

## Preventing pediatric antibiotic-associated diarrhea and *Clostridium difficile* infections with probiotics: A meta-analysis

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### Abstract

**AIM:** To assess the efficacy and safety of probiotics for preventing pediatric: (1) antibiotic associated diarrhea and (2) *Clostridium difficile* (*C. difficile*) infections.

**METHODS:** On June 3, 2013, we searched PubMed (1960-2013), EMBASE (1974-2013), Cochrane Database of Systematic Reviews (1990-2013), CINAHL (1981-2013), AMED (1985-2013), and ISI Web of Science (2000-2013). Additionally, we conducted an extensive grey literature search including contact with National Institutes of Health Clinical Trials Registry, abstracts from annual infectious disease and gastroenterology meetings, experts in the field and correspondence with authors. The primary outcomes were the incidence of antibiotic-associated diarrhea (AAD) and *C. difficile* infections (CDI). Dichotomous outcomes (e.g., incidence of AAD or CDI) were pooled using a random-effects model to calculate the relative risk and corresponding 95% confidence interval (95%CI) and weighted on study quality. To explore possible explanations for heterogeneity, a *priori* subgroup analysis were

conducted on probiotic strain type, daily dose, quality of study and safety of probiotics. The overall quality of the evidence supporting each outcome was assessed using the grading of recommendations, assessment, development and evaluation criteria.

**RESULTS:** A total of 1329 studies were identified with 22 trials (23 treatment arms and 4155 participants) meeting eligibility requirements for our review of prevention of AAD and 5 trials (1211 participants) for the prevention of CDI. Trials in adult populations, trials of uncertain antibiotic exposure or studies which did not provide incidence of AAD were excluded. We found 12 trials testing a single strain of probiotic and 10 trials testing a mixture of probiotic strains. Probiotics (all strains combined) significantly reduced the incidence of pediatric AAD (pooled RR = 0.42, 95%CI: 0.33-0.53) and significantly reduced pediatric CDI (pooled RR = 0.35, 95%CI: 0.13-0.92). Of the two strains with multiple trials, both significantly reduced pediatric AAD: *Saccharomyces boulardii* lyo (pooled RR = 0.43, 95%CI: 0.32-0.60) and *Lactobacillus rhamnosus* GG (pooled RR = 0.36, 95%CI: 0.19-0.69). There was no significant effect by type of antibiotic, or by duration or dose of probiotic. No adverse events associated were found in the 22 controlled trials relating to the use of probiotics.

**CONCLUSION:** This meta-analysis found that probiotics significantly prevented pediatric antibiotic associated diarrhea and pediatric CDI, but the efficacy varies significantly by the strain of the probiotic.

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**Key words:** Probiotics; Pediatric; Antibiotic-associated diarrhea; *Clostridium difficile*; *Saccharomyces boulardii*; *Lactobacillus rhamnosus*; Safety; Meta-analysis; Randomized clinical trials

**Core tip:** A meta-analysis was conducted (1985-2013) for clinical trials testing probiotics for the prevention of pediatric antibiotic-associated diarrhea (AAD) or *Clostridium difficile* infections (CDI). Overall, probiotics significantly reduced the incidence of pediatric AAD (pooled from 22 trials RR = 0.42, 95%CI: 0.33-0.53) and significantly reduced pediatric CDI (pooled from five trials RR = 0.35, 95%CI: 0.13-0.92). Of the two strains with multiple trials, both significantly reduced pediatric AAD: *Saccharomyces boulardii* lyo (RR = 0.43, 95%CI: 0.32-0.60) and *Lactobacillus rhamnosus* GG (RR = 0.36, 95%CI: 0.19-0.69). There was no significant effect by type of antibiotic, or by duration or dose of probiotic.

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## INTRODUCTION

The use of antibiotics, while effective in treating a precipitating infection, may cause diarrheal disease as a common side effect, termed antibiotic associated diarrhea (AAD), caused by the unintended disruption of normal intestinal flora. Normal microbiota is a complex interaction of bacterial and fungal species that produce a phenomenon called "colonization resistance", which acts as a barrier to opportunistic pathogens. The most commonly known etiology of AAD is *Clostridium difficile* (*C. difficile*), which takes advantage of the disruption of colonization resistance and overgrows the intestines, producing toxins and resulting in an inflammatory intestinal disease called *C. difficile* infection (CDI)<sup>[1]</sup>. These unintended consequences of antibiotic use are well-studied phenomena in adults, but less attention has been focused on the pediatric population. Pediatric patients present unique challenges for the clinical management of disease due to differences in their immune development, susceptibility to dehydration and their response to treatments.

Pediatric AAD and pediatric CDI were recognized as important clinical concerns as the incidence of both continues to increase over time and serious consequences of infection are reported<sup>[2]</sup>. The incidence of pediatric AAD varies widely from 6%-11% in pediatric outpatients to 23%-33% in pediatric inpatients<sup>[1,3]</sup>. Data collected from national United States surveys of pediatric inpatients shows the incidence of pediatric CDI has increased 2.5 fold over three years, from 12.8/10000 in 2006<sup>[4]</sup> to 31.5/10000 in 2009<sup>[5]</sup>. A more recent study in 41 children's hospitals found pediatric CDI at 73/10000<sup>[6]</sup>.

Clinical symptoms include asymptomatic carriage of *C. difficile* (typically 65% of neonates carry *C. difficile* but do not develop symptoms), mild-moderate diarrhea is most common in infants and older children (typically

peaking at age 2-6 years old), to more severe disease (colitis or pseudomembranous colitis) less frequently and rarely toxic megacolon<sup>[7,8]</sup>. As with adults, nearly 20% of children with one episode may develop recurrent episodes of CDI<sup>[7]</sup>. Consequences of pediatric AAD or CDI may include a 2-3 fold increase in length of hospital stay<sup>[5,6]</sup>, a 6-fold increase in the risk of mortality<sup>[5,6]</sup>, and the need for colectomy (approximately 2%)<sup>[8,9]</sup>.

Current recommended treatments for pediatric AAD and CDI include discontinuation of the inciting antibiotic if possible (for mild diarrhea) or treatment with metronidazole or vancomycin, however treatment failure is common (18% with metronidazole) and vancomycin is used with caution in children due to toxicity<sup>[7,10]</sup>. Cases of moderate-severe pediatric diarrhea often require the administration of oral rehydration therapy or parental fluids to reduce dehydration associated with diarrhea. Alternative strategies are currently being sought to prevent pediatric AAD and CDI, rather than delaying until the children are ill.

Probiotics are living microorganisms, which when administered in adequate amounts, confer a health benefit to the host<sup>[11]</sup>. The use of probiotics may be especially suited for AAD and CDI, as they are linked by a common mechanism of action, namely interactions with the normal microflora<sup>[2]</sup>. When antibiotics disrupt colonization resistance, overgrowth of pathogens may occur and disease erupts. Probiotics act as surrogate normal flora to protect the intestine until the normal microbiota can recover (typically 1-2 mo, after antibiotics are discontinued)<sup>[11]</sup>. Some probiotics also have other mechanisms of action (production of bacteriocins, stimulation of the immune response, production of toxin-destroying proteases, attachment site interference, *etc.*) that are also beneficial to the pediatric patient<sup>[12]</sup>. While over 60 clinical trials testing probiotics for AAD and/or CDI have been reported, most (66%) have been done in the adult population, so the efficacy of probiotics for children is less well documented. Evidence from meta-analyses of AAD and CDI have indicated probiotics, in general, may be efficacious for the prevention of these diseases, but they have based their results on mixed adult-pediatric populations, or have been based on adults only<sup>[13-15]</sup>. Because the efficacy to prevent AAD and CDI has been determined to be specific by probiotic strain, it is imperative that data are analyzed by separate strains.

The purpose of this meta-analysis to evaluate the efficacy and safety of similar probiotic strains for the prevention of antibiotic associated diarrhea and *C. difficile* infections in the pediatric population.

## MATERIALS AND METHODS

### Study objectives

**Primary aims:** (1) to systematically assess whether probiotics co-administered with antibiotics (any agent) reduces the incidence of AAD in children; and (2) to systematically assess whether probiotics co-administered

with antibiotics (any type) reduces the incidence of CDI in children.

**Secondary aims:** (1) to assess the efficacy by specific strain of probiotic for the prevention of AAD and CDI in children; (2) to systematically assess if there is a dose effect for probiotics in the prevention of AAD and CDI in children; (3) to determine if study quality is associated with a change in the estimate of outcome effect; and (4) to assess the safety of the use of probiotics in children receiving antibiotics.

### Criteria for study selection

Abstracts of all citations and retrieved studies were reviewed and rated for inclusion. Full articles were retrieved if probiotics were given to prevent AAD or CDI in a pediatric population, or if the population age range was unclear from the abstract. Inclusion criteria included randomized (well described or partially) controlled trials (either placebo, standard active treatments, or no treatment given), blinded or open trials in pediatric populations (inpatient or outpatients) published in peer-reviewed journals or on clinical trial websites. Non-English language trials were translated and included whenever possible. Exclusion criteria included pre-clinical studies, safety or phase 2 studies, adult patients or healthy volunteer populations, diarrhea not associated with antibiotic use, case reports or case series, duplicate reports, trials of unspecified types of probiotics, incomplete or no diarrheal outcomes reported, no data on incidence rates of AAD or CDI, mixed pediatric and adult patient populations or if translation could not be obtained.

### Interventions

The type of probiotic intervention included probiotics in any form (*e.g.*, capsule, sachet, yogurt, wafer). Trials investigating non-specific probiotics or yogurts (*e.g.*, products that do not label the probiotic strain and dose) were excluded. Trials combining probiotics with prebiotics were included if the prebiotic dose was less than 2.5 g, as this was judged to be of limited impact to alter the intestinal microflora<sup>[16,17]</sup>. Trials not providing the dose of the prebiotic in the product were excluded. The type of control group may include: placebo, active treatment currently used as standard practice, or no treatment control.

### Outcomes and definitions

The primary outcome for AAD is defined as diarrhea (typically definition varied from > 2-3 loose or watery stools/day for > 2 consecutive days) occurring within 2 mo of antibiotic use<sup>[14]</sup>. The primary outcome for CDI is defined as a new episode of diarrhea associated with a positive culture or toxin (A or B) assay within 1 mo of antibiotic use<sup>[14,18]</sup>.

