidence (Merenstein 2009); one or more abnormally loose bowel movements per day (Tankanow 1990); two or more liquid stools per day on at least two occasions during the course of the study (Vanderhoof 1999); three or more liquid/watery stools per day (Erdeve 2004; Benhamou 1999), three or more watery/loose/liquid stools per day for two consecutive days (Conway 2007; Correa 2005; Kotowska 2005; Arvola 1999); change in bowel habits with the passage of three or more liquid stools per day for at least 2 consecutive days 48 hours after initiation of antibiotic therapy (Destura unpublished); to greater than or equal to 3 loose or watery stools per day for a minimum of 48 hrs, occurring during and/or up to 2 weeks after the end of the antibiotic therapy (Szajewska 2009; Ruszczynski 2008; Szymanski 2008).

Three studies reported on viral and bacterial analysis of fecal samples (Kotowska 2005; Arvola 1999; Destura unpublished). Along with viral and bacterial fecal analysis, one trial reported on the metabolic activity of gut microflora: fecal urease, β-glucosidase and β-glucuronidase activity (Arvola 1999). Two trials reported on frequencies of retroviral diarrhea, salmonellosa diarrhea, shigella diarrhea and C. difficile diarrhea (Ruszczynski 2008; Kotowska 2005). Other outcomes of potential interest included mean diarrhea incubation and percentage suffering from dehydration reported in one study (Correa 2005), fecal lactoferrin (Destura unpublished), and the need for IV rehydration, hospitalization of outpatients, and/or discontinuation of antibiotic treatment (Ruszczynski 2008; Szymanski 2008). Additionally, one study reported on H. pylori outcomes such as positive rapid urea test, positive histopathology for H. pylori, and positive C13 urea breath test (Szajewska 2009). No studies reported on cost-effectiveness related to absenteeism from the workplace, daycare or school between treatment and control groups.

Risk of bias in included studies
Five studies reported information concerning a priori sample size calculations (Merenstein 2009; Ruszczynski 2008; Conway 2007; Kotowska 2005; Vanderhoof 1999). Loss to follow-up was substantial (i.e. > 20%) in 5/15 trials reporting the incidence of diarrhea (Szajewska 2009; Erdeve 2004; Arvola 1999; Benhamou 1999; Tankanow 1990). In particular, LTFU was 37% in Tankanow 1990 and 29% in Arvola 1999. Six trials provided a flow diagram to track participants some of which included details regarding drop-outs (Merenstein 2009; Szajewska 2009; Ruszczynski 2008; Correa 2005; Conway 2007; Kotowska 2005). All studies were conducted in parallel-group designs. Conclusions: studies included participants some of which included details regarding drop-outs (Merenstein 2009; Szajewska 2009; Ruszczynski 2008; Correa 2005; Conway 2007; Kotowska 2005). All studies were conducted in parallel-group designs. Conclusions:
A priori subgroup analyses were performed using a "double-blind" procedure. However, the risk of bias assessment determined that patients in the Conway 2007 and Tankanow 1990 studies were likely unblinded during treatment. Two trials were open label (Contardi 1991; Destura unpublished). The validated risk of bias instrument categorizes risk into three categories: high risk of bias, low risk of bias and unclear. Eight trials were categorized as low risk (Merenstein 2009; Szajewska 2004; Ryczynski 2008; Szymanski 2005; Correa 2005; Kotowska 2005; La Rosa 2003; Vanderhoof 1999) and eight trials were categorized as high risk (Erdeve 2004; Jirapinyo 2002; Arvola 1999; Benhamou 1999; Contardi 1991; Tankanow 1990; Destura unpublished; Conway 2007). See and for the overall results of the risk of bias assessment. The chance-adjusted agreement between reviewers on the application of risk of bias criteria was very good (kappa 0.77; 95% CI 0.50 to 1.03).

