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Meta-Analysis of Probiotics for the Prevention of Antibiotic Associated Diarrhea and the Treatment of *Clostridium difficile* Disease

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CONTEXT: Antibiotic-associated diarrhea (AAD) is a common complication of most antibiotics and *Clostridium difficile* disease (CDD), which also is incited by antibiotics, is a leading cause of nosocomial outbreaks of diarrhea and colitis. The use of probiotics for these two related diseases remains controversial.

OBJECTIVE: To compare the efficacy of probiotics for the prevention of AAD and the treatment of CDD based on the published randomized, controlled clinical trials.

DATA SOURCES: PubMed, Medline, Google Scholar, NIH registry of clinical trials, metaRegister, and Cochrane Central Register of Controlled Trials were searched from 1977 to 2005, unrestricted by language. Secondary searches of reference lists, authors, reviews, commentaries, associated diseases, books, and meeting abstracts.

STUDY SELECTION: Trials were included in which specific probiotics given to either prevent or treat the diseases of interest. Trials were required to be randomized, controlled, blinded efficacy trials in humans published in peer-reviewed journals. Trials that were excluded were pre-clinical, safety, Phase 1 studies in volunteers, reviews, duplicate reports, trials of unspecified probiotics, trials of prebiotics, not the disease being studied, or inconsistent outcome measures. Thirty-one of 180 screened studies (totally 3,164 subjects) met the inclusion and exclusion criteria.

DATA EXTRACTION: One reviewer identified studies and abstracted data on sample size, population characteristics, treatments, and outcomes.

DATA SYNTHESIS: From 25 randomized controlled trials (RCTs), probiotics significantly reduced the relative risk of AAD (RR = 0.43, 95% CI 0.31, 0.58, $p < 0.001$). From six randomized trials, probiotics had significant efficacy for CDD (RR = 0.59, 95% CI 0.41, 0.85, $p = 0.005$).

CONCLUSION: A variety of different types of probiotics show promise as effective therapies for these two diseases. Using meta-analyses, three types of probiotics (*Saccharomyces boulardii*, *Lactobacillus rhamnosus* GG, and probiotic mixtures) significantly reduced the development of antibiotic-associated diarrhea. Only *S. boulardii* was effective for CDD.

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Antibiotic-associated diarrhea (AAD) is a common complication of antibiotic use. The frequency of AAD can be high (26–60%) during hospital outbreaks or moderate (13–29%) during endemic periods and is relatively infrequent in outpatient settings (<0.1%) (1–3). Risk factors for AAD include broad-spectrum antibiotics, host factors (age, health status, gender), hospitalization status, and exposure to nosocomial pathogens (2, 4). AAD usually occurs 2–8 wk after exposure to antibiotics as a result of disrupting intestinal microflora. One of the roles of intestinal microflora is to act as a protective barrier that resists the colonization of intestinal pathogens (5). Without this protective barrier, patients are susceptible to infection by opportunistic pathogens.

Hospitalized patients exposed to antibiotics may develop *Clostridium difficile* disease (CDD). Recently, this pathogen caused nosocomial outbreaks in Canadian hospitals that resulted in over 100 deaths (6). Consequences of AAD and CDD include extended hospital stays (3–7 days), increased rates of subsequent infections (20–65%), higher hospital costs (up to $1 billion/yr) and 2–3 times increased rates of mortality (7–11).

Current therapies for AAD are lacking. Strategies to date include discontinuing the inciting antibiotic, restricting the

use of high-risk antibiotics and specific antibiotic treatments if the etiology is known. For CDD, 80% respond well to the initial treatment of vancomycin or metronidazole. The remaining 20% may develop subsequent episodes of CDD, which may persist over several years, despite repeated antibiotic treatments (9).

Probiotic therapy is well suited to these two types of microbial induced diseases. Probiotics assist in reestablishing the disrupted intestinal microflora, enhancing immune responses and clearing pathogens and their toxins from the host (12–14). Research using probiotics has been reported for the past 28 yr, but the studies have been variable in trial design, type of probiotic, had differing doses and durations of treatment, and thus have yielded contradictory results. The lack of definitive evidence regarding efficacy and safety is limiting the use of this type of treatment strategy. There is a growing interest in probiotics for the treatment of AAD and CDD due to the wide availability of probiotics as dietary supplements and the concern over recent outbreaks of severe CDD in Canada and the United Kingdom (6, 15). Two meta-analyses done in 2002 presented results on only 11 trials and failed to discuss possible reasons for the conflicting findings and did not present detailed study information (16, 17). A recent Cochrane review on treatments for CDD did not include probiotics (18). The need for a meta-analysis of probiotics for these two antibiotic associated diseases is apparent.

METHODS

Objectives

The objectives of this meta-analysis are to assess the efficacy and safety of probiotics for (i) the prevention of AAD and (ii) the treatment of CDD.

Criteria for Study Selection

Abstracts of all citations and retrieved studies were reviewed and rated for inclusion. Full articles were retrieved if specific treatments were given to either prevent or treat the disease of interest. Inclusion criteria include randomized, controlled, blinded efficacy trials in humans published in peer-reviewed journals. Exclusion criteria include pre-clinical studies, case reports or case series, Phase 1 safety studies in volunteers, reviews, duplicate reports, trials of unspecified probiotics, trials of prebiotics, not the disease being studied, or inconsistent outcome measures. External and internal validity is strengthened by including only randomized controlled trials (RCTs).

