REVIEW | Annals of Internal Medicine

# Probiotics for the Prevention of *Clostridium difficile*–Associated Diarrhea

## A Systematic Review and Meta-analysis

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**Background:** Antibiotic treatment may disturb the resistance of gastrointestinal flora to colonization. This may result in complications, the most serious of which is *Clostridium difficile*–associated diarrhea (CDAD).

**Purpose:** To assess the efficacy and safety of probiotics for the prevention of CDAD in adults and children receiving antibiotics.

**Data Sources:** Cochrane Central Register of Controlled Trials, MEDLINE, EMBASE, CINAHL, Allied and Complementary Medicine Database, Web of Science, and 12 gray-literature sources.

**Study Selection:** Randomized, controlled trials including adult or pediatric patients receiving antibiotics that compared any strain or dose of a specified probiotic with placebo or with no treatment control and reported the incidence of CDAD.

**Data Extraction:** Two reviewers independently screened potentially eligible articles; extracted data on populations, interventions, and outcomes; and assessed risk of bias. The Grading of Recommendations Assessment, Development and Evaluation guidelines were used to independently rate overall confidence in effect estimates for each outcome.

**Data Synthesis:** Twenty trials including 3818 participants met the eligibility criteria. Probiotics reduced the incidence of CDAD by 66% (pooled relative risk, 0.34 [95% CI, 0.24 to 0.49]; $I^2 = 0\%$). In a population with a 5% incidence of antibiotic-associated CDAD (median control group risk), probiotic prophylaxis would prevent 33 episodes (CI, 25 to 38 episodes) per 1000 persons. Of probiotic-treated patients, 9.3% experienced adverse events, compared with 12.6% of control patients (relative risk, 0.82 [CI, 0.65 to 1.05]; $I^2 = 17\%$).

**Limitations:** In 13 trials, data on CDAD were missing for 5% to 45% of patients. The results were robust to worst-plausible assumptions regarding event rates in studies with missing outcome data.

**Conclusion:** Moderate-quality evidence suggests that probiotic prophylaxis results in a large reduction in CDAD without an increase in clinically important adverse events.

**Primary Funding Source:** None.

*Ann Intern Med.* 2012;157:878-888. www.annals.org
For author affiliations, see end of text.
This article was published at www.annals.org on 13 November 2012.

Antibiotics are among the most prescribed medications worldwide. Antibiotic treatment may disturb the colonization resistance of gastrointestinal flora, resulting in a range of symptoms—most notably, antibiotic-associated diarrhea. *Clostridium difficile* is the pathogen most often associated with opportunistic proliferation after breakdown of colonization resistance due to antibiotic administration. The spectrum of *C. difficile*–related disease ranges from asymptomatic intestinal colonization to diarrhea, colitis, pseudomembranous colitis, and death (1).

*Clostridium difficile*–associated diarrhea (CDAD) occurs most often in older, hospitalized adults who are exposed to broad-spectrum antibiotics; approximately one third of cases of antibiotic-associated diarrhea can be attributed to *C. difficile* (2, 3). In high-income countries, CDAD is the most common cause of hospital-acquired infectious diarrhea (4–6), and more than 300 000 hospitalized patients in the United States are affected each year (7–9).

*See also:*

**Web-Only**
CME quiz (preview on page I-34)

Beginning in 2002, hospitals in several high-income countries have experienced a dramatic rise in both the incidence and severity of CDAD (10–14). Reports from the United States have demonstrated an almost 2-fold increase in the fatality rate attributable to CDAD (9). A Canadian study of 136 877 hospital admissions showed that regardless of baseline factors, 1 of every 10 patients who acquire *C. difficile* will die (15).

Probiotics are microorganisms that are believed to counteract disturbances in intestinal flora and thereby reduce the risk for colonization by pathogenic bacteria (16). The rationale for probiotic administration includes reinoculation of disturbed indigenous microflora secondary to antibiotic use and inhibition of pathogen adhesion, colonization, and invasion of the gastrointestinal mucosa (17, 18). Probiotics are becoming increasingly available as capsules and dairy-based food supplements sold in health food stores and supermarkets (19). If probiotics are effective, their low cost and low incidence of associated adverse events (20) would make them an attractive choice for the prevention of CDAD. We conducted a systematic review to determine the efficacy and safety of probiotics (any strain or dose) for the prevention of CDAD in adults and children receiving antibiotics.