Effects of interventions

See: Summary of findings for the main comparison Probiotics as an adjunct to antibiotics for the prevention of pediatric antibiotic-associated diarrhea in children

Incidence of diarrhea

To allow for a heterogeneous definition of diarrhea, data (as a binary outcome) were included based on the primary authors' definition of the presence/absence of diarrhea. Fifteen studies (n = 2882) reported incidence of diarrhea. Using an available case (i.e. patients who did not complete the studies were not included in the analysis) approach as the primary analysis, four placebo-controlled studies showed a statistically significant reduction in the incidence of AAD (P < 0.05) (Ryczynski 2008; Correa 2005; Kotowska 2005; La Rosa 2003; Vanderhoof 1999); one active-controlled study (formula) was statistically significant (Correa 2005), and one 'no treatment-control' study demonstrated statistical significance (Erdeve 2004). Six placebo-controlled studies (Merenstein 2009; Szajewska 2004; Szymanski 2008; Jirapinyo 2002; Arvola 1999; Tankanow 1990), two no treatment-control studies (Conway 2007; Destura unpublished), and one active-control (diosmectite) study (Benhamou 1999) did not show a statistically significant difference, but seven of these nine trials showed a non-significant trend favouring probiotics. The overall pooled results using an available case analysis showed that the use of probiotics produced a statistically significant reduction in the incidence of AAD. The incidence of AAD in the probiotic group was 9% compared to 18% in the active, placebo or no treatment control group (2874 participants; RR 0.52; 95% CI 0.38 to 0.72; random-effects). However, statistically significant heterogeneity was detected (P = 0.004) and this was moderate with respect to percent variability due to between (or inter-) study variability (I² = 56%) (Higgins 2003).

Adverse events

None of the studies specifically defined adverse events a priori. Three trials reported no adverse events (Kotowska 2005; Jirapinyo 2002; Vanderhoof 1999). Four trials reported adverse events (Merenstein 2009; Szajewska 2009; Correa 2005; Tankanow 1990). Tankanow 1990 reported 14 adverse events including rash, gas, vomiting, increased phlegm and chest pain. However, for each of the 14 events it was not clear in which group (treatment or control) the adverse events occurred. It appears that the 14 adverse events occurred in three participants (Tankanow 1990). It was assumed for the meta-analysis that the adverse events were in the treatment group. Correa 2005 reported five participants with adverse events in the treatment group. These adverse events were related to the tolerability of the formula supplemented with probiotics. Merenstein 2009 reported a case of emesis in the treatment group and a case of constipation in the control group. Szajewska 2009 reported 18 adverse events in the treatment group and 13 in the control group. In both groups adverse events included nausea, vomiting, constipation, flatulence, taste disturbance, and low appetite. No statistically significant difference in adverse events was found between groups. Meta-analysis of all trials that included adverse events demonstrated no statistically significant differences in the incidence of adverse events (RD 0.00; 95% CI -0.01 to 0.02).

Mean duration of diarrhea

Five studies recorded the mean duration of diarrhea (Correa 2005; La Rosa 2003; Arvola 1999; Vanderhoof 1999; Destura unpublished). The standard deviation (SD) for one of the trials was not reported and this information was requested from authors with no response (Vanderhoof 1999). The SD was imputed for Vanderhoof 1999 from a study reporting a similar mean duration of diarrhea for treatment and control (Arvola 1999). A post hoc sensitivity analysis was conducted to test the robustness of the mean duration results both before and after imputing data. The WMD was not statistically significant before including Vanderhoof 1999 (WMD -0.42; 95% CI -1.01 to 0.16), although it was statistically significant after imputing the SD data (WMD -0.60; 95% CI -1.18 to -0.02). Statistically significant heterogeneity was detected (P = 0.008) and this was high with respect to percent variability due to between (or inter-) study variability (I² = 79%) (Higgins 2003).

Mean stool frequency

Four RCTs reported mean stool frequency (Szymanski 2008; Arvola 1999; Vanderhoof 1999; Contardi 1991). SD data were imputed for one study (Arvola 1999). This study reported a range for the mean stool frequency for both treatment and control which was used to impute a SD for each study arm. A post hoc sensitivity analysis was conducted to test the robustness of the mean stool frequency results both before and after imputing data. The WMD excluding Arvola 1999 was -0.36 (95% CI -0.70 to -0.02). After imputing SD data the WMD was not statistically significant -0.30 (95% CI -0.60 to -0.00; I² = 78%).