Outcomes and Definitions

The primary outcome for AAD is defined as diarrhea ($\geq$3 loose stools/day for at least 2 days or $\geq$5 loose stools/48 h) within 2 months of antibiotic exposure (2, 11). The primary outcome of CDD is defined as a new episode of diarrhea associated with a positive culture or toxin (A or B) assay within 1 month exposure to antibiotics. The outcome for prevention of CDD is a new episode of *C. difficile* positive diarrhea within 1 month of a previous CDD episode (19). Documentation of diarrhea is based on clinical assessment and self-report of symptoms by daily symptom diaries.

Data Sources

PubMed, Medline, and Google Scholar were searched from 1977 to 2005 for articles unrestricted by language. Non-English articles were translated. Three on-line clinical trial registers 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). Secondary and hand searches of reference lists, authors, reviews, commentaries, associated diseases, books, and meeting abstracts were also performed. Six search terms for RCTs (RCT, human, blinding, Phase 2, Phase 3, efficacy) were combined with 15 terms for probiotics. Search terms included probiotics, microflora, antibiotics, *Clostridium difficile*, colitis, PMC, diarrhea, Saccharomyces, Lactobacilli, Bifidobacteria, Enterococci, Bacilli, VSL#3, synbiotics, and Lactinex. Search strategies were broad-based initially, then narrowed to the disease of interest (20). The procedure for this meta-analysis was designed as suggested by Egger *et al*. and MOOSE guidelines using clearly delineated parameters, *a priori* inclusion and exclusion criteria, and standardized data extraction methods (21–23).

Data Extraction

Information on study design, methods, interventions, outcomes, adverse effects, and treatments was extracted from each article. Data on patient inclusion and exclusion criteria, number of completed subjects, attrition, treatment dose and duration, and outcome was extracted into a standardized table. In some cases, the primary or secondary author was contacted for data not reported in the original article. The data abstraction was completed individually, but verified using historic searches with two other researchers for previous review articles (24, 25). A few trials had multiple probiotic arms with a common control group. Each probiotic arm and control group was analyzed separately.

Assessment of Methodological Quality

Studies that met the inclusion criteria were graded for quality using a scale reported by the U.S. Preventive Services Task Force (26). Quality of evidence is rated from 1 to 3 (poor, fair, and good) based on randomization, study design, sample size, generalizability, study biases, and outcome assessment. Study quality was not integrated with the model weights, as trials of poor quality were excluded from review and this practice is not uniformly recommended (27). Weights for this analysis are based solely on sample sizes.

Statistical Analysis

Statistical analysis was performed using Stata software version 8.0 (Stata Corporation, College Station, TX). Relative

risks with 95% confidence intervals were computed as summary statistics. Heterogeneity across trials was evaluated using Cochran Q test based on pooled relative risks by the Mantel-Haenszel method. If the studies were homogeneous, a fixed-effects model was used and a pooled relative risk was calculated with the Mantel-Haenszel method for fixed effects. If the studies were heterogeneous a random effect was employed and a pooled relative risk was calculated using the DerSimonian and Laird method (28). If significant heterogeneity was detected, a subgroup analysis was conducted. *A priori* subgroups were by type of probiotic, dose and indication (AAD or CDD). A funnel plot as well as an adjusted rank correlation test using the Begg and Mazumdar method were used to assess publication bias (29, 30). p Values less than 0.05 were considered significant.

RESULTS

Overview of Included Studies

AAD. The literature search yielded 940 citations, of which 104 were selected from retrieval. Twenty-five (24%) of the screened articles met inclusion criteria and provided data on 2,810 treated patients with AAD. The number of patients in each of these studies was generally moderate (median, 79; range 18–388). A QUOROM (Quality of Reporting of Meta-analysis) flow diagram (Fig. 1) shows an overview of the study selection process (22).

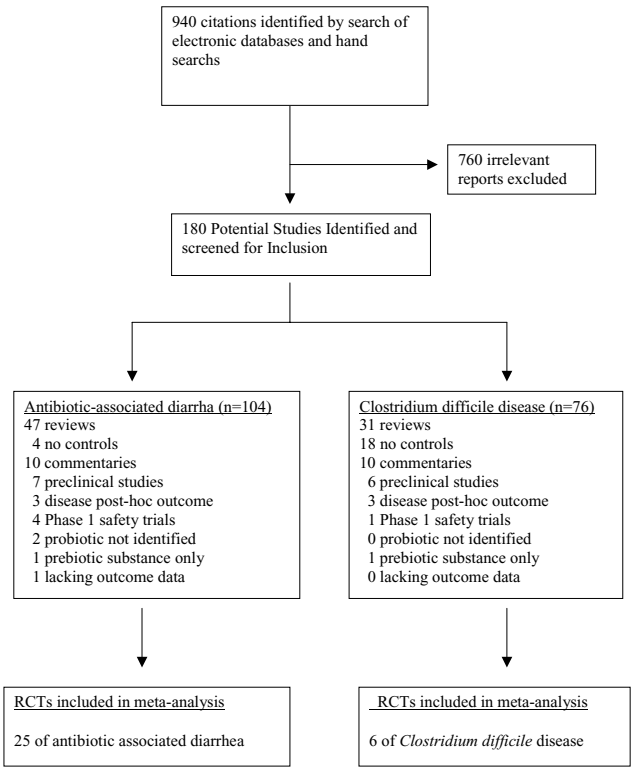

Figure 1. QUOROM flow diagram of included and excluded studies. RCT indicates randomized, controlled trial.