## Methods

### Data Sources and Searches

In June 2012, we searched 6 primary databases, regardless of publication status or language: the Cochrane Central Register of Controlled Trials from the Cochrane Library (2012, Issue 6), MEDLINE (1966 to 2012), EMBASE (1980 to 2012), CINAHL (1982 to 2012), Allied and Complementary Medicine Database (1985 to 2012), and Web of Science (1945 to 2012). The search strategies are available upon request. Our gray-literature search included BIOSIS Previews, Canadian Agency for Drugs and Technology in Health, Dissertation Abstracts, Google Scholar, British Society of Gastroenterology Annual General Meeting, McGill University Technology Assessment Unit, IBD/FBD Group Specialized Register, the Turning Research Into Practice database, and HighWire.

We reviewed bibliographies of review articles and eligible trials for additional studies not identified by the electronic searches. We contacted companies that manufacture probiotic agents and individuals working in the field to identify additional unpublished or ongoing trials. The search for ongoing trials included ClinicalTrials.gov and the metaRegister of Controlled Trials.

### Study Selection

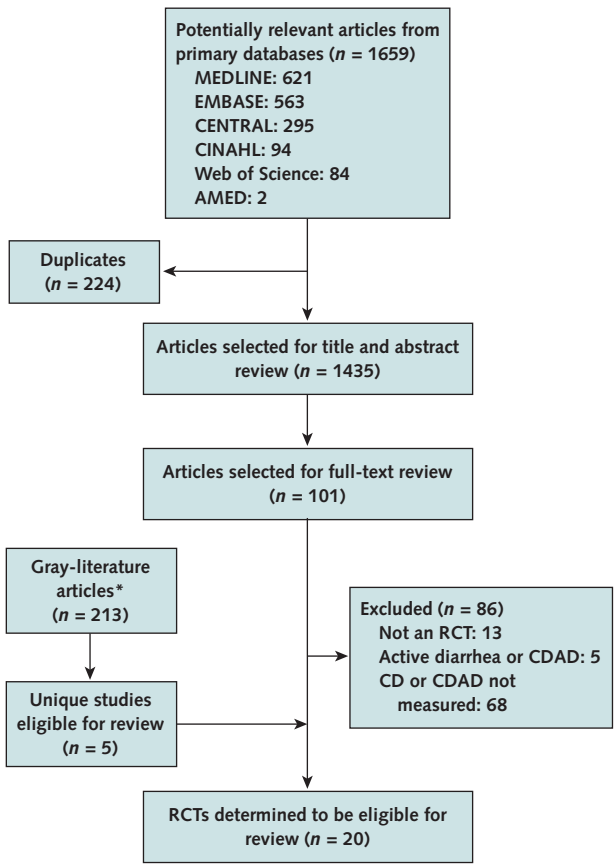
We included randomized, controlled trials in adult or pediatric patients treated with antibiotics that compared the effect of any dose of a specified probiotic of any strain with placebo or no treatment and reported the incidence of diarrhea with associated positive stool cytotoxin assay or culture for *C. difficile*. Two reviewers independently screened the titles and abstracts of articles. The full text of any title or abstract deemed potentially eligible by either reviewer was retrieved. Subsequently, 2 reviewers independently assessed the eligibility of each full-text article and resolved disagreements by consensus.

### Data Extraction and Quality Assessment

Two reviewers independently extracted data on patients, methods, interventions, outcomes, missing outcome data (for example, loss to follow-up), and results by using standardized, pretested, data extraction forms with accompanying instructions. For articles published in abstract form only, we sought further information from authors. We accepted the primary authors' definition of the presence or absence of CDAD.

Two reviewers independently assessed the risk of bias, including sequence generation, allocation concealment, blinding, number of patients with missing outcome data, selective outcome reporting, and other sources of bias (21). We also independently rated the overall quality of evidence (confidence in effect estimates) for each of the outcomes by using the Grading of Recommendations Assessment, Development and Evaluation (GRADE) approach, in which randomized trials begin as high-quality evidence but may be rated down by 1 or more of 5 categories of limitations: risk of bias, inconsistency, indirectness, imprecision, and reporting bias (22).