A priori subgroups

Probiotic species

Four of 15 trials administered Lactobacillus rhamnosus species (three using strain Lactobacillus GG: Szajewska 2009; Arvola 1999; Vanderhoof 1999; and one using strains E/N, Oxy, and Pen: Ryczynski 2008), while three studied the yeast Saccharomyces boulardii (strain lyo) (Kotowska 2005; Erdeve 2004; Benhamou 1999). Combined results from four L. rhamnosus studies (n = 611) were statistically significant indicating a protective effect (RR 0.35; 95% CI 0.22 to 0.56. I² = 0%). The summary statistic for the Saccharomyces boulardii trials (n = 1378) was not statistically significant (RR
Incidence of diarrhea analysis

Imputation for missing data analysis

A sensitivity analysis using random-effects models (RR 0.52; 95% CI 0.38 to 0.72) versus fixed-effect models (RR 0.51; 95% CI 0.42 to 0.62) for the incidence of diarrhea, indicated limited differences between the relative risk and corresponding 95% confidence intervals. Nonetheless, because the I² statistic demonstrated moderate heterogeneity within and between studies, a random-effects model was used for all statistical analyses.

Imputation for missing data analysis

Incidence of diarrhea analysis

There were 3392 pediatric participants originally randomized in the 15 trials reporting on the primary outcome (incidence of diarrhea). Eleven of 15 trials reported LTFU of which five reported substantial attrition concerns. Loss to follow-up was 20%, 21%, 28%, 28% and 36% in the Szajewska 2009; Arvola 1999; Benhamou 1999; Erdeve 2004; and Tankanow 1990 studies respectively. We elected to make assumptions about the missing data which were extreme but still plausible.
for two separate analyses: i) involving the seven trials having employed high dose probiotics (≥ 5 billion CFU/day) for the primary efficacy analysis, and ii) involving all trials that reported on the primary efficacy analyses. Six of seven trials using ≥ 5 billion CFU/day had loss to follow-up (6 to 28%), whereas 11 of 15 reporting on the primary efficacy outcome had loss to follow-up. If no information on the number of patients randomized to each group, or the number LTFU from each group (e.g. not reported in the published trial or unsuccessful contact with authors) was available, it was assumed that the LTFU in the treatment and control groups were as even as possible (block randomization). After imputing data for the missing responses, an extreme-plausible intention to treat (ITT) analysis (60% of children loss to follow-up in probiotic group and 20% loss to follow-up in the control group had diarrhea) involving the seven high-dose trials showed a marginally significant benefit favouring probiotics. For the high dose studies the pooled incidence of AAD in the probiotic group was 17% compared to 22% in the active, placebo or no treatment control group (RR 0.72; 95% CI 0.53 to 0.99; I² = 57%). The extreme-plausible ITT analysis based on all 15 trials reporting probiotics for preventing the onset of diarrhea was not statistically significant. The pooled incidence of AAD in the probiotic group was 16% compared to 18% in the active, placebo or no treatment control group (RR 0.81; 95% CI 0.63 to 1.04; I² = 59%).

Adverse event analysis

Assuming that patients LTFU in each of the trials may have had adverse events, we conducted a sensitivity analysis to test the robustness of the primary available case analysis. To do so, we suspected that the most reasonable assumption to make for those who were LTFU was that LTFU had the same adverse event rate as those followed up in their respective randomization groups. In particular, among the four trials that did report adverse events, the proportion of adverse events was 14.4% (27/187) in the treatment group and 14/192 (7.29%) in the control group. For all eleven trials that reported LTFU, we assigned the same adverse event rate as those followed up in their respective randomization groups (14.4% and 7.29% assumed to have adverse events among treatment and control groups respectively) to those who were LTFU.

Similar to our primary available case harms analysis (RD 0.00; 95% CI -0.01 to 0.02), the same event rate assumptions analysis which included all 16 trials yielded a pooled estimate of effect (RD 0.01; 95% CI -0.00 to 0.02).