CDD. The literature search yielded 940 citations, of which 76 were selected from retrieval. Only six (8%) of the screened articles met inclusion criteria and provided data on 354 treated patients with CDD. The number of patients in each of these studies was generally small (median, 25; range 15–138).

Excluded Studies

AAD. Of the AAD studies, 79 failed to meet one or more of the inclusion criteria. Most were reviews or commentaries (n = 57), pre-clinical or Phase 1 safety studies done in healthy volunteers (n = 11) or had no control group (n = 4). AAD was a *post hoc* outcome in three trials and one study failed to provide outcome data. Some studies were excluded because the type of probiotic was not specified (31, 32) or only a prebiotic (oligosaccharide with no living probiotic) was given as the intervention (33).

CDD. Of the CDD studies, 70 failed to meet one or more of the inclusion criteria. Most were reviews or commentaries (n = 41), pre-clinical or Phase 1 safety studies done in healthy volunteers (n = 7) or had no control group (n = 18). Some studies were excluded because CDD was a *post hoc* outcome (n = 3) or only a prebiotic (oligosaccharide with no living probiotic) was given as the intervention (34).

Study Quality

The quality of the studies is presented in Tables 1 and 3, indicating generally good methodological quality. Most studies of poor quality were excluded from the data extraction in the preliminary steps of this study.

Efficacy Studies

AAD. Twenty-five RCTs provided adequate data regarding efficacy in a total of 2,810 patients with AAD, as shown in Table 1. Of the 25 trials, 13 (52%) reported a significant reduction of AAD in the probiotic-treated group compared with the placebo group in their study. Twelve studies did not reject the null hypothesis of no difference in the incidence of AAD for probiotic treated *versus* controls.

These contradictory results may be due to differences in the study population enrolled, the type of probiotic, the dose of probiotic given, or the duration of treatment. Typically, these trials were done in adults given broad-spectrum antibiotics (64%), while 36% of the trials were done in children taking antibiotics. Of 16 RCTs of AAD in adult patients, 7 (44%) showed significant efficacy for probiotics. Of nine RCTs of AAD in children, six (67%) had significant efficacy. There was not a significant difference in efficacy of probiotics according to whether adults or children were enrolled (Fisher's $p = 0.41$). The types of probiotics varied from single strains (*Saccharomyces boulardii*, *Lactobacillus rhamnosus* GG, *Bacillus clausii*, *Bifidobacterium longum*, *Clostridium butyricum* miyairir, *Lactobacillus acidophilus*, *Enterococcus faecium* SF68), to mixtures of two types of probiotic and to a synbiotic (a probiotic combined with a pre-biotic substance). Daily doses of probiotics ranged from 1×10^7 to 1×10^{11}, with a mean of 3×10^9. Use of a high dose ($\geq 10^{10}$/day) of

Table 1. Description of 25 Randomized Controlled Trials of Probiotics for the Prevention of Antibiotic Associated Diarrhea

						Probiotic-Treated		Control Group				
N	Subjects	Probiotic	Dose/Day	Txt Duration	Follow-Up	No. Failed	No. Cured	No. Failed	No. Cured	Quality	Weight	Reference
388	Adults, b-lactam or tetracycline	SB	4×10^9	1 wk	0	9 (4.5)	190	33 (17.5)	156	3	4.9	(35)
180	Adults, varied	SB	2×10^{10}	On abx	2 wk	11 (9.5)	105	14 (21.8)	50	3	4.9	(36)
193	Adults, b-lactams	SB	2×10^{10}	4 wk	7 wk	7 (7.2)	90	14 (14.6)	82	3	4.4	(37)
246	Peds, varied	SB	1×10^{10}	1–2 wk	2 wk	4 (3.4)	115	22 (17.3)	105	3	3.8	(38)
69	Elderly, varied	SB	4×10^9	2 wk	0	7 (21)	26	5 (13.9)	31	2	3.8	(39)
43	*H. pylori* + 3 abx	SB	5×10^9	7 days	0	1 (5)	21	6 (30)	15	2	1.7	(40)
119	Peds, varied	LGG	4×10^{10}	2 wk	12 wk	3 (5)	58	9 (16)	49	3	3.2	(41)
188	Outpatient peds, varied oral abx	LGG	$1–2 \times 10^{10}$	10 days	0	7 (7.5)	86	25 (26)	70	3	4.6	(42)
81	Hosp peds, varied	LGG	1×10^{10}	Varied	0	3 (6.7)	42	12 (33.3)	24	2	3.4	(43)
267	Hosp adults 70% b-lactam/varied	LGG	2×10^{10}	2 wk	1 wk	39 (29.3)	94	40 (29.9)	94	3	6.1	(44)
42	*H. pylori* + 3 abx	LGG	6×10^9	7 days	0	1 (5)	20	6 (30)	15	2	1.7	(40)
120	*H. pylori* + 3 abx	LGG	1×10^{10}	2 wk	0	2 (3.4)	58	16 (26.6)	44	3	2.8	(45)
100	*H. pylori* + 3 abx	BC	6×10^9	2 wk	0	15 (30)	35	17 (34)	33	2	5.4	(46)
20	Adults, clindamycin	BL	5×10^{10}	21 days	0	4 (40)	6	7 (70)	3	2	4.4	(47)
110	Peds, varied	CB	$1–4 \times 10^7$	6 days	0	6 (7)	77	16 (59)	11	2	4.5	(48)
45	Adults, varied	EF	1.5×10^7	7 days	0	2 (8.7)	21	6 (27)	16	2	2/6	(49)
200	Adults with TB	EF	Ng	8 wk	0	5 (5)	95	18 (18)	82	3	4.1	(50)
27	Adults amoxicillin	LA	1.2×10^8	Varied	0	10 (83)*	2	10 (67)	5	2	5.9	(51)
79	Hosp adults, ampicillin	Lactinex	2×10^9	5 days	0	3 (8.3)	33	9 (21)	34	2	3.2	(52)
38	Peds, amoxicillin	Lactinex	2×10^9	10 days	0	10 (66)	5	16 (69.5)	7	2	5.8	(53)
20	Adults, clindamycin	LABLa	1×10^{11}	21 days	0	2 (20)	8	7 (70)	3	2	3.0	(47)
42	*H. pylori* + 3 abx	LABL	5×10^9	7 days	0	1 (5)	20	6 (30)	15	2	1.7	(40)
77	Children, varied	BLST	1×10^7	15 days	15 days	6 (16)	32	12 (31)	27	2	4.3	(54)
98	Children, varied	LS FOS	$5.5 \times 10^8 + 250$ mg	10 days	0	14 (29)	34	31 (62)	19	2	5.7	(55)
18	Infants 1–36 months on antibiotics	LABI	6×10^9	7 days	0	3 (37.5)	5	8 (80%)	2	2	4.1	(56)