*Figure 1.* **Summary of evidence search and selection.**




AMED = Allied and Complementary Medicine Database; CD = *Clostridium difficile*; CDAD = *Clostridium difficile*–associated diarrhea; CENTRAL = Cochrane Central Register of Controlled Trials; RCT = randomized, controlled trial.

* Sources were BIOSIS Previews, Canadian Agency for Drugs and Technology in Health, Dissertation Abstracts, Google Scholar, British Society of Gastroenterology Annual General Meeting, McGill University Technology Assessment Unit, Inflammatory Bowel Disease/Functional Bowel Disease register, the Turning Research Into Practice database, HighWire, clinical trial registries industry contact, and bibliographies.

### Data Synthesis and Analysis

Data were analyzed by using the RevMan Analyses statistical package in Review Manager, version 5.1 (Cochrane Collaboration, Copenhagen, Denmark). Using the DerSimonian–Laird random-effects model, we calculated relative risks and 95% CIs. To test the precision of the estimate of effect, we calculated the optimal information size (OIS) using $\alpha$ (0.05) and $\beta$ (0.20) values, a relative risk reduction of 30% for both the CDAD analysis and adverse events analysis, and the median control group incidence (23). For calculating risk differences, we used the median control group risk estimate from the included studies.

*Table 1.* **Population, Intervention, and Follow-up in Included Trials**

| Study, Year (Reference) | Population | Treatment Group | | Control Group | |
|---|---|---|---|---|---|
| | | Participants, *n* | Mean Age (SD or Range) | Participants, *n* | Mean Age (SD or Range) |
| Arvola et al, 1999 (32) | 5 inpatients and 114 outpatients | 89 | 4.7 y (2 wk–11.8 y) | 78 | 4.4 y (2 wk–12.8 y) |
| Beausoleil et al, 2007 (33) | 89 inpatients | 44 | 68.8 y (14.5 y) | 45 | 72.9 y (13.4 y) |
| Bravo et al, 2008 (34) | 86 outpatients | 41 | 49.8 y (20.5 y) | 45 | 51.0 y (17.9 y) |
| Can et al, 2006 (35) | 151 inpatients | 73 | NS* | 78 | NS* |
| Duman et al, 2005 (36) | NS (14-d triple therapy for *Helicobacter pylori* eradication) | 204 | 45.7 y (12.7 y) | 185 | 44.7 y (13.9 y) |
| Gao et al, 2010 (37) | 255 inpatients | 171 | 60.0 y (6.0 y) | 84 | 60.0 y (6.0 y) |
| Hickson et al, 2007 (38) | 135 inpatients | 69 | 73.7 y (11.1 y) | 66 | 73.9 y (10.5 y) |
| Kotowska et al, 2005 (39) | 72 inpatients and 197 outpatients (total, 269) | 132 | 4.9 y (6.2 mo–14.8 y) | 137 | 4.7 y (5.2 mo–15.2 y) |
| Lönnermark et al, 2010 (40) | 137 inpatients and 102 outpatients (total, 239) | 118 | 47 y | 121 | 43 y |
| McFarland et al, 1995 (41) | 193 inpatients | 97 | 40.7 y (16.0 y) | 96 | 42.3 y (17.7 y) |
| Miller et al, 2008 (47)† | 189 inpatients | 95 | ≥18 y | 94 | ≥18 y |
| Miller et al, 2008 (47)†‡ | 316 inpatients | 157 | ≥18 y | 159 | ≥18 y |
| Plummer et al, 2004 (42) | 138 inpatients | 69 | Elderly | 69 | Elderly |
| Psaradellis et al, 2010 (48)† | 248 inpatients and 189 outpatients (total, 437) | 233 | 59.5 y (18.1 y)§ | 239 | 58.1 y (19.1 y)§ |
| Rafiq et al, 2007 (49)† | 100 inpatients | 45 | NS | 55 | NS |
| Ruszczyński et al, 2008 (43) | 134 inpatients and 106 outpatients (total, 240) | 120 | 4.5 y (3.7 y) | 120 | 4.6 y (3.8 y) |
| Safdar et al, 2008 (44) | 40 inpatients | 23 | 66.6 y (14.5 y) | 17 | 72.5 y (11.0 y) |
| Selinger et al, 2011 (50)† | 124 inpatients | 62 | NS | 62 | NS |
| Surawicz et al, 1989 (45) | 318 inpatients | 212 | 48.6 y§ | 106 | 45.4 y§ |
| Thomas et al, 2001 (46) | 302 inpatients | 152 | 57.2 y (18.0 y)§ | 150 | 54.4 y (17.4 y)§ |