Publication bias

A funnel plot analysis provides no compelling indication of publication bias showing general symmetry of the funnel for the relationship between risk ratio and standard error. (See ). Although there are a limited number of trials reporting on the incidence of diarrhea (n = 15), additional tests based on Begg’s test of correlation (P = 0.55) and Egger’s regression test (P = 0.46), also failed to suggest evidence of publication bias. Although our tests for publication bias fail to demonstrate that negative studies remain unpublished, the literature suggests that these tests are, at best, subjective. The validity of these tests has not been established and as a result strong claims for or against publication bias should be avoided if there is not clear evidence suggesting the presence or absence of negative results remaining unpublished (Ioannidis 2008; Ioannidis 2007; Lau 2006).

DISCUSSION

Discussion

The primary objective of this review was to determine if the co-administration of probiotics with antibiotics prevents or ameliorates AAD in children. Sixteen eligible studies included treatment with Bacillus spp., Bifidobacterium spp., Lactobacillus spp., Lactococcus spp., Leuconostoc cremoris, Saccharomyces spp., or Streptococcus spp., alone or in combination. Seven of 16 trials tested S. boulardii or Lactobacillus rhamnosus spp. Available case (i.e. patients who did not complete the studies were not included in the analysis) results from 15/16 trials reporting on the incidence of diarrhea, demonstrated a relatively large, precise benefit from probiotics (RR 0.52; 95% CI 0.38 to 0.72, I² = 56%). An a priori hypothesis testing for heterogeneity indicated that high dose (≥ 5 billion CFUs/day; RR 0.40 95% CI 0.29 to 0.55; I² = 29%) is significantly more effective than low probiotic dose (< 5 billion CFUs/day; RR 0.80 95% CI 0.53 to 1.21). Based on high-dose probiotics, the number needed to treat (NNT) to prevent one case of diarrhea is seven (NNT 7; 95% CI 6 to 10).

To test the robustness of our available case analysis, we elected to make assumptions about the missing data from the trials having employed high dose probiotics (≥ 5 billion CFU/day) which were less extreme and arguably more plausible. Six of seven trials using ≥ 5 billion CFU/day had loss to follow-up (6 to 28%). After imputing data for the missing responses, an extreme-plausible ITT analysis (60% of children loss to follow-up in probiotic group and 20% loss to follow-up in the control group had diarrhea) indicated a marginally significant benefit for high dose probiotics (1776 participants; RR 0.72; 95% CI 0.53 to 0.99; I² = 57%). An extreme-plausible ITT analysis of the 15 trials reporting on the incidence of diarrhea was not statistically significant (RR 0.81; 95% CI 0.63 to 1.04; I² = 59%).

Statistical heterogeneity was moderate in both of the available case analyses. We specified a priori subgroup hypotheses to explore the heterogeneity in our results that included probiotic species, probiotic dose, risk of bias (e.g., blinding, allocation concealment), antibiotic agent and definition of diarrhea. A test for heterogeneity was significant for one subgroup - probiotic dose - providing evidence that a dose-response gradient is the most likely explanation for the observed statistical heterogeneity. The test for interaction for potential dose related heterogeneity was statistically significant (P = 0.010).

Using 11 criteria to evaluate the credibility of the subgroup analysis, the results indicate that the subgroup effect based on dose (≥ 5 billion CFU/day) was convincing (Sun 2010; See Appendix 1, Table 1). This represents an important finding as dosage recommendations for products containing probiotics available in pharmacies and health food stores have a wide range (e.g. 1 to 450 billion CFU/day); and dosages approaching the lower range may not confer a therapeutic benefit (Raza 1995). Given our review included trials testing 10 different probiotics either alone or in combination, amongst a diverse clinical population, with nearly all demonstrating favourable results; for the purposes of clinical recommendations and future research, our findings suggest that the minimal effective dose may be 5 billion CFU/day.