N = number of subjects with evaluable outcome; SB = *Saccharomyces boulardii*; LGG = *Lactobacillus rhamnosus* GG; BC = *Bacillus clausii*; BL = *Bifidobacterium longum*; CB = *Clostridium butyricum* MIYAIRI; EF = *Enterococcus faecium* SF68; LA = *Lactobacillus acidophilus*; LALB = Lactinex = *L. acidophilus* and *L. bulgaricus*; LABL = *Lactobacillus acidophilus* and *Bifidobacterium longum*; LABLa = *Lactobacillus acidophilus* and *Bifidobacterium lactis*; BLST = *Bifidobacterium lactis* and *Streptococcus thermophilus*; LSFOS = *Lactobacillus sporogenes* and fructo-oligosaccharide; LABI = *Lactobacillus acidophilus* and *Bifidobacterium infantis*; Txt = treatment; Abx = antibiotic.
Quality: 1 = poor; 2 = fair; 3 = good (26).
*Any gastrointestinal complaint including diarrhea.

Table 2. Meta-Analyses of Relative Risks Stratified by Type of Probiotic for the Prevention of Antibiotic Associated Diarrhea

Probiotic	Number of RCT	Combined RR	95% CI	*p* Value	Type of Model	References
Saccharomyces boulardii	6	0.37	0.26, 0.52	<0.0001	Fixed	(35–40)
Lactobacillus rhamnosus GG	6	0.31	0.13, 0.72	0.006	Random	(41–45)
Single strains of probiotics	6	0.46	0.21, 1.03	0.06	Random	(46–51)
Mixtures of two probiotics	7	0.51	0.38, 0.68	<0.0001	Fixed	(40, 47, 52–56)

Single strains included: *Clostridium butyricum* MIYAIRI; *Enterococcus faecium* SF68; *Lactobacillus acidophilus*; *Bifidobacterium longum*; *Bacillus clausii*; *Bifidobacterium lactis*; *Streptococcus thermophilus*; or *Lactobacillus sporogenes*.

Mixtures included: Lactinex = *L. acidophilus* and *L. bulgaricus*; *Lactobacillus acidophilus* and *Bifidobacterium lactis*; *Lactobacillus acidophilus* and *Bifidobacterium infantis*. *p* Value for null hypothesis that RR = 1.

probiotic was associated with a significant efficacy for AAD. Eight (67%) of 12 RCTs with a positive efficacy for AAD used a high daily dose of probiotic compared with only 2 (17%) of 12 RCTs that showed no significant difference yet used a high daily dose ($p = 0.04$). The duration of probiotic treatment also varied widely from 5 days to 8 wk (median of 2 wk), but the duration of probiotic did not significantly differ in trials showing protective efficacy compared to no difference.

Data from 25 RCTs were combinable for a meta-analysis, as they reported frequencies of outcomes in treated and controls (35–56). As the χ^2 test for heterogeneity was 82.5 ($p < 0.001$), indicating a low degree of homogeneity between studies, a random-effects model was utilized. The combined efficacy shows probiotics have a significant protective effect for AAD (Fig. 2). The relative risk for AAD was 0.43 (95% CI 0.31, 0.58), z = 5.4, $p < 0.001$. A funnel plot (Fig. 3) may indicate the modest presence of some publication bias or may reflect the differences due to the type of probiotic. No significant evidence of publication bias was found using the Begg rank correlation test (z = –1.05, $p = 0.29$).

As the efficacy may vary by the probiotic strain being tested, additional meta-analyses were done, stratified *a priori* by the probiotic type. Two single probiotic strains showed significant efficacy for AAD: *S. boulardii* and *L. rhamnosus* GG (Table 2), as well as mixtures composed of two different types of probiotics. The meta-analysis for other single probiotic strain preparations than those above was not significantly protective for AAD, but as the strains were diverse, clinical conclusions should be made with caution.