CFU = colony-forming units; NS = not specified; Pro1 = probiotic group 1; Pro2 = probiotic group 2.
* Adult population (eligibility criteria, 25–50 y).
† Abstract.
‡ Miller and colleagues reported on 2 studies; the dosage of probiotic used was 3 times greater than in the study above.
§ For complete cases.

Heterogeneity was investigated by using the chi-square test and $I^2$ statistic (24). A priori, we specified the following possible explanations for heterogeneity: age (adult vs. pediatric) with a postulated larger effect in adults; dosage of probiotic (≤10 billion vs. >10 billion colony-forming units of bacteria or yeast per day), with a postulated larger effect in trials administering a larger dose (25); probiotic species when 2 or more trials administered the same species, with an expected larger effect in trials of *Lactobacillus rhamnosus* or *Saccharomyces boulardii* (25); and risk of bias, with an expected larger effect in trials at high or unclear risk of bias versus trials at low risk of bias. In addition, a reviewer suggested the effect may be larger in trials administering multiple versus single species.

For each subgroup analysis, we tested for interaction by using a chi-square significance test (26). For subgroups with more than 2 variables, we performed random-effects meta-regression of odds ratios by using the statistical package R, version 2.14 (University of Vienna, Vienna, Austria). For trials with cells with zero events, a continuity correction of 0.5 was used for our primary analysis. Using a trial sequential analysis software (27), we conducted sensitivity analyses with and without double-zero cell trials, using 3 adjustments: continuity correction of 0.5, continuity correction of 0.005, and inverse sample size continuity correction. For each subgroup, we applied published criteria to evaluate the credibility of subgroup effects (28).