### Data sources

On June 3, 2013, we searched PubMed (1960-2013), EMBASE (1974-2013), Cochrane Database of Systematic

Reviews (1990-2013), CINAHL (1981-2013), AMED (1985-2013), and ISI Web of Science (2000-2013). Three on-line clinical trial registries were searched: Cochrane Central Register of Controlled trials (<http://www.cochrane.org>), MetaRegister of Controlled Trials (<http://www.controlled-trials.com/mrct>) and National Institutes of Health (<http://www.clinicaltrials.gov>). Additionally, we conducted an extensive grey literature search including abstracts from annual infectious disease and gastroenterology meetings, experts in the field and communication with published authors on pediatric AAD or CDI. Search terms included: Antibiotic-associated diarrhea, *C. difficile* disease and/or infection, pediatric, randomized controlled trial and probiotics and specific probiotic strains. Search strategies were broad-based initially, then narrowed to the disease and population of interest. The procedure of this meta-analysis follows MOOSE guidelines using clearly delineated parameters, a *priori* inclusion and exclusion criteria and standardized data extraction tools<sup>[19,20]</sup>.

### Data extraction

Two authors independently and in duplicate extracted data and assessed risk of bias using pre-constructed, and piloted, data extraction forms. Any disagreements were resolved by discussion. For articles published in abstract form only, further information was sought by contacting principal authors. Articles not published in the English language were translated. Using a standardized data extraction form, we systematically collected the following data: authors, year of publication and journal, pediatric population data (age range, setting, antibiotic given for disease, types of antibiotics given), study aims and outcomes, study methods (study design, eligibility criteria, sample size calculations, interim analysis, statistical methods used, recruitment methods, subgroup analysis done), randomization (method of randomization allocation, randomization method), degree of blinding (open, single or double), intervention data (probiotic strains used, daily dose, duration of treatment, duration of follow-up, type of control used, treatment concealment), results (balanced randomization achieved, attrition rate and reasons, comparison of treatment groups by demographics, *etc.*, CONSORT flow-chart provided), AAD outcome data (incidence of AAD by group, ITT or APP analysis used, method to assess AAD), CDI outcome data (incidence of CDI by group, ITT or APP analysis used, method to assess CDI), safety data (adverse events reported by group), discussion points (limitations, generalizability and comparison of study results to published papers), clinical trial registration, location of protocol, and source of funding.

### Assessment of methodological quality

Quality components for each trial were assessed for selection, detection, performance, reporting and loss to follow-up bias. Each of the included studies was evaluated using 33 items collected with the standardized data extraction form. Each item was graded as: present, ab-

sent, or not applicable (for example studies done in countries not requiring clinical trial registration, CONSORT flow-chart not present if trial was published before this became a standard, *etc.*)<sup>[19]</sup>. The overall quality score for the trial was calculated as the percent of items present divided by the total items present and absent (not applicable items were excluded from the calculation). Each of the 33 quality items were analyzed within one of six categories of potential of bias: study design bias (trial title, setting, early stoppage, background, study aims, prospective design, eligibility criteria, sample size calculation, interim analysis, statistical methods, recruitment methods, subgroup methods, probiotic well described by strain, daily dose and duration), selection bias (randomization allocation method, balanced groups resulted), detection bias (double blinded, treatments concealment), attrition bias (rates provided and reasons by each group), reporting bias (baseline group comparison, CONSORT flow-chart, intent to treat analysis done for each AAD and CDI outcome, incidence of each outcome provided, adverse event data provided and sub-group analysis provided, if applicable) and miscellaneous sources of bias (limitations, generalizability and comparison with other studies in discussion, trial registration, location of protocol for access and source of funding, if appropriate). Trials were classified as high quality if > 75% of the quality items were present, moderate quality if 50%-75% were present and low quality of < 50% were present.

We also employed the GRADE (grading of recommendations, assessment, development and evaluation) system for rating overall quality of evidence for each of the outcomes (prevention of AAD or prevention of CDI) by probiotic strain or type (single strain compared to mixtures of strains)<sup>[21,22]</sup>. Recommendation for use of each probiotic strain or mixture can be assessed by the overall strength of the evidence (“strong”, many randomized controlled trials show significant protection, more benefit than risk, cost-effective or “weak”, only case series or reports, limited number of small trials, *etc.*). Quality of the evidence is graded as “high quality” (further research is unlikely to change our confidence in the estimate of the effect), or “moderate quality” (further research is likely to have an important impact on our confidence and may change the estimate of the effect), or “low quality” (further research is very likely to change our confidence in the estimate and may change the direction of the estimate of the effect).

### Statistical analysis

Statistical analysis was performed using Stata software version 12 (Stata Corporation, College Station, Texas). The primary outcomes were the incidence of AAD and CDI. Univariate analysis of bivariate parameters were analyzed using  $\chi^2$  test or Fisher’s exact test for small cell sizes (< 5) with a significance level of  $P \leq 0.05$ . Meta-analysis was conducted for dichotomous outcomes (*e.g.*, incidence of AAD or CDI) using models to calculate the pooled relative risk and corresponding 95% confidence

interval (95%CI) and weighted by study quality score. Heterogeneity across trials was evaluated using Cochran Q test based on pooled relative risks by the Mantel-Haenszel method<sup>[23]</sup>. If the studies were homogenous, a fixed effects model was used, if studies were heterogeneous, a random effect model was employed. If significant heterogeneity was detected, a subgroup analysis was conducted to determine the source of heterogeneity. To explore possible explanations for heterogeneity, a *priori* subgroup analyses were conducted on study size, probiotic strain type, daily dose [ $\geq 1 \times 10^{10}$  colony-forming units (cfu) per day or  $< 1 \times 10^{10}$  cfu/d] and by quality of study.

### Publication bias

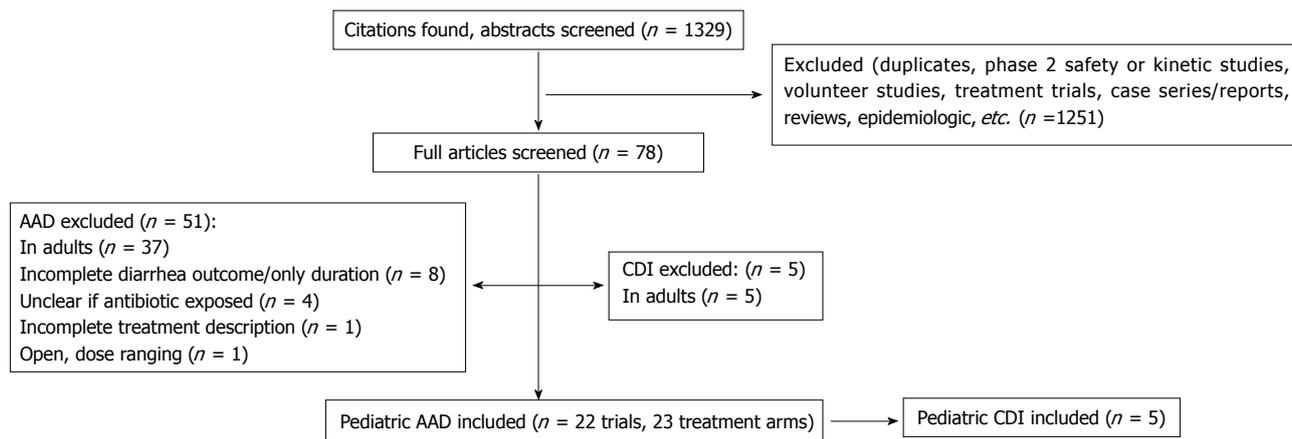
To assess for publication bias, a funnel plot, as well as a weighted regression (Egger’s test) and a rank correlation test (Begg’s test for small study effects) were conducted<sup>[20,24]</sup>. If publication bias was apparent, adjustment of the pooled estimates was considered using the trim and fill method<sup>[25]</sup>. Funnel plots show graphically that as sample sizes of trials increase, the precision is estimating the underlying treatment effect increases, which results in the effect estimates (relative risks) from small trials scattering more widely at the bottom of the graph and narrower scattering among larger studies. In the absence of publication bias, the funnel plot resembles a symmetrical inverted funnel. Reporting bias (smaller studies showing no protective effect) often are not published, and are indicated by an asymmetrical appearance with a gap in the bottom left of a funnel plot<sup>[26,27]</sup>.

## RESULTS

### Overview of included studies

The literature review yielded 1329 abstracts that were screened for inclusion. Of those 1251 were excluded according to our exclusion criteria (see Figure 1) and 78 full articles or meeting abstracts were pulled for full review. Of the 78, 51 were excluded relating to the prevention of AAD (37 were in adult patients, 8 did not provide sufficient diarrhea outcome data, 4 were not associated with antibiotic use or it was unclear if the patients had been exposed to antibiotics, one did not describe the product sufficiently and one was an open dose-ranging study) and 5 articles were also excluded relating to CDI, as they were in adult patients. As a result of the review, 22 pediatric trials were included in this meta-analysis<sup>[28-49]</sup>. The majority of the trials designated AAD as the primary outcome, while three (14%) trials designated AAD as a secondary outcome<sup>[28,41,44]</sup>.

Of the 22 included clinical trials in pediatric populations, two trials had two treatment arms<sup>[34,42]</sup> and one trial had two types of controls (a placebo yogurt and a ‘no treatment’ control)<sup>[31]</sup>. Erdevé *et al.*<sup>[34]</sup> compared *Saccharomyces boulardii* (*S. boulardii*) to controls using two different antibiotic arms, thus these were analyzed separately. Seki *et al.*<sup>[42]</sup> had two probiotic arms, with *C. butyricum* starting



**Figure 1** Flow chart of included and excluded trials for pediatric antibiotic-associated diarrhea and *Clostridium difficile* infections. AAD: Antibiotic-associated diarrhea; CDI: *Clostridium difficile* infections.

half-way through the antibiotic exposure and the other arm starting the probiotic from time of antibiotic initiation. As there was no significant difference in the efficacy by the timing of the probiotic, these two arms were combined into one probiotic group. One paper did not present pediatric AAD data separately from adults, and this information was obtained directly from the author<sup>[51]</sup>. Two controlled trials with uncertain randomization protocols were included to decrease potential publication bias, but were downgraded in their quality score as a result. Four articles were translated from the original language (French, Italian, Persian, or Chinese)<sup>[30,37,41,49]</sup>.

There were no separate randomized controlled trials using probiotics for the prevention of pediatric CDI as their primary outcome, but five trials for the prevention of pediatric AAD included CDI as a secondary outcome in their trial and were thus included<sup>[29,33,36,40,43]</sup>. We included 22 randomized clinical trials (RCT) evaluating the use of probiotics in a pediatric population for the prevention of AAD and 5 RCT for CDI.

### Excluded pediatric studies

Of the 78 articles screened, 42 were in adult populations and were excluded. Of the 14 excluded trials in pediatric populations (Table 1), eight had incomplete documentation of diarrhea outcomes: the outcome was given as days of diarrhea, not AAD incidence<sup>[50]</sup>, outcome was mixed “any GI effects” or “disorders of defecation”, which grouped diarrhea and/or nausea and/or discomfort<sup>[51,52]</sup>, or no data on diarrhea outcome was reported<sup>[53-57]</sup>. Four other trials evaluating probiotics in children aimed at the prevention of nosocomial diarrhea were excluded as they either did not document if antibiotic exposure occurred or specifically excluded antibiotic-exposed children<sup>[58-61]</sup>. One study was excluded as it was an early dose-ranging study, which was not randomized nor used a placebo<sup>[62]</sup>. One study was excluded as their investigational probiotic also included unknown doses of inulin and lactoferrin<sup>[63]</sup>. The inter-rater agreement on inclusion and exclusion of trials was 100%.