CDD. Six RCTs provided adequate data regarding efficacy in a total of 354 patients with CDD, as shown in Table 3 (57–62). Of the six trials, two (33%) reported a significant reduction of CDD recurrences in the probiotic-treated group compared with the placebo group. Four studies did not reject the null hypothesis of no difference in the incidence of CDD recurrences for probiotic treated *versus* controls.

These contradictory results may be due to differences in the study population enrolled, the type of probiotic, the dose of probiotic given or the duration of treatment. All these trials were done in adults with prior antibiotic exposure and three studies were done exclusively in patients with recurrent CDD. The types of probiotics included *S. boulardii*, *L. rhamnosus* GG, *L. plantarum* 299v, and a mixture of *L. acidophilus* and *Bifidobacterium bifidum*. Five of the six RCTs were treating patients with established CDD and the probiotic was combined with standard antibiotics (either vancomycin or metronidazole) for treating CDD. Unfortunately, the type or dose of the antibiotic was not randomized along with the probiotic arm for any of the studies. Daily doses of probiotics ranged from 2×10^{10} to 6×10^{11}, with a mean daily dose of 5×10^{10}. The duration of probiotic treatment also varied from 3 to 5 wk, with a median of 3 wk. The number of trials was too small to determine if a dose-response or duration-response effect was present.

Data from six RCTs were combinable for a meta-analysis, as they reported frequencies of outcome in treated and controls. As the χ^2 test for heterogeneity was 4.6 ($p = 0.5$), indicating a high degree of homogeneity between studies, a fixed-effects model was utilized. The combined efficacy shows probiotics have a significant protective effect for CDD (Fig. 4). The relative risk for CDD was 0.59 (95% CI 0.41, 0.85), z = 2.8, $p = 0.005$. A funnel plot (Fig. 5) indicates the absence of publication bias. No significant evidence of publication bias was found using the Begg rank correlation test (z = 0.56, $p = 0.57$).

Of the three different probiotics tested for the treatment of CDD, only *S. boulardii* showed significant reductions in recurrences of CDD (57, 58). *L. rhamnosus* GG and *L. plantarum* 299v did not show significant differences in CDD recurrence rates in probiotic *versus* control treated groups. The one trial testing a probiotic mixture (*L. acidophilus* and *B. bifidum*) for the prevention of CDD did not show significant efficacy (62).

Adverse Events

Twenty-six (84%) of the 31 trials presented data on adverse reactions, but five trials did not (35, 48, 52, 60, 62). In 24 trials, no adverse reactions were associated with the probiotic treatments. McFarland *et al*. reported significantly more subjects taking *S. boulardii* reported thirst (9%) or constipation (14%) compared with controls (57). Wullt *et al*. reported mild bloating (25%) or gas (37%) was associated with *L. rhamnosus* GG (60). No cases of bacteremia or fungemia or other serious adverse events were reported in the 31 RCTs.

COMMENT

Antibiotic-induced diseases present unique treatment challenges for the health-care provider. Treatment with additional

Table 3. Description of Six RCT of Probiotics for the Treatment or Prevention of *Clostridium difficile* Disease

N	Subjects	Probiotic	Probiotic Dose Per Day	Antibiotic Dose Per Day	Txt Duration	Follow-Up	Probiotic-Treated (recurred) No. Failed	Control Group (recurred) No. Cured	No. Failed	No. Cured	Quality	Weight	Reference
124	Adults CDD/RCDD	SB + V/M	2×10^{10}	Varied	4 wk	4 wk	15 (26)	42	30 (44.8)	37	3	51.5	(57)
32	Adults RCDD	SB + V	2×10^{10}	2 g	4 wk	4 wk	3 (16.7)	15	7 (50)	7	2	14.7	(58)
25	Adults CDD/RCDD	LGG + V or M	nr	nr	3 wk	0	4 (36.4)	7	5 (35.7)	9	2	8.2	(59)
20	Adults RCDD	LP 299v + M	5×10^{10}	nr	38 days	0	4 (36)	7	6 (67)	3	2	12.3	(60)
15	Adults RCDD	LGG + V or M	6×10^{11}	Varied	3 wk	4 wk	3 (37.5)	5	1 (14.3)	6	2	2.0	(61)
138	Inpatients varied antibiotics	LABB, no V or M	2×10^{10}	None	20 days	0	2 (2.9)	67	6 (8.6)	63	3	11.2	(62)

N = number of subjects with evaluable outcome; SB = *Saccharomyces boulardii*; LGG = *Lactobacillus rhamnosus* GG; LP = *Lactobacillus plantarum* 299v; V = vancomycin; M = metronidazole, nr = not reported, LABB = *Lactobacillus acidophilus* and *Bifidobacterium bifidum*; nr = not reported; Quality: 1 = poor; 2 = fair; 3 = good (26).

antibiotics for diarrhea symptoms can worsen the condition by further disrupting the intestinal microflora. Efforts to prevent AAD by restricting antibiotic use in hospitals or reducing inappropriate antibiotic prescriptions has met with only limited success (63–65). Probiotics have shown promise in antibiotic mediated diseases as they are not disruptive of intestinal microflora and have multiple mechanisms of action in which to combat opportunistic pathogens *in situ* (12, 24). Acceptance of probiotics in routine formularies has been slow due to the lack of a consensus on the efficacy and safety of probiotics.

The result of the literature search on probiotics found that the majority of articles (54%) screened for inclusion were reviews or commentaries. Unfortunately, the prevalence of RCTs is not frequent in this area. Only 31 (17%) were included in this meta-analysis. This is the largest number of RCT analyzed by meta-analysis to date.