*Table 1*—Continued

| Probiotic | Control (Risk for CDAD, %) | Duration of Follow-up |
|---|---|---|
| *L. rhamnosus* GG 53103, 40 × $10^9$ CFU/d for the duration of the antibiotic course | Placebo (2) | 3 mo after first antibiotic dose |
| *L. acidophilus* CL1285 and *L. casei,* 25 × $10^9$ CFU/d for 2 d, then 50 × $10^9$ CFU/d for the duration of the antibiotic course | Placebo (16) | 21 d after last study drug dose |
| *S. boulardii,* 10.2 × $10^9$ CFU/d for 12 d; duration of antibiotic course, 5–10 d | Placebo (0) | 9 d after last study drug dose |
| *S. boulardii* lyophilized 20 × $10^9$ CFU/d ≤ 48 h of antibiotic start dose (duration of study drug course NS) | Placebo (3) | 4 wk after last antibiotic dose |
| *S. boulardii,* 30 × $10^9$ CFU/d for 14 d (duration of antibiotic course 14 d) | No treatment (1) | 4 wk after last study drug dose |
| Pro1: *L. acidophilus* CL1285 and *L. casei* LBC80R, 50 × $10^9$ CFU/d<br>Pro2: *L. acidophilus* CL1285 and *L. casei* LBC80R, 100 × $10^9$ CFU/d, within 36 h of starting antibiotic therapy until 5 d after discontinuation | Placebo (24) | 21 d after last study drug dose |
| *L. casei immunitas* DN-114 001, 19 × $10^9$ CFU/d; *L. bulgaricus,* 1.9 × $10^9$ CFU/d; and *S. thermophilus,* 19 × $10^9$ CFU/d within 48 h of starting antibiotic therapy until 7 d after discontinuation | Placebo (17) | 4 wk after last antibiotic or study drug dose |
| *S. boulardii,* 10 × $10^9$ CFU/d for the duration of the antibiotic course | Placebo (8) | 2 wk after last study drug dose |
| *L. plantarum* 299v, 10 × $10^9$ CFU/d, within 48 h of starting antibiotic therapy until 7 d after discontinuation | Placebo (0) | ≥1 wk after last study drug dose |
| *S. boulardii* lyophilized, 30 × $10^9$ CFU/d within 72 h of starting antibiotic therapy until 3 d after discontinuation | Placebo (4) | 7 wk after last study drug dose |
| *L. rhamnosus* GG, 40 × $10^9$ CFU/d within 72 h of starting antibiotic therapy, then for 14 d (duration of antibiotic course ≤14 d) | Placebo (7) | 30 d after last study drug dose |
| *L. rhamnosus* GG, 120 × $10^9$ CFU/d within 72 h of starting antibiotic therapy, then for 14 d (duration of antibiotic course ≤14 d) | Placebo (0) | 30 d after last study drug dose |
| *L. acidophilus* and *B. bifidum,* 20 × $10^9$ CFU/d within 36 h of starting antibiotic therapy, then for 20 d | Placebo (7) | Last day of study drug dose |
| *L. acidophilus* CL1285 and *L. casei,* 25 × $10^9$ CFU/d for 2 d then 50 × $10^9$ CFU/d until 5 d after discontinuation of antibiotic | Placebo (2) | 21 d after last study drug dose |
| *L. acidophilus,* 80%; *L. bulgaricus,* 10%; *B. bifidum,* 5%, and *S. thermophilus,* 5%, 3 g/d with start of antibiotic therapy until hospital discharge | NS (40) | NS |
| *L. rhamnosus* GG (2593, 2594, 2595), 2 × $10^{10}$ CFU/d for the duration of the antibiotic course | Placebo (6) | 2 wk after last study drug dose |
| *L. acidophilus,* 60 × $10^9$ CFU/d during and 14 d after antibiotic course | Placebo (6) | NS |
| VSL #3 (*B. breve, B. longum, B. infantis, L. acidophilus, L. plantarum, L. paracasei, L. bulgaricus, S. thermophilus*), 900 × $10^9$ CFU/d during and 7 d after the antibiotic course | Placebo (0) | 21 d after last study drug dose |
| *S. boulardii* lyophilized, 20 × $10^9$ CFU/d within 48 h of starting antibiotic therapy until 2 wk after discontinuation | Placebo (8) | Mean, 17.3 d (SD, 8.6)‡ |
| *L. rhamnosus* GG, 20 × $10^9$ CFU/d within 24 h of starting antibiotic therapy, then for 14 d | Placebo (2) | 7 d after last study drug dose |

To explore the effect of missing outcome data, we collected information on all missing CDAD outcome data from the included studies, including missing *C. difficile* assays, and compared our primary complete case analysis for CDAD to a series of sensitivity analyses. For the sensitivity analyses, we assumed that the event rate was the same among control participants for whom data were missing and among those who were successfully followed. For the probiotic group, we calculated effects by using assumed ratios of event rate in persons with missing data compared with those who were successfully followed: 1.5:1, 2:1, 3:1, and 5:1 (29). We then determined whether the results withstood the range of assumptions, including the worst-plausible assumption (5:1).

To evaluate the potential for publication bias, we applied funnel plots, the rank correlation test (30), and weighted regression (31) to the main efficacy outcome, CDAD.

**Role of the Funding Source**

The study did not receive external funding.

## RESULTS

**Figure 1** shows the results of the literature search. Of 1659 studies identified from the primary electronic databases—621 from MEDLINE, 563 from EMBASE, 295 from the Cochrane Central Register of Controlled trials, 94 from CINAHL, 84 from Web of Science, and 2 from the Allied and Complementary Medicine Database—224 were duplicates, leaving 1435 abstracts or titles identified as original publications. Of these, 101 were potentially relevant for full-text review, and 15 were ultimately eligible (32–46). The gray-literature search identified 8 additional studies, 5 of which were eligible (47–50).