### Patient population

The characteristics of the enrolled study populations by trial arm are presented in Table 2. The age of enrolled pediatric patients ranged from 1 mo to 18 years old and usually included both genders. Race or ethnicity was not reported in most clinical trials. The trials were carried out in a wide array of countries: Poland ( $n = 4$ ), United States ( $n = 3$ ), Finland ( $n = 2$ ), Iran ( $n = 2$ ), China ( $n = 2$ ) and one each in Brazil, Bulgaria, France, Italy, Japan, Philippines, Thailand, Turkey and the United Kingdom. The clinical setting was usually outpatient only ( $n = 11$ , 50%) or inpatient only ( $n = 6$ , 27%) or a combination of inpatient and outpatients ( $n = 4$ , 18%) and the type of practice was not reported in one trial.

The type of infection for which the antibiotic(s) were prescribed included mixed types of infections (respiratory and/or urinary tract and/or otitis media) in 10 trials (45%), or were restricted to one type of infection [respiratory, in 6 trials (27%) or *Helicobacter pylori* (*H. pylori*) in three trials (14%) or otitis media in one trial (4%)] and the type of infection was not reported in two trials, as shown in table 2<sup>[34,38]</sup>.

### Antibiotic exposure

**Type of antibiotics:** Three trials (four treatment arms) limited inclusion due to a single type of antibiotic: amoxicillin<sup>[46,47]</sup>, or sulbactam-ampicillin<sup>[34]</sup>, or azithromycin<sup>[34]</sup>. Three trials limited antibiotic exposure to the two contained in the standard triple therapy components for *H. pylori* infections (amoxicillin and clarithromycin or furazolidone)<sup>[28,41,44]</sup>. The majority of trials ( $n = 15$ , 68%) included a mixture of eligible antibiotic types<sup>[29-33,35-37,39,40,42,43,45,48,49]</sup> and one trial did not report the type of antibiotic<sup>[38]</sup>. Most common types of antibiotics were in these mixed-type antibiotic trials included: amoxicillin (19%-66%), ampicillin (76%), penicillin (47%-71%), cephalosporins (11%-89%).

**Duration of antibiotic use:** While most trials did not provide the time of antibiotic exposure prior to study, the

**Table 1 Excluded studies from pediatric antibiotic-associated diarrhea and *Clostridium difficile* infections by year of publication and reason**

Probiotic strain	Outcome	Reason for exclusion	Ref.
<i>C. butyricum</i>	AAD	Open, dose ranging study	Kurata <i>et al</i> <sup>[62]</sup> , 1988
<i>L. acidophilus</i> + <i>Bifido bifidum</i>	Prevent AAD	Outcome measured by days of diarrhea. No data on AAD incidence	Contardi <i>et al</i> <sup>[50]</sup> , 1991
<i>Bifido bifidum</i> _ <i>Strept thermo</i>	Prevention of nosocomial diarrhea	Unclear how many were exposed to antibiotics	Saavedra <i>et al</i> <sup>[58]</sup> , 1994
<i>L. acidophilus</i> + <i>L. bulgaricus</i>	Prevent GI effects	No data on AAD, just "any GI effects"	Witsell <i>et al</i> <sup>[51]</sup> , 1995
<i>L. rhamnosus</i> GG	Prevention of nosocomial diarrhea	Unclear how many were exposed to antibiotics	Szajewska <i>et al</i> <sup>[59]</sup> , 2001
<i>L. acidophilus</i> + <i>Bifido infantis</i> + FOS	Increase in body weight for children on antibiotics	No data on diarrhea provided	Schrezenmeir <i>et al</i> <sup>[56]</sup> , 2004
<i>L. casei</i>	Eradicate <i>H. pylori</i> in children given triple therapy	No data on diarrhea provided	Sýkora <i>et al</i> <sup>[57]</sup> , 2005
<i>L. reuteri</i>	Reduce side effects of triple therapy for pediatric <i>H. pylori</i> infections	No data on diarrhea provided, only "disorders of defecation", mixed diarrhea and upper GI	Lionetti <i>et al</i> <sup>[52]</sup> , 2006
<i>L. acidophilus</i> + <i>L. rhamnosus</i>	Eradicate <i>H. pylori</i> in children given triple therapy	No data on diarrhea provided	Plewinska <i>et al</i> <sup>[55]</sup> , 2006
<i>Bifido animalis</i> + <i>L. casei</i>	Eradication of <i>H. pylori</i> in children	Side effects (diarrhea) not documented	Goldman <i>et al</i> <sup>[53]</sup> , 2006
<i>S. boulardii</i>	Eradication of <i>H. pylori</i> in children	No data on diarrhea provided	Hurdac <i>et al</i> <sup>[54]</sup> , 2009
<i>L. rhamnosus</i> GG	Prevention of nosocomial pediatric respiratory or GI infections	None were exposed to antibiotics	Hojsak <i>et al</i> <sup>[60]</sup> , 2010
<i>L. reuteri</i> DSM 17938	Prevent nosocomial diarrhea in children	Unclear how many were exposed to antibiotics	Wanke <i>et al</i> <sup>[61]</sup> , 2012
Mix of 9 bacterial strains and inulin and lactoferrin	Improve <i>H. pylori</i> eradication and reduce AAD in children	Poorly described product (unknown concentrations of inulin and lactoferrin)	Tolone <i>et al</i> <sup>[63]</sup> , 2012

*S. boulardii*: *Saccharomyces boulardii*; *L. rhamnosus*: *Lactobacillus rhamnosus*; *H. pylori*: *Helicobacter pylori*; AAD: Antibiotic-associated diarrhea; CDI: *Clostridium difficile* infection; GI: Gastrointestinal.

trial intervention typically started as soon as possible after the antibiotic was initiated. Overall, the mean duration of antibiotic use during the trial averaged between 7 and 10 d, but the range was broad (3-30 d).

**Antibiotic route:** Most trials ( $n = 12$ , 54%) included children using oral antibiotics<sup>[28-31,34,37,41,42,44,46-48]</sup>. Mixed intravenous and oral antibiotics were given in six trials<sup>[32,36,39,40,45,49]</sup>. One trial was limited to solely intravenous antibiotics<sup>[43]</sup>. In three trials, it was unclear what antibiotic route was used<sup>[33,35,38]</sup>.

### Definition of AAD and CDI

Most trials defined AAD as diarrhea associated with the use of antibiotics (any type, route, or duration). The standard definition of AAD in adults, ( $\geq 3$  loose or watery stools per day for  $\geq 2$  consecutive days) was used in 10 (45%) of the trials (as shown in Table 2). Other trials just required either  $\geq 3$ <sup>[30,42]</sup> or  $\geq 2$ <sup>[34,37,48,49]</sup> or  $\geq 1$ <sup>[46]</sup> loose or watery stools per day, but did not require a specific number of days to be considered as defined diarrhea. Less stringent definitions were used by two trials: "parent report"<sup>[47]</sup> or "otherwise unexplainable diarrhea"<sup>[35]</sup> and three trials did not report a definition for AAD<sup>[35,38,39]</sup>.

Of 22 trials, only 8 (36%) tested diarrheal stools for viral (adenovirus, rotavirus, calicivirus or astrovirus) and bacterial (*Salmonella*, *Shigella*, *Yersinia*, *Campylobacter*, *Staphylococcus aureus*, *C. difficile* and yeasts) enteric pathogens<sup>[28,29,32,36,39,40,43,44]</sup>. *C. difficile* was diagnosed using standard enzyme immunoassays (EIA) for toxins A/B in four trials for children who developed diarrhea<sup>[29,36,40,43]</sup>, and in two trials, the type of *C. difficile* assay was not report-

ed<sup>[33,42]</sup>.

Most of the trials (16, 73%) used daily diaries given to the parents or the child to document gastrointestinal symptoms and adverse events, but one trial in inpatient children had hospital staff chart symptoms<sup>[35]</sup> and one had staff call parents<sup>[48]</sup>, while four trials did not describe the method used to collect gastrointestinal symptoms<sup>[33,34,39,42]</sup>.

### Intervention

Details of the intervention for the 22 trials (23 trial arms) are given in Tables 3 and 4.

**Randomization:** Of the 22 trials, 20 were randomized, but two did not clearly report if they were randomized<sup>[39,42]</sup>. Seki *et al*<sup>[42]</sup> only stated "the subjects were divided into three groups" and does not provide a method for randomization, but does provide data showing that the three treatment groups were not significantly different by gender, age, type of antibiotic distribution or treatment group assignment. The other trial was from a published meeting abstract and a full paper was never found in the literature nor were we successful in contacting the authors, which might have provided more details on the methods used<sup>[39]</sup>.

**Degree of blinding:** Of the 22 trials, 15 (68%) were double-blinded, one was single-blinded and 6 (27%) were open trials (due to the nature of the control group used), as shown in Table 3. One trial used two types of controls, an identical looking and smelling yogurt (double-blinded comparison) and a "no treatment" control arm (open)<sup>[31]</sup>.