In this meta-analysis of 31 randomized, controlled trials, a clear message is seen supporting the use of probiotics for two antibiotic-associated diseases. Probiotics given for the prevention of AAD have a pooled relative risk of 0.43 (0.31, 0.58), using a random-effects model. This is similar to pooled estimates of risk from three earlier meta-analysis based on fewer (5–9) RCTs (16, 17, 66). D'Souza *et al*. pooled nine trials and found probiotics significantly reduced the odds of AAD (OR = 0.37, 95% CI 0.26, 0.52), but did not provide data on heterogeneity testing or publication bias (16). Cremonini *et al*. analyzed seven trials and found a pooled relative risk of 0.40 (95% CI 0.27, 0.57) (17). Although it was not stated if this was a random- or fixed-effects model, no significant heterogeneity was found ($p = 0.42$) and no publication bias was seen in a funnel plot. Publication bias was not assessed by either Begg's or Egger's test in this meta-analysis. Szajewska and Mrukowicz analyzed five trials and found a pooled relative risk of 0.43 (95% CI 0.23, 0.78) using a random-effects model (66). No significant publication bias was found. However, this meta-analysis was restricted to one type of probiotic (*S. boulardii*) and did not examine other types of probiotics. These three meta-analyses were small and did not consistently provide full information on heterogeneity and publication bias. Despite these limitations, the three meta-analyses demonstrated a significant efficacy of probiotics for the prevention of AAD and validated our findings. The current meta-analysis included a large number of RCTs and fully described potential biases.

The etiologies of AAD are diverse and largely not identified. Approximately one-third of AAD is due to *C. difficile*, while another 10–20% is due to bacterial and viral etiologies (2). Of the 25 RCTs in this analysis, only 9 (36%) attempted to determine the etiologies of AAD in their trials. Only four trials reported treatment-specific efficacies stratified on *C. difficile* status and none were significant (36–38, 43). This is not surprising, as the original trials were powered for AAD and not *C. difficile* AAD. As the proportion of *C. difficile* is variable and may only account for one-third of the enrolled patients, trials for the prevention of *C. difficile* AAD typically

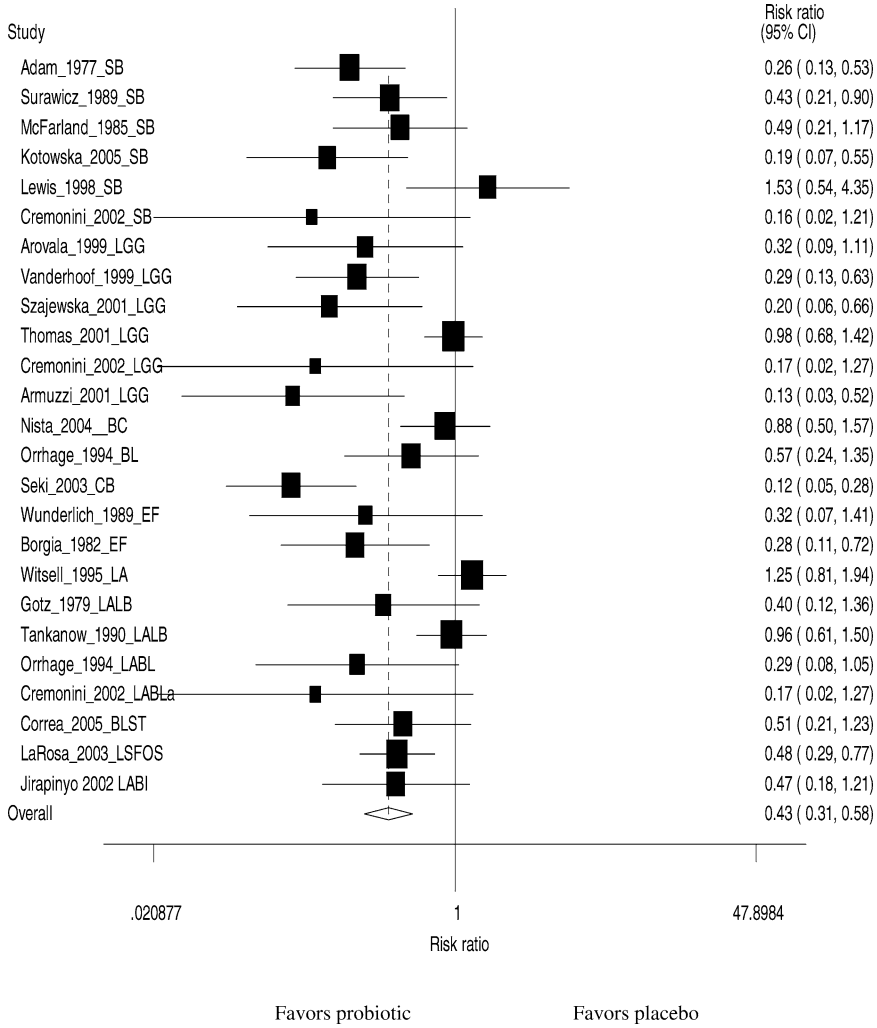

Figure 2. Forest Plot of 25 randomized controlled trials of probiotics for the prevention of antibiotic associated diarrhea showing crude and pooled risk ratios. SB = *Saccharomyces boulardii*; LGG = *Lactobacillus rhamnosus* GG; BC = *Bacillus clausii*; BL = *Bifidobacterium longum*; CB = *Clostridium butyricum* MIYAIRI; EF = *Enterococcus faecium* SF68; LA = *Lactobacillus acidophilus*; LALB = Lactinex = *L. acidophilus* and *L. bulgaricus*; LABL = *Lactobacillus acidophilus* and *Bifidobacterium longum*; LABLa = *Lactobacillus acidophilus* and *Bifidobacterium lactis*; BLST = *Bifidobacterium lactis* and *Streptococcus thermophilus*; LSFOS = *Lactobacillus sporogenes and* fructo-oligosaccharide; LABI = *Lactobacillus acidophilus* and *Bifidobacterium infantis.*