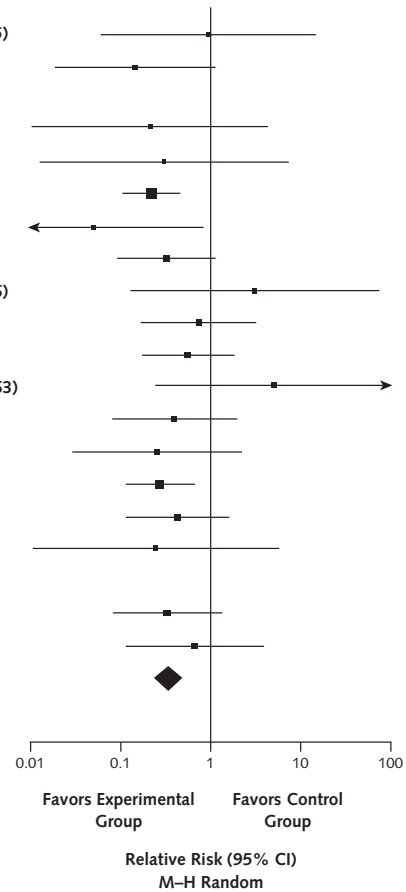
**Table 1** and the **Appendix Table** (available at www.annals.org) show the characteristics of included trials. The

*Figure 2.* **Probiotics for the prevention of *Clostridium difficile*–associated diarrhea.**

| Study, Year (Reference) | Experimental Group, *n* | | Control Group, *n* | | Weight, % | Relative Risk (95% CI) M–H Random |
|---|---|---|---|---|---|---|
| | Events | Total | Events | Total | | |
| Arvola et al, 1999 (32) | 1 | 61 | 1 | 58 | 1.7 | 0.95 (0.06–14.85) |
| Beausoleil et al, 2007 (33) | 1 | 44 | 7 | 45 | 3.0 | 0.15 (0.02–1.14) |
| Bravo et al, 2008 (34) | 0 | 41 | 0 | 45 | – | Not estimable |
| Can et al, 2006 (35) | 0 | 73 | 2 | 78 | 1.4 | 0.21 (0.01–4.37) |
| Duman et al, 2005 (36) | 0 | 196 | 1 | 180 | 1.2 | 0.31 (0.01–7.47) |
| Gao et al, 2010 (37) | 9 | 171 | 20 | 84 | 23.0 | 0.22 (0.11–0.46) |
| Hickson et al, 2007 (38) | 0 | 56 | 9 | 53 | 1.6 | 0.05 (0.00–0.84) |
| Kotowska et al, 2005 (39) | 3 | 119 | 10 | 127 | 7.9 | 0.32 (0.09–1.14) |
| Lönnermark et al, 2010 (40) | 1 | 80 | 0 | 83 | 1.3 | 3.11 (0.13–75.26) |
| McFarland et al, 1995 (41) | 3 | 97 | 4 | 96 | 5.9 | 0.74 (0.17–3.23) |
| Miller et al, 2008 (47) | 4 | 95 | 7 | 94 | 8.9 | 0.57 (0.17–1.87) |
| Miller et al, 2008 (47)* | 2 | 157 | 0 | 159 | 1.4 | 5.06 (0.25–104.63) |
| Plummer et al, 2004 (42) | 2 | 69 | 5 | 69 | 4.9 | 0.40 (0.08–1.99) |
| Psaradellis et al, 2010 (48) | 1 | 216 | 4 | 221 | 2.7 | 0.26 (0.03–2.27) |
| Rafiq et al, 2007 (49) | 5 | 45 | 22 | 55 | 16.1 | 0.28 (0.11–0.67) |
| Ruszczyński et al, 2008 (43) | 3 | 120 | 7 | 120 | 7.2 | 0.43 (0.11–1.62) |
| Safdar et al, 2008 (44) | 0 | 23 | 1 | 17 | 1.3 | 0.25 (0.01–5.79) |
| Selinger et al, 2011 (50) | 0 | 62 | 0 | 62 | – | Not estimable |
| Surawicz et al, 1989 (45) | 3 | 116 | 5 | 64 | 6.5 | 0.33 (0.08–1.34) |
| Thomas et al, 2001 (46) | 2 | 133 | 3 | 134 | 4.0 | 0.67 (0.11–3.96) |
| Total (95% CI) | | 1974 | | 1844 | 100.0 | 0.34 (0.24–0.49) |
| Total events, *n* | 40 | | 108 | | | |

Heterogeneity: $\tau^2 = 0.00$; chi-square = 12.09; $P = 0.79$; $I^2 = 0\%$

Test for overall effect: $Z = 5.87$; $P < 0.001$




M–H = Mantel–Haenszel.