**Table 2** Characteristics of enrolled pediatric population and probiotic therapies for 22 clinical trials of pediatric antibiotic-associated diarrhea and *Clostridium difficile* infections

Probiotic strain	Age range	Country	Setting (inpatient or outpatient)	Type of inciting infection	Type of antibiotic (s)	Diarrhea defined <sup>1</sup>	Ref.
<i>S. boulardii</i> Iyo	1-5 yr	France	Out	Resp	Mixed: amox (19%), ceph (11%)	> 3	Benhamou <i>et al</i> <sup>[30]</sup> , 1999
<i>S. boulardii</i> Iyo	1-15 yr	Turkey	Out	Nr	Sulbactam-ampicillin only	> 2	Erdeve <i>et al</i> <sup>[34]</sup> , 2004
<i>S. boulardii</i> Iyo	1-15 yr	Turkey	Out	Nr	Azithromycin only	> 2	Erdeve <i>et al</i> <sup>[34]</sup> , 2004
<i>S. boulardii</i> Iyo	6 mo-14 yr	Poland	In and Out	OM, Resp	Mixed: ceph (41%), amox (29%)	> 3/2 d	Kotowska <i>et al</i> <sup>[36]</sup> , 2005
<i>S. boulardii</i> Iyo	6 mo-14 yr	China	In	Resp	IV only: ceph (52%), amox (26%)	> 3/2 d	Shan <i>et al</i> <sup>[43]</sup> , 2014
<i>L. rhamnosus</i> GG	5 mo-11 yr	Finland	Out	OM	Amox only	"by parents"	Vaisanen <i>et al</i> <sup>[47]</sup> , 1998
<i>L. rhamnosus</i> GG	2 wk-12.8 yr	Finland	Out	Resp	Mixed: amox (66%)	> 3/2 d	Arvola <i>et al</i> <sup>[29]</sup> , 1999
<i>L. rhamnosus</i> GG	6 mo-10 yr	United States	Out	Resp, UTI, skin	Mixed: amox (52%)	> 2	Vanderhoof <i>et al</i> <sup>[48]</sup> , 1999
<i>L. rhamnosus</i> GG	5-17 yr	Poland	In	<i>H. pylori</i> +	Amox and clarithromycin only	> 3/2 d	Szajewska <i>et al</i> <sup>[44]</sup> , 2009
<i>L. sporogenes</i> (aka <i>Bacillus sporogenes</i> ) + FOS	4 mo-15 yr	Italy	Out	Resp	Mixed: ceph (41%), amox (30%)	> 2	La Rosa <i>et al</i> <sup>[37]</sup> , 2003
<i>C. butyricum</i> MIYAIRI	1 mo-15 yr	Japan	Nr	Resp, GI	Mixed: ceph (48%)	> 3	Seki <i>et al</i> <sup>[42]</sup> , 2003
<i>L. acidophilus</i>	1 mo-18 yr	Bulgaria	In	Resp, Pyel	Mixed: b-lactams, clinda, amino	Nr	Pancheva-dimitrova <i>et al</i> <sup>[39]</sup> , 2004
<i>B. clausii</i>	6 mo-12 yr	Philippines	In and Out	Resp, GU, Skin	Mixed beta-lactams: pen (47%), ceph (35%)	"otherwise unexplained"	Destura <i>et al</i> <sup>[33]</sup> , 2008
Mixes							
<i>L. acidophilus</i> + <i>L. bulgaricus</i>	5 mo-6 yr	United States	Out	OM, pharyn, etc.	Amox only	> 1	Tankanow <i>et al</i> <sup>[46]</sup> , 1990
<i>L. acidophilus</i> + <i>Bifido infantis</i>	1-36 mo	Thailand	In	Sepsis or meningitis	Mixed: cefotaxime (89%)	Nr	Jirapinyo <i>et al</i> <sup>[35]</sup> , 2002
<i>Bifido lactis</i> + <i>Strept thermophilus</i>	6-36 mo	Brazil	In	Nr	Mixed: amp (76%), amox (58%)	> 3/2 d	Corrêa <i>et al</i> <sup>[32]</sup> , 2005
<i>Bifido longum</i> PL03 + <i>L. rhamnosus</i> KL53A + <i>L. plantarum</i> PL02	5 mo-16 yr	Poland	In and Out	Resp, OM, UTI	Mixed: amox (43%), ceph (26%)	> 3/2 d	Szymański <i>et al</i> <sup>[45]</sup> , 2008
<i>L. rhamnosus</i> (3 strains) E/N, Pen and Oxy	3 mo-14 yr	Poland	In and Out	Resp, OM, UTI, skin	Mixed: amp or pen (50%), ceph (37%)	> 3/2 d	Ruszczynski <i>et al</i> <sup>[40]</sup> , 2008
Kefir (mix of 9 strains) <sup>1</sup>	1-5 yr	United States	Out	Resp	Nr	Nr	Merenstein <i>et al</i> <sup>[38]</sup> , 2009
<i>C. butyricum</i> + <i>Bifido. infantis</i>	3 mo-3 yr	China	In	Pneumonia	Mixed: ceph (46%)	> 2	Investigating Group for Prevention of AAD in Children with Pneumonia by <i>Clostridium Butyricum</i> and <i>Bifidobacterium</i> <sup>[49]</sup> , 2012
<i>Bifido animalis</i> + <i>L. acidophilus</i> + <i>Strept thermophilus</i>	1-17 yr	United Kingdom	Out	Resp, skin, UTI	Mixed: pen (71%)	> 3/2 d	Conway <i>et al</i> <sup>[31]</sup> , 2007
<i>L. casei</i> + <i>L. acidophilus</i> + <i>L. reuteri</i> + <i>L. bulgaricus</i> + <i>Strept. cremoris</i> + <i>Bifido. bifidum</i> + <i>Bifido. infantis</i> + FOS	4-14 yr	Iran	Out	<i>H. pylori</i> +	Amox and clarithromycin only	> 3/2 d	Saneeyan <i>et al</i> <sup>[41]</sup> , 2011
<i>L. casei</i> + <i>L. rhamnosus</i> + <i>L. bulgaricus</i> + <i>L. acidophilus</i> + <i>Strept. thermophilus</i> + <i>Bifido. breve</i> + <i>Bifido. infantis</i>	3-14 yr	Iran	Out	<i>H. pylori</i> +	Amox and furazolidone only	> 3/2 d	Ahmad <i>et al</i> <sup>[28]</sup> , 2013

Diarrhea defined as > 3/2 d: indicates 3 or more loose/watery stools for at least 2 consecutive days; > 3: 3 or more loose or watery stools/day; > 2: 2 or more watery stools/day; "by parents": Defined by parents; "otherwise unexplained": Diarrhea with no other explanation associated with antibiotic use. Amino: Aminoglycosides; Amp: Ampicillin; Amox: Amoxicillin +/- clavulanic acid; Ceph: Cephalosporins; Clinda: Clindamycin; GU: Genito-urinary; FOS: Fructooligosaccharide (990 mg/d); *H. pylori*: *Helicobacter pylori*; In: Inpatient; Nr: Not reported; OM: Otitis media; Out: Outpatient; Pharyn: Pharyngitis; Pyel: Pyelonephritis; Resp: Upper or lower respiratory tract infection; UTI: Urinary tract infection. <sup>1</sup>Kefir contains: *Lactococcus plantarum*, *L. rhamnosus*, *L. acidophilus*, *L. casei*, *L. lactis* subspecies *diacetylactis*, *Leuconostoc cremoris*, *Bifido. longum*, *Bifido. breve*, *Saccharomyces florentinus*.

Of the 15 double-blinded trials, most (11, 73%) described how treatments were concealed (*e.g.*, identical appearance

and taste), but four trials did not provide any further details, other than the trial was double blinded<sup>[28,35,46,47]</sup>.

**Table 3** Description of the interventions for 22 clinical trials of pediatric antibiotic-associated diarrhea and *Clostridium difficile*

Probiotic strain	Randomized	Blinding	Type of controls	Formulation	Daily dose (cfu/d)	Duration treatment	Follow-up post-treatment	Ref.
<i>S. boulardii</i> lyo	Yes	Double	Active (diosmectite)	Capsules	$4.5 \times 10^9$	6-10 d	None	Benhamou <i>et al</i> <sup>[30]</sup> , 1999
<i>S. boulardii</i> lyo	Yes	Open	No treatment	Sachet	$5 \times 10^9$	duration	2 wk	Erdeve <i>et al</i> <sup>[34]</sup> , 2004
<i>S. boulardii</i> lyo	Yes	Open	No treatment	Sachet	$5 \times 10^9$	duration	2 wk	Erdeve <i>et al</i> <sup>[34]</sup> , 2004
<i>S. boulardii</i> lyo	Yes	Double	Placebo	Wafers	$1 \times 10^{10}$	5-7 d	2 wk	Kotowska <i>et al</i> <sup>[36]</sup> , 2005
<i>S. boulardii</i> lyo	Yes	Open	No treatment	Powder	$1 \times 10^{10}$	2 wk	2 wk	Shan <i>et al</i> <sup>[43]</sup> , 2014
<i>L. rhamnosus</i> GG	Yes	Double	Milk control	Whey drink	$8 \times 10^{10}$	7 d	None	Vaisanen <i>et al</i> <sup>[47]</sup> , 1998
<i>L. rhamnosus</i> GG	Yes	Double	Placebo	Capsules	$4 \times 10^{10}$	7-10 d	3 mo	Arvola <i>et al</i> <sup>[29]</sup> , 1999
<i>L. rhamnosus</i> GG	Yes	Double	Placebo	Capsules	$1-2 \times 10^{10}$	10 d	None	Vanderhoof <i>et al</i> <sup>[48]</sup> , 1999
<i>L. rhamnosus</i> GG	Yes	Double	Placebo	Capsules	$2 \times 10^9$	7 d	6 wk	Szajewska <i>et al</i> <sup>[44]</sup> , 2009
<i>L. sporogenes</i> [aka <i>Bacillus sporogenes</i> ] + FOS	Yes	Double	Placebo	Capsules	$5.5 \times 10^8$	10 d	None	La Rosa <i>et al</i> <sup>[37]</sup> , 2003
<i>C. butyricum</i> MIYAIRI	Nr	Open	No treatment	Capsules	$1-4 \times 10^7$	6 d	None	Seki <i>et al</i> <sup>[42]</sup> , 2003
<i>L. acidophilus</i>	Nr	Open	No treatment	Nr	$2 \times 10^9$	duration	None	Pancheva-Dimitrova <i>et al</i> <sup>[39]</sup> , 2004
<i>B. clausii</i>	Yes	Open	No treatment	Powder	$4 \times 10^9$	7-21 d	6 wk	Destura <i>et al</i> <sup>[33]</sup> , 2008
Mixes								
<i>L. acidophilus</i> + <i>L. bulgaricus</i>	Yes	Double	Placebo	Sachets	$2 \times 10^9$	10 d	None	Tankow <i>et al</i> <sup>[46]</sup> , 1990
<i>L. acidophilus</i> + <i>Bifido infantis</i>	Yes	Double	Placebo	Capsules	$6 \times 10^9$	7 d	None	Jirapinyo <i>et al</i> <sup>[35]</sup> , 2002
<i>Bifido lactis</i> + <i>Strept thermophilus</i>	Yes	Double	Placebo	Formula	$4 \times 10^8$	15 d	15 d	Corrêa <i>et al</i> <sup>[32]</sup> , 2005
<i>Bifido longum</i> PL03 + <i>L. rhamnosus</i> KL53A + <i>L. plantarum</i> PL02	Yes	Double	Placebo	Capsules	$2 \times 10^8$	3-14 d	2 wk	Szymański <i>et al</i> <sup>[45]</sup> , 2008
<i>L. rhamnosus</i> (3 strains) E/N, Pen and Oxy	Yes	Double	Placebo	Capsules	$4 \times 10^{10}$	3-30 d	2 wk	Ruszczynski <i>et al</i> <sup>[40]</sup> , 2008
Kefir (mix of 9 strains)	Yes	Double	Heat-killed drink	Drink	$7-10 \times 10^9$	10 d	4 d	Merenstein <i>et al</i> <sup>[38]</sup> , 2009
<i>C. butyricum</i> + <i>Bifido. infantis</i>	Yes	Open	No treatment	Sachet	$5 \times 10^9$	7 d	None	Investigating Group for Prevention of AAD in Children with Pneumonia by <i>Clostridium Butyricum</i> and <i>Bifidobacterium</i> <sup>[49]</sup> , 2012
<i>Bifido animalis</i> + <i>L. acidophilus</i> + <i>Strept thermophilus</i>	Yes	Double, open	Control yogurt and no treatment groups	Yogurt	$1 \times 10^9$	12 d	None	Conway <i>et al</i> <sup>[31]</sup> , 2007
<i>L. casei</i> + <i>L. acidophilus</i> + <i>L. reuteri</i> + <i>L. bulgaricus</i> + <i>Strept. cremoris</i> + <i>Bifido. bifidum</i> + <i>Bifido. infantis</i> + FOS	Yes	Single	Placebo	Sachet	$1 \times 10^9$	14 d	2 wk	Saneeyan <i>et al</i> <sup>[41]</sup> , 2011
<i>L. casei</i> + <i>L. rhamnosus</i> + <i>L. bulgaricus</i> + <i>L. acidophilus</i> + <i>Strept. thermophilus</i> + <i>Bifido. breve</i> + <i>Bifido. infantis</i>	Yes	Double	Placebo	Sachet	$1 \times 10^9$	28 d	4-8 wk	Ahmad <i>et al</i> <sup>[28]</sup> , 2013

cfu/d: Colony-forming units/day; Diosmectite: An anti-spasmodic; Duration: Treatment given for the duration of the antibiotic; Nr: Not reported; *S. boulardii*: *Saccharomyces boulardii*; *L. rhamnosus*: *Lactobacillus rhamnosus*.