have not been done due to the large sample sizes required. As an alternative, probiotic treatment trials for patients with existing CDD have usually been done. Of the six RCTs, five were for treatment and only one was for the prevention of CDD. The pooled relative risk from this meta-analysis for CDD was 0.59 (0.41, 0.85). As no significant heterogeneity was found ($p = 0.5$) even for this limited number of trials, a fixed effect model was used. Both a funnel plot and Begg's test did not find significant publication bias ($p = 0.57$). The CDD meta-analysis relies heavily upon two studies done by the author, but the potential for bias has been limited by including only trials published in peer-reviewed journals and by the use of quantitative outcomes. The trials were weighted by study size and not a qualitative measure of quality, which has the potential for bias, especially if the author is evaluating their own studies. We did not find any other meta-analyses of probiotics for CDD for comparison. There are also too few trials in the literature to analyze the efficacy of probiotics for carriers *versus* diseased patients or by the history of the patient (initial cases compared to recurrent CDD), although these types of trials would be useful.

The most frequent limitation of these RCTs was that studies may have suffered from insufficient power to detect a significant difference. Few studies reported sample size calculations in their methods section and three authors reported that slow recruitment caused premature termination of the trial (56, 59, 62). Calculating a mean sample size based on a 50% reduction of AAD for probiotic treated *versus* 37% AAD in controls (mean taken from 25 AAD trials) with an alpha of 0.05 and a power of 80%, the required number of patients would be 204 per trial. Few (3, 10%) of the 31 RCTs reached this enrollment goal. Future trials need to calculate required sample sizes before initiating the study and strive to recruit sufficient numbers of patients.

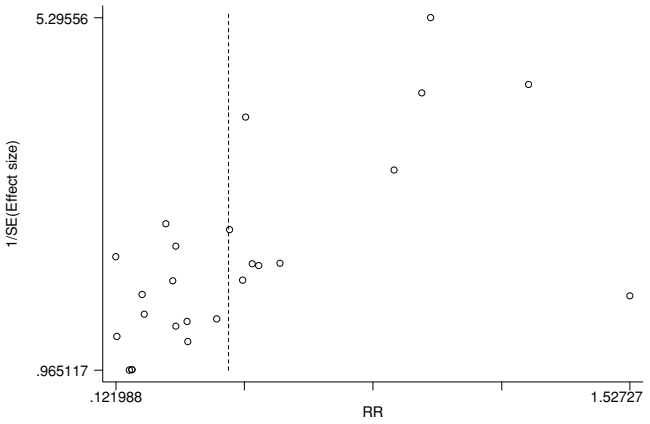

Figure 3. Funnel Plot of 25 randomized controlled trials of probiotics for antibiotic associated diarrhea. Dashed line indicates pooled relative risk of 0.43.

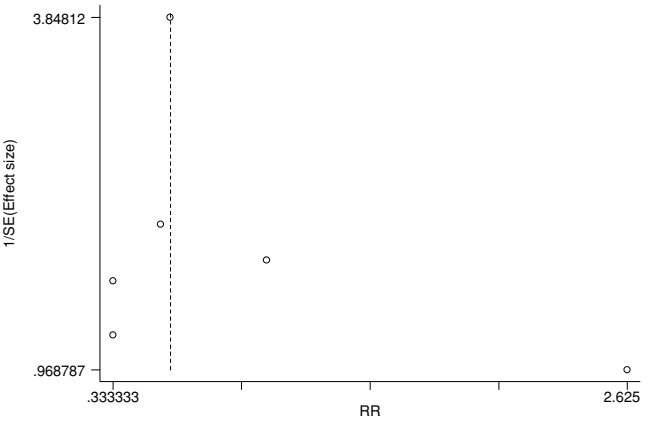

Figure 5. Funnel Plot of six randomized controlled trials of probiotics for *Clostridium difficile* associated disease. Dashed line indicates pooled relative risk of 0.59.

Heterogeneity between studies can be a limiting factor for meta-analyses. It may arise from differences in study populations, type of probiotic being investigated or differences in probiotic doses and duration of treatment. For AAD trials, the heterogeneity may be due to the different populations enrolled, as both adults and children given a variety of different types of antibiotics were studied. However, the efficacy was not found to differ for adult and pediatric subjects. For RCT involving CDD, all trials enrolled adults exposed to antibiotics.

Another source of heterogeneity for probiotic trials is the type of probiotic itself. Significant differences in effectiveness have been reported for different species and strains of similar species of bacteria and yeasts (24, 67, 68). Unfortunately, many trials only report the genus and species and do not provide strain designations. A few studies (excluded from the analysis) even failed to provide the identity of the probiotic and only stated the treatment was "living yogurt" or "protective lactobacilli." Future studies need to provide the complete identity of the probiotic being tested.