Rea andardino safetv. 11/16 trials reported on adverse events. none havinino reported a serious adverse event. Meta-analysis

http://cochrane.bvsalud.org/cochrane/show.php?db=reviews&mfn=&id=CD004827&lang=&dblang=&lib=COC#
Concerning the secondary outcome mean duration of diarrhea (five trials, n = 897), using an available case analysis, probiotics decreased the mean duration of diarrhea by almost three quarters of a day (WMD -0.60; 95% CI -1.18 to -0.02), a statistically significant difference. With respect to differences in mean stool frequency (four trials, n = 425), the available case results were not statistically significant (WMD -0.30 95% CI -0.60, 0.00). For the two secondary outcomes, mean duration of diarrhea and mean stool frequency, the quality of evidence was categorized as low owing to inconsistency (i.e. large statistical heterogeneity with I² of > 77%, low P value [P < 0.06], point estimates and confidence intervals vary considerably) and imprecision (e.g. confidence intervals include effect estimates that are of questionable patient importance). Furthermore, results for mean duration of diarrhea may be misleading given our suspicion of selective reporting bias. In particular, the majority of studies fail to report results for this key outcome that otherwise would be expected to have been evaluated. A previous review of RCTs of acute diarrhea reported that duration of diarrhea was the most common primary outcome (72/138 trials, 52% of trials) and this was reported in almost all trials as either a primary or secondary outcome (Johnston 2010). In this review, only 5 of 16 trials reported duration of diarrhea as a primary or secondary outcome.

This systematic review has several strengths. We asked a clear clinical question and the search strategy for this review was comprehensive including any relevant trials irrespective of language or publication status (i.e. we included unpublished data from Destura unpublished and obtained pediatric specific data from Conway 2007). Additional strengths of the review include its rigorous application of the GRADE criteria for each of the outcomes (Guyatt 2008) and the rigorous evaluation of each of the five subgroups (i.e. probiotic species, probiotic dose, antibiotic class, risk of bias and definition of diarrhea) using the 11 criteria for assessing subgroup credibility (Sun 2010).

This review also has limitations. The United European Gastroenterology Week, North American Society for Pediatric Gastroenterology, and Hepatology and Nutrition conference proceedings were not searched. In addition, although we previously did a more comprehensive search of the grey literature, for our update search we did not search conference proceedings or dissertation abstracts. Some readers may question the pooling of different probiotic species. In keeping with the justification for the combining of probiotic species used in two trials included in this review (Tankanow 1990 administered both L acidophilus with L bulgaricus; Jirapinyo 2002 administered both L acidophilus with B infantis; Szymanski 2008 administered a cocktail of B longum, L rhamnosus and L plantarum), data were pooled because the probiotics used in each trial share the recommended characteristics of a viable probiotic: non-pathogenic properties (noting that further study is needed on L sporogenes), the ability to survive transit through the gastrointestinal tract, adherence to intestinal epithelium, colonization in the intestinal tract, production of antimicrobial substances, and a good shelf life in food or powdered form (Goldin 1998).

AUTHORS’ CONCLUSIONS

Implications for practice

Despite heterogeneity in probiotic strain, dose, and duration, and study quality issues (risk of bias), the overall evidence suggests a protective effect of probiotics in preventing AAD. A test for heterogeneity indicates that a dose-response gradient explains some of the statistical heterogeneity. Using 11 criteria to evaluate the credibility of the subgroup analysis on probiotic dose, available case results indicate that the subgroup effect based on dose (≥5 billion CFU/day) was credible, demonstrating a large, precise benefit of high dose probiotics (RR 0.40; 95% CI 0.29 to 0.55). Based on high-dose probiotics, the number needed to treat (NNT) to prevent one case of diarrhea is seven (NNT 7; 95% CI 6 to 10). However, the extreme plausible ITT analysis was marginally significant for high dose probiotics (RR 0.72; 95% CI 0.53 to 0.99). The use of Lactobacillus rhamnosus or Saccharomyces boulardii at 5 to 40 billion colony forming units/day may be appropriate given the modest NNT and the likelihood that adverse events are very rare. It is premature to draw conclusions about the efficacy and safety of other probiotic agents for pediatric AAD. Although no serious adverse events were observed amongst the otherwise healthy children included in this pool of trials, moderate to serious adverse events have been observed in severely debilitated or immuno-compromised children with underlying risk factors including central venous catheter use and disorders associated with bacterial/fungal translocation. Until further research has been conducted, probiotic use should be avoided in pediatric populations at risk for adverse events.