**Type of controls:** Of the 22 trials, two studies had two separate control groups. One trial had two control groups (a placebo yogurt and a “no treatment/no yogurt” group)<sup>[31]</sup>. Another study paired *S. boulardii* and placebo groups for each of two different types of antibiotics<sup>[34]</sup>. Of the 24 control arms (Table 3), 15 (62%) used a placebo comparison, 8 (33%) used a ‘no treatment’ control consisting of just the antibiotic used in both groups; one trial compared the probiotic to a standard anti-spasmodic (diosmectite) treatment<sup>[30]</sup>.

**Formulation used:** Most of the 23 treatment arms used a capsule (9 arms, 39%), while six (26%) used sachets, two trials (9%) used fermented drinks<sup>[38,47]</sup>, and two trials used powder<sup>[33,43]</sup>, as shown in table 3. Less frequent formulations used in single trials included: wafers<sup>[36]</sup>,

yogurt<sup>[31]</sup>, infant formula<sup>[32]</sup>, while one trial did not report the type of formulation used<sup>[39]</sup>.

### Probiotic used

**Type of probiotic strain(s):** In the 23 treatment arms, 13 (57%) tested a single strain of probiotic and 10 (43%) had 2-9 strains in their test probiotic treatment. Only two probiotic strains, *S. boulardii* lyo and *Lactobacillus rhamnosus* (*L. rhamnosus*) GG were tested in multiple controlled trials, as shown in Table 3.

**Probiotic dose:** The daily dose of probiotics varied widely from  $10^7$  cfu/d to  $10^{10}$  cfu/d, as shown in table 3. The most common daily doses were  $1-6 \times 10^9$ /d (54% of trials), while only one trial used  $10^7$ /d<sup>[42]</sup>, three trials used  $10^8$ /d<sup>[32,37,45]</sup>, and seven trials (32%) used a higher

**Table 4 Outcomes for 22 clinical trials of pediatric antibiotic-associated diarrhea and *Clostridium difficile* infections trials (total 23 treatment arms)**

Probiotic strain	Attrition	ITT or APP	Incidence A-AD probiotic	Incidence AAD controls	P value (% power)	Incidence CDI probiotic	Incidence CDI controls	P value (% power)	Ref.
<i>S. boulardii</i> Iyo	21%	APP	25/327 (7.6%)	16/289 (5.5%)	0.29 14%	--	--	--	Benhamou <i>et al</i> <sup>[30]</sup> , 1999
<i>S. boulardii</i> Iyo + SAM	29%	APP	7/117 (5.7%)	30/117 (25.6%)	< 0.001 98%	--	--	--	Erdeve <i>et al</i> <sup>[34]</sup> , 2004
<i>S. boulardii</i> Iyo + AZT	29%	APP	7/127 (5.5%)	12/105 (11.4%)	0.15 29%	--	--	--	Erdeve <sup>[34]</sup> , 2004
<i>S. boulardii</i> Iyo	8.60%	APP	9/119 (8.0%)	29/127 (23.0%)	0.001 87%	3/119 (2.5)	10/127 (7.9%)	0.09 36%	Kotowska <i>et al</i> <sup>[36]</sup> , 2005
<i>S. boulardii</i> Iyo	15%	APP	6/139 (4.3%)	28/144 (19.4%)	< 0.001 96%	1/139 (0.7%)	8/144 (5.6%)	0.04 42%	Shan <i>et al</i> <sup>[43]</sup> , 2014
<i>L. rhamnosus</i> GG	0%	ITT	6/23 (26%)	8/36 (22%)	0.76 3%	--	--	--	Vaisanen <i>et al</i> <sup>[47]</sup> , 1998
<i>L. rhamnosus</i> GG	28.70%	APP	3/61 (5%)	9/58 (16%)	0.07 38%	1/61 (1.6%)	1/58 (1.7%)	1 10%	Arvola <i>et al</i> <sup>[29]</sup> , 1999
<i>L. rhamnosus</i> GG	6.90%	APP	7/93 (7.5%)	25/95 (26%)	0.001 90%	--	--	--	Vanderhoof <i>et al</i> <sup>[48]</sup> , 1999
<i>L. rhamnosus</i> GG	20%	ITT	2/34 (6%)	6/30 (20%)	0.13 26%	--	--	--	Szajewska <sup>[44]</sup> , 2009
<i>L. sporogenes</i> [Bac. sporogenes] + FOS	18%	ITT	14/48 (29%)	31/50 (62%)	0.001 88%	--	--	--	La Rosa <i>et al</i> <sup>[37]</sup> , 2003
<i>C. butyricum</i> MIYAIRI	Nr	Nr	6/86 (7%)	16/27 (59%)	< 0.001 99%	0/86	0/27	--	Seki <i>et al</i> <sup>[42]</sup> , 2003
<i>L. acidophilus</i>	Nr	Nr	10/215 (4.6%)	30/139 (21.6%)	< 0.001 99%	--	--	--	Pancheva-Dimitrova <i>et al</i> <sup>[39]</sup> , 2004
<i>B. clausii</i>	0%	ITT	3/162 (1.8%)	7/161 (4.3%)	0.22 16%	0/162 (0%)	1/161 (0.6%)	0.5 3%	Destura <i>et al</i> <sup>[33]</sup> , 2008
Mixes									
<i>L. acidophilus</i> + <i>L. bulgaricus</i>	37%	APP	10/15 (66%)	16/23 (69.5%)	1 3%	--	--	--	Tankanow <i>et al</i> <sup>[46]</sup> , 1990
<i>L. acidophilus</i> + <i>Bifido infantis</i>	0%	ITT	3/8 (37.5%)	8/10 (80%)	0.14 25%	--	--	--	Jirapinyo <i>et al</i> <sup>[35]</sup> , 2002
<i>Bifido lactis</i> + <i>Strept thermophilus</i>	7.10%	APP	13/80 (16%)	24/77 (31.2%)	0.04 54%	--	--	--	Corrêa <i>et al</i> <sup>[32]</sup> , 2005
<i>Bifido longum</i> PL03 + <i>L. rhamnosus</i> KL53A + <i>L. plantarum</i> PL02	0%	ITT	1/40 (2.5%)	2/38 (5.3%)	0.61 3%	--	--	--	Szymański <i>et al</i> <sup>[45]</sup> , 2008
<i>L. rhamnosus</i> (3 strains) E/N, Pen and Oxy	1.20%	ITT	9/120 (7.5%)	20/120 (17%)	0.046 53%	3/120 (2.5%)	7/120 (5.8%)	0.33 16%	Ruszczynski <i>et al</i> <sup>[40]</sup> , 2008
Kefir (mix of 9 strains)	6.40%	ITT	11/61 (18%)	14/64 (21.9%)	0.66 5%	--	--	--	Merenstein <i>et al</i> <sup>[38]</sup> , 2009
<i>C. butyricum</i> + <i>Bifido. infantis</i>	2.10%	APP	15/193 (7.8%)	30/179 (16.8%)	0.01 70%	--	--	--	Investigating Group for Prevention of AAD in Children with Pneumonia by <i>Clostridium Butyricum</i> and <i>Bifidobacterium</i> <sup>[49]</sup> , 2012
<i>Bifido animalis</i> + <i>L. acidophilus</i> + <i>Strept thermophilus</i>	12%	ITT	2/48 (4%)	3/34 (9%)	0.64 8%	--	--	--	Conway <i>et al</i> <sup>[31]</sup> , 2007
<i>L. casei</i> + <i>L. acidophilus</i> + <i>L. reuteri</i> + <i>L. bulgaricus</i> + <i>Strept. cremoris</i> + <i>Bifido. bifidum</i> + <i>Bifido. infantis</i> + FOS	0%	ITT	3/25 (12%)	13/25 (52%)	0.005 80%	--	--	--	Saneeyan <i>et al</i> <sup>[41]</sup> , 2011
<i>L. casei</i> + <i>L. rhamnosus</i> + <i>L. bulgaricus</i> + <i>L. acidophilus</i> + <i>Strept. thermophilus</i> + <i>Bifido. breve</i> + <i>Bifido. infantis</i>	0%	ITT	2/33 (6.1%)	8/33 (24.2%)	0.04 40%	--	--	--	Ahmad <i>et al</i> <sup>[28]</sup> , 2013

AAD: Antibiotic-associated diarrhea; CDI: *Clostridium difficile* disease; *S. boulardii*: *Saccharomyces boulardii*; *L. rhamnosus*: *Lactobacillus rhamnosus*; Nr: Not reported.