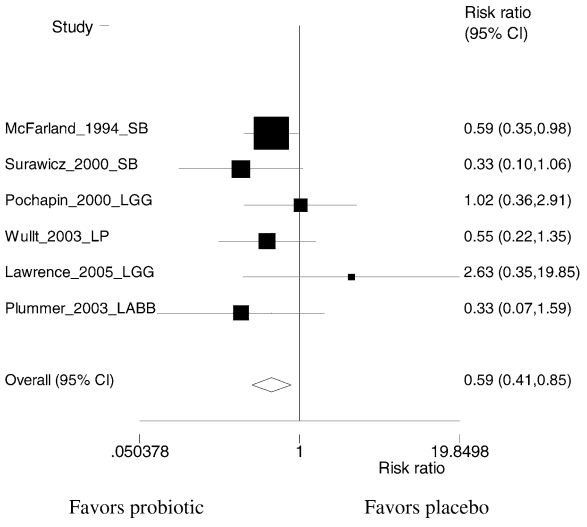

Figure 4. Forest Plot of six randomized controlled trials of probiotics for the treatment of *Clostridium difficile* disease showing crude and pooled risk ratios. SB = *Saccharomyces boulardii*; LGG = *Lactobacillus rhamnosus* GG; LP = *Lactobacillus plantarum* 299v; LA = *Lactobacillus acidophilus*; BB = *Bifidobacterium bifidum.*

Trials for AAD failing to show significant efficacy may have used sub-therapeutic doses of probiotics ($<10^{10}$ organisms/day) or failed to provide the probiotic during the entire period of susceptibility when normal intestinal microflora is becoming reestablished (usually 6–8 wk) (2). This meta-analysis found a dose-response for probiotics used to prevent AAD. Trials for the treatment of CDD were more homogeneous in terms of probiotic treatments than trials for AAD. Probiotic doses and durations were similar (100% of those reporting doses used $>10^{10}$/day) and all studies treated patients for at least 3 wk.

Another limitation in these trials was the lack of standardization when a combination treatment regimen was used. The common strategy for treating CDD infections is to combine the investigational probiotic with one of the standard antibiotics (vancomycin or metronidazole) given to treat *C. difficile*. The hypothesis of the combination therapy is that the antibiotic kills vegetative *C. difficile* organisms in the intestine, which would clear the pathogenic toxins, and the probiotic would assist in reestablishing the protective intestinal microflora so that when residual spores germinate, colonization is rebuffed by the newly restored microflora barrier. Unfortunately, only the probiotic component of these trials was randomized. The standard antibiotic varied by type and dose and was not controlled in most trials. This is an important consideration because Surawicz *et al*. found only a high dose of vancomycin (2 g/day) completely cleared *C. difficile* toxins by the end of 10 days of therapy, whereas a lower dose (500 mg/day) of vancomycin failed to clear toxins in 10% and metronidazole (1.5 g/day) only cleared toxins in 40% of the patients (58). Future studies should randomize the combination treatment with a standard dose of both the

standard antibiotic and the probiotic to test the complete regimen.

Potential biases in review process may be due to publication bias. Sutton *et al*. reviewed 48 meta-analyses and found 30 (63%) made no reference to publication bias or reported funnel plots (69). In this meta-analysis, publication bias was minimized by conducting extensive searches through multiple databases and receiving original data from the authors. Funnel plots for AAD and CDD showed there may be some publication bias present for AAD, but the discovery of numerous negative trials for AAD may have minimized this bias. Selection and ascertainment bias was minimized by including only RCTs with validated outcomes.

Concerns about the safety of probiotics have been raised. As probiotics are living organisms given to ill patients, the potential for adverse reactions exists. Some intestinal bacteria have been shown to translocate from the intestine to other organs and antibiotic-resistance gene acquisition also is a potential concern. These two problems have yet to be observed in clinical trials using probiotics. Although case reports and case series of bacteremia and fungemia have been reported in the literature, no incidents occurred in patients enrolled in the 31 RCTs reviewed for this meta-analysis (70, 71). Caution should be exercised for patients who are severely ill and receiving nutrition or antibiotics through a potentially open portal (catheter or nasogastric tube). Infrequent blood-stream infections have been reported, most probably due to contamination of the environment as the probiotic capsule is opened at bedside and mixed with food (72). Rare complications including endocarditis and liver abscess have been associated with *L. rhamnosus* use (73, 74). Bacteremia and fungemia have been associated with probiotics, but respond well to antibiotics or anti-fungal medications (75–78).

Considering that millions of doses of probiotics are taken per year globally, the risk of complications due to probiotics is extremely low. However, prolonged safety issues have not been addressed in studies.

CONCLUSION

In summary, the present meta-analyses suggest that probiotics can significantly reduce the incidence of AAD and are an effective treatment for CDD. Future studies should expand the types of probiotics tested and pay careful attention to proper study design and sample size considerations.

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STUDY HIGHLIGHTS

What Is Current Knowledge

- Treatment strategies for diseases that involve the disruption of intestinal microflora by antibiotics may be particularly effective when probiotics are used.
- Studies of the efficacy of probiotics in *C. difficile* colitis are conflicting and a consensus has yet to be reached.

What Is New Here

- This meta-analysis pooled together 31 randomized controlled trials to determine if probiotics are efficious overall for these types of diseases.
- Three different probiotics (Saccharomyces boulardii, Lactobacillus rhamnosus GG and probiotic mixtures) helped prevent antibiotic-associated diarrhea but only S. boulardii appeared useful for *Clostridium difficile* disease.

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