Implications for research

The GRADE analysis indicated that the overall quality of the evidence for the primary endpoint for high dose probiotic studies (incidence of diarrhea) was low due to a high risk of bias in the pooled analysis (high loss to follow-up: 29% in two studies and 16% across the seven studies) and imprecision (sparse data, 225 events). The benefit for high dose probiotics (Lactobacillus rhamnosus or Saccharomyces boulardii) needs to be confirmed by a large well designed RCT. More refined trials are also needed that test strain specific probiotics and evaluate the efficacy (e.g. incidence and duration of diarrhea) and safety of probiotics with limited losses to follow-up. In particular, trials should look to determine the effect of strain...
specific probiotics on: 1. incidence of diarrhea and duration of diarrhea using a appropriate definitions of diarrhea onset (incidence of diarrhea) and offset (duration of diarrhea), 2. define potential adverse events a priori and monitor for these adverse reactions according to available guidelines (Ioannidis 2004) and 3. given the relatively short follow-up time in trials of this nature - limit losses to follow-up. In addition, future trials would benefit from a standard, core set of outcomes in acute diarrhea trials that are valid and reliable (Clarke 2007; Johnston 2010). This would make trials more comparable and help avoid selective reporting bias.

ACKNOWLEDGEMENTS

Acknowledgements

We would like to thank John K MacDonald (Cochrane IBD/FBD Review Group) for his ongoing support and Nancy Santesso for translation of Italian articles.

BCJ holds a SickKids Foundation Post-doctoral Fellowship (Complementary and Alternative Health Care & Paediatrics Fellowship Award). XS is supported by the Ontario Graduate Scholarship, and the National Natural Science Foundation of China (70703025).

Funding for the IBD/FBD Review Group (September 1, 2010 - August 31, 2015) has been provided by the Canadian Institutes of Health Research (CIHR) Knowledge Translation Branch (CON - 105529) and the CIHR Institutes of Nutrition, Metabolism and Diabetes (INMD); and Infection and Immunity (III) and the Ontario Ministry of Health and Long Term Care (HLTC3968FL-2010-2235).

Miss Ila Stewart has provided support for the IBD/FBD Review Group through the Olive Stewart Fund.

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Schrezenmeir 2001

Sun 2010
Sun X, Briel M, Walter S, Guyatt GH. Is a subgroup effect believable? Updating criteria to evaluate the credibility of subgroup analyses. BMJ 2010;340:c117-.

Sussman 1986

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Turck 2003

Wenus 2008

Whelan 2010

Wistrom 2001

GRAPHS

Graphs and Tables

To view a graph or table, click on the outcome title of the summary table below.

### Incidence of Diarrhea

<table>
<thead>
<tr>
<th>Outcome title</th>
<th>No. of studies</th>
<th>No. of participants</th>
<th>Statistical method</th>
<th>Effect size</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Incidence of Diarrhea: Complete case</td>
<td>15</td>
<td>2874</td>
<td>Risk Ratio (M-H, Random, 95% CI)</td>
<td>0.52 [0.38, 0.72]</td>
</tr>
<tr>
<td>1.1 Incidence of Diarrhea: Active controlled trials</td>
<td>2</td>
<td>773</td>
<td>Risk Ratio (M-H, Random, 95% CI)</td>
<td>0.85 [0.33, 2.21]</td>
</tr>
<tr>
<td>1.2 Incidence of Diarrhea: Placebo controlled trials</td>
<td>10</td>
<td>1206</td>
<td>Risk Ratio (M-H, Random,</td>
<td>0.48 [0.33, 0.68]</td>
</tr>
</tbody>
</table>
1.3 Incidence of Diarrhea: No treatment control