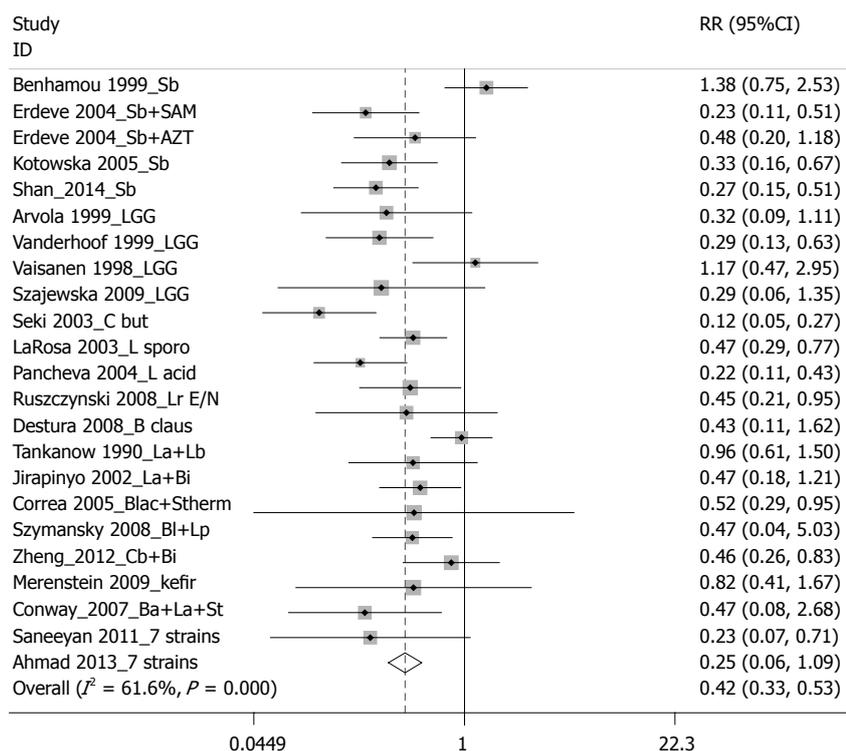


Figure 2 Forest plot of 23 probiotic treatment arms for the prevention of pediatric antibiotic associated diarrhea.

daily dose of probiotic ( $\geq 10^{10}$ /d). As there is no standard recommended dose of probiotics for the pediatric population, doses varied, even for the same strain of tested probiotic. The daily doses in the four trials testing *S. boulardii* lyo ranged from  $4.5 \times 10^9$  to  $1 \times 10^{10}$  cfu/d. The daily doses in the four trials testing *L. rhamnosus* GG ranged from  $2 \times 10^9$  to  $8 \times 10^{10}$  cfu/d.

**Duration of probiotic treatment:** Typically, the probiotic/control treatments are started soon after the inciting antibiotic is begun, but only three trials stated they required the study intervention to begin within 24 h of the antibiotic initiation<sup>[35,36,43]</sup>, while the remaining trials did not specify a minimum time. As the probiotic and control treatment were to be given concurrently with the antibiotic, the time of probiotic/control treatments varied according to the duration of the antibiotic given and ranged from 5 to 30 d, with the most duration of 7-10 d, as shown in Table 3.

**Duration of follow-up post-antibiotic:** Of the 23 treatment arms, 10 (43%) did not follow the pediatric subjects after the antibiotics and investigational treatments were discontinued. Only four trials followed children for an adequate time (6-12 wk) to capture delayed-onset AAD<sup>[28,29,33,44]</sup>, while nine arms had very short follow-up times, ranging from 4 d to 2 wk, as shown in Table 3.

**Attrition:** Lost-to-follow up data was reported in 20 (91%) of the 22 trials (Table 4), but was not reported in two trials<sup>[39,42]</sup>. Six trials (27%) did not report any loss to follow-up<sup>[28,33,35,41,45,47]</sup>, six (27%) had low attrition rates (< 10%)<sup>[32,36,38,40,48,49]</sup>, while eight (36%) had higher attrition

rates ranging from 12%-37%<sup>[29-31,34,37,43,44,46]</sup>. Only 11 (50%) of the trial arms included all enrolled patients in their intent-to-treat analysis, while nine (41%) excluded dropped patients from their as-per-protocol analysis and two (9%) did not report how many dropped from their studies<sup>[39,42]</sup>.

**Efficacy of probiotics for AAD**

**Incidence of pediatric AAD:** The incidence of AAD for each treatment arm is presented in table 4. The incidence of AAD in pediatric controls ranged from 4.3%-80%. Of the 23 probiotic treatment arms analyzed separately, 12 (52%) significantly protected children from AAD. As there is significantly heterogeneity in these trials by study size, type of probiotic strain(s) tested, formulation, dose and study design quality, further investigation and analysis was required. A meta-analysis of the 23 treatment arms weighted on study quality score revealed a significant efficacy for probiotics (in general) of a pooled RR for the prevention of pediatric AAD of 0.42 (95%CI: 0.33-0.53), as shown in the forest plot in Figure 2. When the model was run weighted on study size, the pooled results were similar: RR = 0.43 (95%CI: 0.33-0.56,  $P < 0.001$ ). As significant heterogeneity was found ( $\chi^2_{22} = 57.4, P < 0.001$ ), a randomized effect model was used in all meta-analysis models. The number needed to treat to prevent one case of pediatric AAD was 8.5.

**Incidence of pediatric CDI:** The incidence of CDI for each treatment arm analyzed separately is presented in Table 4. Of the five trials, only one significantly protected children from CDI<sup>[43]</sup>. A meta-analysis of the five treatment arms for the prevention of CDI using probiotics revealed that probiotics are significantly protective for the

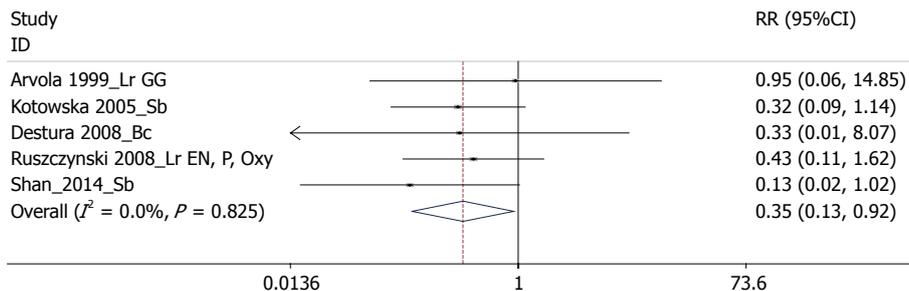


Figure 3 Forest plot of 5 probiotic treatment arms for the prevention of pediatric *Clostridium difficile* disease.

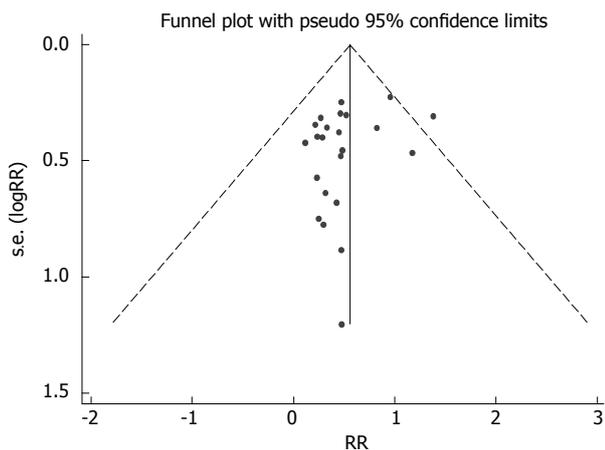


Figure 4 Funnel plot for publication bias assessment from 22 clinical trials for the prevention of pediatric antibiotic associated diarrhea.

prevention of *C. difficile* disease, but only when all strains are pooled (pooled RR = 0.35, 95%CI: 0.13-0.92,  $P = 0.03$ ), as shown in Figure 3. The number needed to treat to prevent one case of pediatric CDI was 34.8.

**Publication bias**

A funnel plot analysis (Figure 4) provides no compelling indication of publication bias for AAD trials showing general symmetry of the funnel for the relationship between risk ratio and standard error. Although there are a limited number of trials reporting on the incidence of diarrhea ( $n = 22$ ), Egger’s test for small study effects ( $P = 0.17$ ) and Begg’s test ( $P = 0.81$ ) 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 only indication that publication bias might exist is the gap in the funnel plot where small studies having an elevated risk for probiotics would appear.

A similar test for publication bias for publication bias for CDI trials also did not indicate significant publication bias (Egger’s test,  $P = 0.62$  and Begg’s test,  $P = 0.62$ ), but caution is warranted due to the small number of trials published for pediatric CDI.

**Subgroup analysis**

**Probiotic species:** It is well known that not all probiotic

strains are equally effective for the prevention of disease, therefore it is necessary to analyze the efficacy by similar probiotic strains whenever possible. Only two probiotic strains have been tested in multiple trials in the pediatric population: *S. boulardii* lyo and *L. rhamnosus* GG. When the five treatment arms (one trial had two treatment arms) testing *S. boulardii* were pooled in a meta-analysis model weighted by study quality<sup>[30,34,36,43]</sup>, there was a significant protective effect for pediatric AAD (pooled RR = 0.43, 95%CI: 0.32-0.60,  $P < 0.001$ ). When the four trials testing *L. rhamnosus* GG were pooled in a meta-analysis model weighted by study quality<sup>[29,44,47,48]</sup> this strain is also significantly protective for pediatric AAD (pooled RR = 0.36, 95%CI: 0.19-0.69,  $P = 0.002$ ).

A meta-analysis for the prevention of CDI was not possible by probiotic strain, as there are no multiple trials within any probiotic strain, other than the two trials for *S. boulardii*.

**Probiotic dose:** The *a priori* subgroup analyses on dose compared high dose probiotic ( $\geq 1 \times 10^{10}$  cfu/d) *vs* low dose ( $< 1 \times 10^{10}$  cfu/d). Seven of the treatment arms used high daily doses of probiotics and 16 used lower doses (Table 3). For the seven trials using high dose ( $\geq 1 \times 10^{10}$  cfu/d) probiotics, the pooled incidence of AAD was 8.3% for the probiotic group and 20.6% for the control group ( $P < 0.001$ ). For the 16 trials using lower doses, the pooled incidence of AAD was 7.3% for the probiotic group and 15.9% for controls ( $\chi^2_1 = 59.3$ ,  $P < 0.001$ ). A meta-analysis stratifying by low *vs* high dose trials (Figure 5) showed no significant difference by dose (pooled RR by high dose trials, RR = 0.42, 95%CI: 0.31-0.58 and pooled RR by low dose trials, RR = 0.41, 95%CI: 0.30-0.58). If a lower dose threshold was used ( $5 \times 10^9$ ), there was no significant effect on AAD incidence for probiotics given at  $5 \times 10^9$  cfu/d (7.2%) *vs* lower doses of probiotics (7.6%). For the 23 different probiotic treatments given, there was no significant dose-effect on the incidence of AAD in children.