* Miller and colleagues reported on 2 studies; the dosage of probiotic used was 3 times greater than in the study above.

overall risk of bias was low in 7 studies and high or unclear in 13 studies. Ten of these 13 studies (32–34, 36, 41, 45, 47–49) were judged to have either unclear or high risk of bias because of allocation concealment and missing participant data (**Appendix Figure**, available at www.annals.org).

### Incidence of CDAD

*Clostridium difficile*–associated diarrhea is defined as an episode of diarrhea associated with a positive *C. difficile* culture or toxin (A or B) assay. To allow for the varying definitions of diarrhea in the original studies, data were included as a binary outcome on the basis of the primary authors' definition of the presence or absence of diarrhea. Of 20 studies (3818 participants) reporting on the incidence of CDAD, 18 were placebo-controlled (32–35, 37–48, 50); 1 provided no treatment to the control group (36); and 1, published in abstract form only, did not report the control group intervention (49). Control group risk varied from 0% to 40%. Probiotics were found to reduce CDAD (relative risk, 0.34 [95% CI, 0.24 to 0.49]; $I^2 = 0\%$; heterogeneity $P = 0.79$) (**Figure 2**).

In 13 of the 20 trials, data on CDAD were missing for 5% to 45% of patients. When we used the assumed plausible ratios of event rates in participants with missing data compared with those who were successfully followed (29), our results proved robust to all assumptions: Even when a 5:1 ratio of events in intervention group participants with missing data versus those with complete data was assumed, the effect was large and the CI was narrow (relative risk, 0.50 [CI, 0.34 to 0.76]; $I^2 = 28\%$; heterogeneity $P = 0.13$).

We calculated the OIS on the basis of a relative risk reduction of 30%. The OIS (5676 persons) was greater than the total sample size (3818 participants), whereas the number of events across trials was relatively low (148 events). We therefore rated the quality of evidence down for imprecision. We categorized the confidence in esti-

mates of the effect of probiotics on CDAD incidence as moderate; the true effect is likely to be close to the estimate of effect, but there is a possibility that it differs substantially.

### Incidence of Adverse Events

Of 17 studies reporting on adverse events, 4 reported no adverse events in either the treatment group or control group and 3 reported serious adverse events. However, more serious adverse events occurred in the control group in each trial, with investigators reporting that none of these events were related to the probiotic intervention (47, 48). The most common adverse events were abdominal cramping, nausea, fever, soft stools, flatulence, and taste disturbance. The incidence of adverse events was 9.3% (range, 0% to 47.7%) in the probiotic group versus 12.6% (range, 0% to 44.7%) in the control group (relative risk, 0.82 [CI, 0.65 to 1.05]; $I^2 = 18\%$; heterogeneity $P = 0.28$) (**Figure 3**). Zero-cell sensitivity analyses generally yielded a borderline significant relative risk estimate (relative risk, 0.85 [CI, 0.72 to 1.00]) favoring probiotics.

Using standard $\alpha$ (0.05) and $\beta$ (0.20) values, we calculated the OIS on the basis of a relative risk increase of 30%. Although the OIS (5686 persons) was greater than the total sample size (3421 participants), the number of events among trials was high (374 events), and the CI almost excluded an increase in adverse events. However, only 17 of 20 trials reported on adverse events, an outcome that would presumably be documented in all probiotics trials, and so we rated the quality of evidence down for selective reporting bias (51). For the short-term use of probiotics in patients who are not immunocompromised or severely debilitated, we categorized the confidence in the assessment of adverse events as moderate (further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate) (**Table 2**).