| Risk Ratio (M-H, Random, 95% CI) | 3 | 895 |

2 Incidence of Diarrhea: Probiotic dose

2.1 ≥5 billion CFU of probiotic/day

| Risk Ratio (M-H, Random, 95% CI) | 7 | 1474 |

2.2 <5 billion CFU of probiotic/day

| Risk Ratio (M-H, Random, 95% CI) | 7 | 1382 |

3 Incidence of Diarrhea: Probiotic species

3.1 Lactobacillus rhamnosus (strains: GG and E/N, Oxy, Pen)

| Risk Ratio (M-H, Random, 95% CI) | 4 | 611 |

3.2 L. acidophilus & L. bulgaricus

| Risk Ratio (M-H, Random, 95% CI) | 1 | 38 |

3.3 L. acidophilus and Bifidobacterium infantis

| Risk Ratio (M-H, Random, 95% CI) | 1 | 18 |

3.4 L. sporogenes

| Risk Ratio (M-H, Random, 95% CI) | 1 | 98 |

3.5 Saccharomyces boulardii

| Risk Ratio (M-H, Random, 95% CI) | 3 | 1328 |

3.6 B. lactis & S. thermophilus

| Risk Ratio (M-H, Random, 95% CI) | 1 | 157 |

3.7 Bacillus clausii

| Risk Ratio (M-H, Random, 95% CI) | 1 | 323 |

3.8 Lactococcus lactis, L. plantarum, L. rhamnosus, L. casei, L. lactis subsp. diacetylactis, Leuconostoc cremoris, Bifidobacterium longum, B. breve, Lactobacillus acidophilus, and Saccharomyces florentinus

| Risk Ratio (M-H, Random, 95% CI) | 1 | 117 |

3.9 Bifidobacterium longum PL03, Lactobacillus rhamnosus KL53A, and Lactobacillus plantarum PL02

| Risk Ratio (M-H, Random, 95% CI) | 1 | 78 |

3.10 Streptococcus thermophilus, L. acidophilus, B. anamalis subsp. lactis, L. delbrueckii subsp. bulgaris

| Risk Ratio (M-H, Random, 95% CI) | 1 | 106 |
4 Incidence of Diarrhea: Risk of Bias

4.1 Low Risk

4.2 High Risk

5 Incidence of Diarrhea: Antibiotic class (≥5 billion CFU/day)

5.1 Beta-lactams/penicillins

5.2 Cephalosporins

5.3 Macrolides (azithromycin)

6 Incidence of Diarrhea: Definition of diarrhea

6.1 ≥3 loose/watery/liquid stools per day for at least 2 consecutive days

6.2 ≥3 watery/liquid stools per 24 hours

6.3 ≥2 liquid stools per day on at least 2 occasions during study

6.4 ≥2 liquid stools per 24 hr

6.5 ≥1 abnormally loose bowel movement per 24 hrs

7 Incidence of Diarrhea: Sensitivity analysis (complete case - fixed effects)

7.1 Active controlled
### Adverse Events

<table>
<thead>
<tr>
<th>Outcome title</th>
<th>No. of studies</th>
<th>No. of participants</th>
<th>Statistical method</th>
<th>Effect size</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1 Adverse Events: Complete case</strong></td>
<td>11</td>
<td>1578</td>
<td>Risk Difference (M-H, Random, 95% CI)</td>
<td>0.00 [-0.01, 0.02]</td>
</tr>
<tr>
<td><strong>2 Adverse Events: Same event rate assumptions analysis</strong></td>
<td>16</td>
<td>3432</td>
<td>Risk Difference (M-H, Random, 95% CI)</td>
<td>0.01 [-0.00, 0.02]</td>
</tr>
</tbody>
</table>

### Mean Stool Frequency

<table>
<thead>
<tr>
<th>Outcome title</th>
<th>No. of studies</th>
<th>No. of participants</th>
<th>Statistical method</th>
<th>Effect size</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1 High Dose vs Low Dose: Complete case</strong></td>
<td>4</td>
<td>425</td>
<td>Mean Difference (IV, Random, 95% CI)</td>
<td>-0.30 [-0.60, -0.13]</td>
</tr>
<tr>
<td><strong>1.1 ≥5 billion CFU/day</strong></td>
<td>2</td>
<td>307</td>
<td>Mean Difference (IV, Random, 95% CI)</td>
<td>-0.08 [-0.29, 0.14]</td>
</tr>
<tr>
<td><strong>1.2 &lt;5 billion CFU/day</strong></td>
<td>2</td>
<td>118</td>
<td>Mean Difference (IV, Random, 95% CI)</td>
<td>-0.50 [-0.89, -0.10]</td>
</tr>
</tbody>
</table>