**Quality of studies:** Of the 22 trials, 10 were judged to be of high quality<sup>[31,32,36-38,40,43-45,48]</sup>, 10 trials were judged to be of moderate quality<sup>[28-30,33-35,41,42,46,49]</sup>. Two trials that had only meeting abstract data available were judged to be of low quality, largely due to missing information<sup>[39,47]</sup>. The

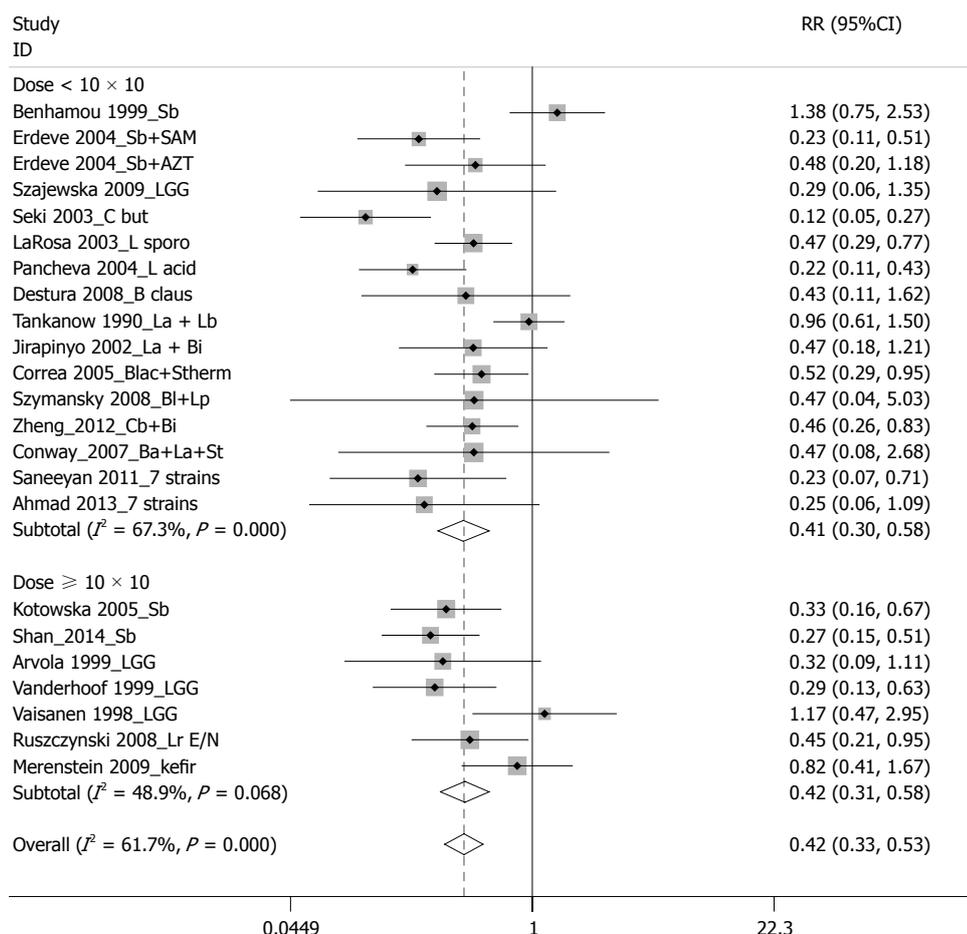


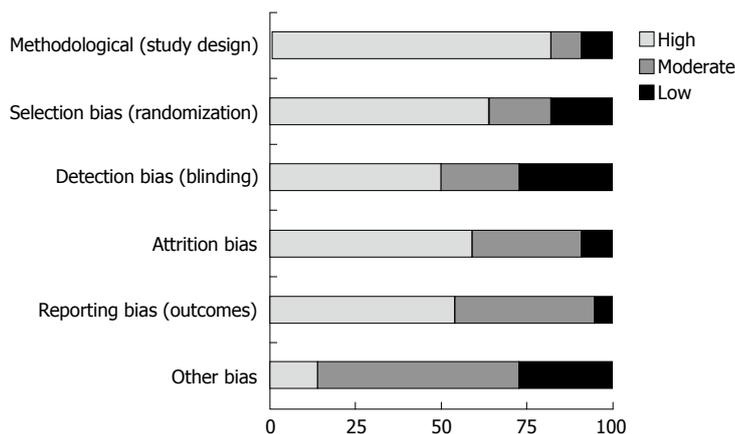
Figure 5 Meta-analysis of pediatric antibiotic-associated diarrhea by high dose (1010 cfu/d) compared to lower doses of daily probiotics given (colony forming units).

33 study items scored on quality were assessed for six sources of potential bias, as shown in Figure 6. Within the study design factors, 82% were scored as high quality, as the studies were typically well described and designed and the interventions were well defined. However, only 36% of trials provided sample size calculations. Within the randomization factors, 64% of the trials were of high quality, but 36% did not describe the method used to generate the randomized treatment allocation numbers. Within the blinding factors, 50% were moderate-low quality, as 32% were not double-blinded and the method of treatment concealment was not well described in 50% of the trials. Within the attrition factors, 41% of the trials were moderate-low quality, and while most (91%) provided attrition rates, only 68% described why children dropped out or were lost-to-follow-up. Within the outcome factors, 54% of the trials were of high quality. Most of the source of reporting bias was due to asper-protocol analyses (excluding attrition) and not using intent-to-treat analyses. In addition, 27% of the trials did not present a CONSORT flow-chart of the study population and 14% did not present any adverse event data by treatment group. Within the ‘other’ categories, only 14% of the trials were scored as high quality, largely due to a lack of two topics in the discussion (only 9% discussed

generalizability and only 50% discussed limitations of their trial). Other areas that could use improvement were to provide clinical trial registry information and to provide a location where the full protocol may be accessed. The agreement between reviewers on the initial calculation of quality scores was good ( $\kappa = 0.68$ , 95%CI: 0.63-0.73) and improved after re-review ( $\kappa = 0.98$ , 95%CI: 0.97-0.99). All disagreements were resolved after further discussion.

**Adverse events**

Of the 22 trials, 19 (86%) planned a *priori* to document any adverse events that might occur during the intervention and follow-up period (if done), while three trials did not document adverse events during their trials<sup>[34,42,47]</sup>. None of the trials reported significantly more adverse events in the probiotic group compared to the control groups, nor were there any reported cases of bacteremia or fungemia. Conway *et al*<sup>[31]</sup> reported 44% abdominal pain and 63% gas in his study, but there was no significant difference by treatment group. La Rosa *et al*<sup>[37]</sup> reported more (64%) abdominal complaints (cramps, gas and other) in the placebo group than the probiotic group (46%,  $P = 0.07$ ). Merenstein *et al*<sup>[38]</sup> reported one case of emesis in the probiotic group and one case of constipation in the



**Figure 6** Frequency of study quality based on six different types of potential bias. Low quality: 0%-50% quality items within category not present; Moderate quality: 51%-75% items not present; High quality: 76%-100% items present.

placebo group ( $P > 0.05$ ). Szajewska *et al*<sup>[44]</sup> reported 18 adverse events in the probiotic group (nausea, vomiting, taste disturbance, loss of appetite, flatulence, constipation), but these were not significantly different than the 13 adverse events reported in the placebo group. Tankanow *et al*<sup>[46]</sup> reported 14 adverse events (including rash, gas, burping, hiccups, constipation, vomiting, *etc.*), but failed to report in which treatment group these occurred. The result<sup>[49]</sup> found fewer adverse events in the probiotic group (27%) compared to the placebo group (57%,  $P = 0.06$ ), which included dehydration, fever and vomiting.

### GRADE criteria for AAD

For the prevention of pediatric AAD, we recommend the following probiotic strains: *S. boulardii* lyo (high quality and strong strength) and *L. rhamnosus* GG (high quality and strong strength). All other strains require additional multiple randomized, controlled trials before a recommendation can be provided.

### GRADE criteria for CDI

For the prevention of pediatric CDI, we are unable to make any recommendations for a specific probiotic strain at the present time due to the limited number of clinical trials performed.

## DISCUSSION

Our meta-analyses found that, while in general, probiotics may be an effective strategy to prevent AAD and CDI in children, only a few probiotic strains (*S. boulardii* lyo and *L. rhamnosus* GG) have sufficient evidence from randomized clinical trials to be confident in their abilities to prevent disease in the pediatric population. The safety of probiotics was excellent, as there were no adverse reactions significantly associated with the use of probiotics in any of the 22 clinical trials.

The evidence from meta-analyses of AAD and CDI in the literature have indicated probiotics, in general, may be efficacious for the prevention of AAD or CDI, but two main issues have limited the conclusions for pediatric populations: either trials did not assess the efficacy by specific probiotic strain<sup>[13,64]</sup> or the authors did not ana-

lyze the pediatric data separately<sup>[14]</sup> or the studies only included adults<sup>[65]</sup>. Hempel *et al*<sup>[13]</sup> reviewed 63 randomized controlled trials in adult and pediatric subjects and found a protective effect for probiotics in the prevention of AAD (pooled RR = 0.58, 95%CI: 0.50-0.68), but did not analyze the data by probiotic strain for just pediatric subjects. When the subgroup of pediatric data only was presented, the authors did not present it by probiotic strain. This is an important consideration, as not all probiotics strains are equally effective for AAD or CDI. Two meta-analyses including adult and pediatric subjects did restrict their analysis to trials using only one type of probiotic (*S. boulardii*), and found a protective effect of this strain (pooled RR = 0.43, 95%CI: 0.23-0.78)<sup>[66]</sup> and (pooled RR = 0.47, 95%CI: 0.35-0.63)<sup>[67]</sup>, but only one of these 10 trials was in a pediatric population. Kale-Pradhan *et al*<sup>[68]</sup> reviewed six trials in adults and four trials in children and found the use of different Lactobacilli probiotic strains were protective (RR = 0.35, 95%CI: 0.19-0.67), but not when only pediatric patients were analyzed. Unfortunately, the pediatric data was not analyzed grouped by identical Lactobacilli strain types. Of ten meta-analyses of probiotics for the prevention of AAD found in the literature, only four analyzed probiotics strains separately for pediatric subjects.

Several meta-analyses in pediatric populations only have limited their inclusion to studies to the same probiotic strain for the prevention of AAD. Szajewska *et al*<sup>[69]</sup> pooled the results from six RCT in children and found *L. rhamnosus* GG was significantly protective in two RCT, but other probiotic strains were not. Johnston *et al*<sup>[70-72]</sup> also conducted a sub-group analysis by probiotic strains for pediatric cases of AAD over a series of three meta-analysis over time and from the most current meta-analysis of 16 RCT, found *L. rhamnosus* GG was significantly protective in three RCT, but *S. boulardii* did not show a significant efficacy in results pooled from three other RCT. In our meta-analysis, we found both *S. boulardii* and *L. rhamnosus* GG had significant efficacy for preventing pediatric AAD. No other probiotics strains have been tested with multiple clinical trials and this is required before any conclusions and recommendations can be made on other probiotic strains.

There have been several meta-analyses investigating the use of probiotics for the prevention of CDI, but they were in adult populations<sup>[65,67,73]</sup>, or used a pediatric subgroup for the treatment, not prevention, of CDI<sup>[74]</sup>. Goldenberg *et al*<sup>[75]</sup> pooled three pediatric trials from their 23 trials in adult and pediatric populations and found a significant protective effect of probiotics for pediatric CDI (RR = 0.37, 95%CI: 0.23-0.60), which was similar to our findings from five randomized controlled trials for CDI (pooled RR = 0.35, 95%CI: 0.14-0.91). Our meta-analysis for the prevention of CDI combined five treatment arms, four with non-significant findings from the individual trials, but overall resulting in a pooled estimate of 65% risk reduction. This finding illustrates a limitation with meta-analytic methods. Although the pooled relative risk indicates a significant protective effect of probiotics, most individual trials did not. This may be interpreted as probiotics, in general, may be an effective strategy for the prevention of pediatric CDI, but the choice of the appropriate tactic (*i.e.* the specific strain of probiotic) has yet to be resolved. As only five randomized clinical trials were found for the prevention of pediatric CDI, but only two trials tested the same strain, we recommend confirmatory clinical trials for these four strains. Clearly, more randomized clinical trials testing specific probiotic strains in multiple trials are required before a conclusion can be reached.

Besides the strain of the probiotic, other factors may either confound the efficacy estimate or be as important as a predictor. These factors may include the dose of probiotic used, the duration used, the formulation and the quality of the study. We investigated the dose of probiotic used and the impact on the efficacy for AAD and CDI by doing sensitivity analyses by different daily doses. Johnston *et al*<sup>[72]</sup> reported higher dose groups ( $\geq 5 \times 10^9$  cfu/d) resulted in a significant reduction in pediatric AAD for probiotics (8%) compared to controls (22%) and compared the high dose groups who those developing AAD assigned to lower doses (8% probiotics *vs* 11% in controls), but the apparent dose-effect was driven solely by differences in AAD rates in the control groups, not by rates in the probiotic groups. We did not find a significant dose effect in our meta-analysis of pediatric AAD, as our rates of AAD were similar in the probiotic groups regardless of the threshold used (8% for  $\geq 10^{10}$  or 7.8% for  $\geq 5 \times 10^9$  cfu/d and 8.2% for  $< 5 \times 10^9$ ), but our AAD rates did not vary significantly in the control groups depending upon the dose group (21% for  $\geq 10^{10}$  or 20% for  $\geq 10^9$  cfu/d and 18% if  $< 5 \times 10^9$ ), unlike the study by Johnston *et al*<sup>[72]</sup>.

The quality of clinical trials varied from a score of 38% to 96%, which was not surprising as some of the trials were done at an earlier time before standardized randomized controlled trial guidelines were widely published and some trials with low quality scores were from meeting abstracts that never resulted in full article publications. The advantage of scoring trials on quality is this allows a meta-analysis model to be run weighing more

heavily on higher quality trials. Another advantage of assessing the quality of the clinical trials is the results allow an assessment of recommendations to improve future studies by assessing the different types of bias present in the studies. The trials included in this meta-analysis had generally low rates of bias relating to study design, attrition and reporting bias, but could show improvements in randomization methods and the degree of blinding.

We did not find any significant adverse events associated with the use of probiotics in the 22 pediatric trials and most of the reviews of probiotic clinical trials have not found adverse reactions associated with probiotic use<sup>[67]</sup>. However, bacteremia and fungemia have been reported in the literature, especially for immunocompromised infants who have a central catheter or have disorders associated with increased bacterial translocation<sup>[76-78]</sup>. Whelan and Myers reviewed the literature from 1950 to 2009 for adverse reactions noted in trials using probiotics in adult and pediatric populations and found only 20 case reports of adverse events. There were five cases of pediatric bacteremia associated with *L. rhamnosus* GG and six cases of fungemia in children taking *S. boulardii* and all eleven children recovered after treatment with antibiotics or anti-fungals were given<sup>[78]</sup>. Salminen *et al*<sup>[79]</sup> reported since the introduction of *L. rhamnosus* GG in Finland in 1990, only 0.02% of blood cultures were positive for Lactobacilli bacteremia and none of the 11 cases were found to have taken the oral probiotic. It is unclear what the absolute risk is for probiotics, as safety data is not routinely collected and reported for children treated with probiotics. The safety of probiotic products is a concern due to the lack of standardized regulations on the quality control of commercial probiotic products and the differing safety regulations depending upon if the probiotic product is an over-the-counter product, dietary supplement or prescribed medication. A review of the field by the World Gastroenterology Organization identified several issues relating to the safety of probiotics, including the inconsistent quality control results due to the failure of some probiotic products to meet their label claims with regard to the numbers and types of viable organisms in their product and the lack of standardization regulations for safety assessment of probiotic products<sup>[80]</sup>. Sanders *et al*<sup>[81]</sup> evaluated the safety of probiotic products and also identified several safety concerns: the presence of unlabeled organisms in some of the retail products and the higher rate of sepsis in immunocompromised patients. However, she also found use of probiotics reduced post-surgery infections in five of seven randomized controlled trials<sup>[81]</sup>. As a consequence, the use of probiotics in severely ill patients should be restricted to probiotic products with strong evidence-based efficacy and beneficial safety profiles.

This systematic review has several strengths. We had specific outcomes selected *a priori* and the search strategy for this review was comprehensive including any relevant trials irrespective of language or publication status (*i.e.* we included published data from meeting abstracts, obtained

pediatric specific data from authors, and translated four non-English trials). Additional strengths of the review include its rigorous application of the GRADE criteria for each of the outcomes<sup>[21]</sup> and the rigorous evaluation of each of the subgroups (*i.e.* probiotic species, probiotic dose, antibiotic class, and risk of bias) using the 33 criteria for assessing subgroup credibility<sup>[82]</sup>. The results of this meta-analysis may be generalizable to the global pediatric population, because we included a wide range of ages, countries and settings (inpatients and outpatient children were included). It should be noted however, that ethnicity and race data were not reported, nor were immunocompromised children included in most of the trials, so the applicability of our results to these types of pediatric populations is not known.

This review also has limitations. While we did a more comprehensive search of the grey literature, we did not search all conference proceedings or dissertation abstracts. One of the main limitations for doing meta-analysis on probiotics is the limited number of probiotic strains that have data from multiple trials. Probiotic strain is the key indicator of efficacy for AAD and CDI, but the limited number of trials on the same strain limits our ability draw robust conclusions on most of the strains used for pediatric studies. Only five trials had data on CDI and only two of those were done using the same strain of probiotic. Clearly, more confirmatory research on probiotic strains is mandated. Combining the results of different clinical trials introduces sources of heterogeneity, which may influence the estimate of efficacy. To control for these differences in trial populations and designs, we performed sensitivity analyses by the influence of different doses, by study quality and did separate models for probiotic strains with sufficient numbers of trials. Vidlock *et al*<sup>[64]</sup> used another technique, meta-regression modeling, to assess the association of study-related variables (age, probiotic type, risk of bias and incidence of diarrhea in the placebo group), but failed to find any significant association between these variables and the risk of AAD in adult and pediatric clinical trials. However, it is possible that differences in efficacies found in our meta-analysis may have been influenced by differences in study population, and other study-related variables that we did not stratify on.

The issues of strain-specific efficacy, study design and safety are not unique to the use of probiotics for the prevention of AAD and CDI. Other reviews and meta-analyses of probiotics have also addressed the issues of identifying an appropriate target population, choice of an effective probiotic strain, which needs to be given at an effective dose and for a sufficiently long duration, even though they have been for a different indications than our meta-analysis, such as the treatment of acute pediatric diarrhea<sup>[83,84]</sup>, treatment of adults with irritable bowel syndrome<sup>[85,86]</sup>, and the prevention of adult AAD and CDI<sup>[65,67,73]</sup>.

The alternatives for therapies to prevent pediatric diarrhea are scarce. Racecadotril and diosmectite have been

used as adjunctive therapy with oral rehydration therapy in children with existing diarrhea<sup>[87]</sup>, but only diosmectite has been tested in one study for the prevention of diarrhea in pediatric patients receiving pelvic radiation<sup>[88]</sup>. Thus probiotics remain one of the few strategies available for the prevention of pediatric AAD and CDI.

### Suggestions for future research

Recommendations for future research include multiple randomized, controlled trials on the same probiotic strains, allowing confirmation of single clinical trial results. Improvements in study design include reducing bias by the use of treatment concealment (double blinding), calculating sample size *a priori* to power a large enough study to detect significant results, use of intent-to-treat analysis to account for patient attrition effects, the collection of adverse event data and having sufficient follow-up time after the treatments are discontinued. While most cases of AAD occur while a person is on antibiotics; because it takes 6-8 wk for the normal intestinal microbial to become re-established, delayed onset AAD may occur up to 8 wk after antibiotics are discontinued<sup>[89,90]</sup>. In our meta-analysis, only four of the trials had sufficient follow-up times (6-12 wk) to capture delayed-onset cases of AAD or CDI. Future clinical trials need to incorporate sufficient follow-up times in their study protocols. As the safety of probiotic products continues to be a concern, safety data needs to be collected and global standards for commercial probiotic products are recommended.

In conclusion, our meta-analyses found probiotics are beneficial and safe in the prevention of pediatric AAD and pediatric CDI and, while only two strains had sufficient evidence to conclude they are efficacious for the prevention of AAD (*S. boulardii* lyo and *L. rhamnosus* GG), other probiotic strains are promising.

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## COMMENTS

### Background

Antibiotic associated diarrhea and *Clostridium difficile* (*C. difficile*) infections are important side-effects of antibiotic use. The frequency of both these diseases is increasing over time and the new therapies are needed to prevent these diseases. The use of probiotics (living organisms that have health benefits) have gained popularity for the prevention and treatment of various diseases, but the evidence can be confusing due to differences in the type of probiotic used and the type of patients treated.

### Research frontiers

Of the many available types of probiotic products, only a few have evidence-

based information on the efficacy and safety for the prevention of antibiotic associated diarrhea and *C. difficile* infections. While there are several reviews of probiotics in the adult population, there are limited meta-analysis for the prevention of these two diseases in the pediatric population. The research hotspot is how to choose the proper probiotic strain(s) for the prevention of these diseases in the pediatric population.

### Innovations and breakthroughs

In the present meta-analysis, the largest number of randomized controlled trials in the pediatric population have been reviewed for the efficacy and safety of probiotics. By analyzing the quality of the studies, recommends on how to improve future clinical trials for probiotics have been discovered.

### Applications

This meta-analysis found two probiotic strains (*Saccharomyces boulardii* lyo and *Lactobacillus rhamnosus* GG) were found to be significantly preventive for pediatric antibiotic-associated diarrhea. These two probiotics are generally safe to use in pediatric patients, but use is cautioned in children who are immunocompromised or are severely ill.

### Terminology

Probiotics are living microorganisms (bacteria or yeasts) that when taken at a sufficient daily dose show a health benefit for the child.

### Peer review

This manuscript on the meta-analysis of the use of probiotics in antibiotic-associated diarrhea and *C. difficile* infections is very well written.

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