---

**7.2 Placebo controlled**

- Risk Ratio (M-H, Fixed, 95% CI): 0.46 [0.36, 0.59]
- Risk Ratio (M-H, Fixed, 95% CI): 0.39 [0.24, 0.64]

**7.3 No treatment control**

- Risk Ratio (M-H, Random, 95% CI): 0.81 [0.63, 1.04]

**8 Incidence of Diarrhea: Sensitivity analysis (extreme-plausible analysis)**

- Risk Ratio (M-H, Random, 95% CI): 0.72 [0.54, 0.95]

**8.1 Active controlled**

- Risk Ratio (M-H, Random, 95% CI): 0.98 [0.69, 1.38]

**8.2 Placebo controlled**

- Risk Ratio (M-H, Random, 95% CI): 0.98 [0.69, 1.38]

**8.3 No treatment control**

- Risk Ratio (M-H, Random, 95% CI): 0.83 [0.64, 1.07]

**9 Incidence of Diarrhea: Probiotic dose (extreme-plausible analysis)**

- Risk Ratio (M-H, Random, 95% CI): 0.72 [0.53, 0.99]

**9.1 ≥5 billion CFU of probiotic/day**

- Risk Ratio (M-H, Random, 95% CI): 1.01 [0.68, 1.51]

**9.2 <5 billion CFU of probiotic/day**

- Risk Ratio (M-H, Random, 95% CI): 1.01 [0.68, 1.51]

---

http://cochrane.bvsalud.org/cochrane/show.php?db=reviews&mfn=&id=CD004827&lang=&&dblang=&lib=COC#
### Mean Duration of Diarrhea

<table>
<thead>
<tr>
<th>Outcome title</th>
<th>No. of studies</th>
<th>No. of participants</th>
<th>Statistical method</th>
<th>Effect size</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 High Dose vs Low Dose: Complete case</td>
<td>5</td>
<td>897</td>
<td>Mean Difference (IV, Random, 95% CI)</td>
<td>-0.60 [-1.18, -0.02]</td>
</tr>
<tr>
<td>1.1 ≥5 billion CFU/day</td>
<td>3</td>
<td>417</td>
<td>Mean Difference (IV, Random, 95% CI)</td>
<td>-0.70 [-1.44, 0.04]</td>
</tr>
<tr>
<td>1.2 &lt;5 billion CFU/day</td>
<td>2</td>
<td>480</td>
<td>Mean Difference (IV, Random, 95% CI)</td>
<td>-0.44 [-1.63, 0.75]</td>
</tr>
</tbody>
</table>

### COVER SHEET

**Probiotics for the prevention of pediatric antibiotic-associated diarrhea**

**Reviewer(s)**

Johnston Bradley C, Goldenberg Joshua Z, Vandvik Per O, Sun Xin, Guyatt Gordon H

**Contribution of Reviewer(s)**

- Issue protocol first published: 2004 issue 3
- Issue review first published: 2007 issue 2
- Date of last minor amendment: Information not supplied by reviewer
- Date of last substantive amendment: Information not supplied by reviewer
- Most recent changes:
  - Date new studies sought but none found: Information not supplied by reviewer
  - Date new studies found but not yet included/excluded: Information not supplied by reviewer
  - Date new studies found and included/excluded: Information not supplied by reviewer
  - Date reviewers’ conclusions section amended: Information not supplied by reviewer

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**Cochrane Library number**

CD004827

**Editorial group**

Cochrane Inflammatory Bowel Disease and Functional Bowel Disorders Group

**Editorial group code**

HM-IBD
SOURCES OF SUPPORT

External sources of support
- Hospital for Sick Kids Foundation, Toronto, Ontario, Canada.

Internal sources of support
- No sources of support supplied

KEYWORDS
Child; Child, Preschool; Female; Humans; Infant; Male; Adolescent; Anti-Bacterial Agents [*adverse effects]; Diarrhea [chemically induced] [*prevention & control]; Probiotics [*therapeutic use]; Randomized Controlled Trials as Topic

HISTORY

History
Protocol first published: Issue 3, 2004
Review first published: Issue 2, 2007

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