### Subgroup Analyses

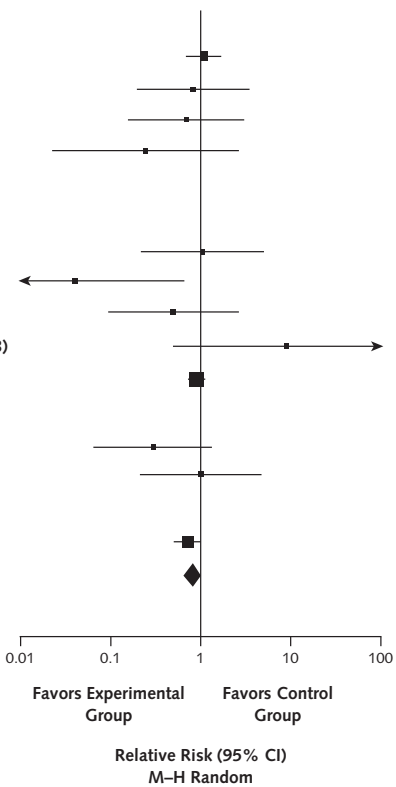
Results of subgroup analyses were similar in adults and children, with lower and higher doses, with different probiotic species (for example, *S. boulardii* vs. *L. rhamnosus*), and in studies at higher and lower risk of bias. The subgroup hypothesis evaluating single versus multiple species suggested a possible increased effect with multiple species (interaction $P = 0.06$) (**Figure 4**).

*Figure 3.* **Risk for adverse effects with probiotics.**

| Study, Year (Reference) | Experimental Group, *n* | | Control Group, *n* | | Weight, % | Relative Risk (95% CI) M–H Random |
|---|---|---|---|---|---|---|
| | Events | Total | Events | Total | | |
| Arvola et al, 1999 (32) | 0 | 61 | 0 | 58 | – | Not estimable |
| Beausoleil et al, 2007 (33) | 21 | 44 | 20 | 45 | 18.9 | 1.07 (0.68–1.68) |
| Bravo et al, 2008 (34) | 3 | 41 | 4 | 45 | 2.6 | 0.82 (0.20–3.46) |
| Duman et al, 2005 (36) | 3 | 196 | 4 | 180 | 2.5 | 0.69 (0.16–3.04) |
| Gao et al, 2010 (37) | 1 | 171 | 2 | 84 | 1.0 | 0.25 (0.02–2.67) |
| Hickson et al, 2007 (38) | 0 | 56 | 0 | 53 | – | Not estimable |
| Kotowska et al, 2005 (39) | 0 | 119 | 0 | 127 | – | Not estimable |
| Lönnermark et al, 2010 (40) | 3 | 80 | 3 | 83 | 2.2 | 1.04 (0.22–4.99) |
| McFarland et al, 1995 (41) | 0 | 93 | 12 | 92 | 0.7 | 0.04 (0.00–0.66) |
| Miller et al, 2008 (47) | 2 | 95 | 4 | 94 | 2.0 | 0.49 (0.09–2.64) |
| Miller et al, 2008 (47)* | 4 | 157 | 0 | 159 | 0.7 | 9.11 (0.49–167.88) |
| Psaradellis et al, 2010 (48) | 87 | 216 | 99 | 221 | 38.8 | 0.90 (0.72–1.12) |
| Ruszczyński et al, 2008 (43) | 0 | 120 | 0 | 120 | – | Not estimable |
| Safdar et al, 2008 (44) | 2 | 23 | 5 | 17 | 2.4 | 0.30 (0.06–1.35) |
| Selinger et al, 2011 (50) | 3 | 62 | 3 | 62 | 2.2 | 1.00 (0.21–4.76) |
| Surawicz et al, 1989 (45) | 0 | 116 | 0 | 64 | – | Not estimable |
| Thomas et al, 2001 (46) | 37 | 133 | 52 | 134 | 26.0 | 0.72 (0.51–1.01) |
| Total (95% CI) | | 1783 | | 1638 | 100.0 | 0.82 (0.65–1.05) |
| Total events, *n* | 166 | | 208 | | | |

Heterogeneity: $\tau^2 = 0.03$; chi-square = 13.27; $P = 0.28$; $I^2 = 17\%$

Test for overall effect: $Z = 1.58$; $P = 0.11$




M–H = Mantel–Haenszel.

* Miller and colleagues reported on 2 studies; the dosage of probiotic used was 3 times greater than in the study above.

*Table 2.* **Probiotics to Prevent *Clostridium difficile*–Associated Diarrhea and Quality of the Evidence***

| Outcome | Assumed Risk: Control Group† | Corresponding Risk: Probiotic Group (95% CI)‡ | RR (95% CI) | Participants (Studies) | Quality of the Evidence§ | Comments |
|---|---|---|---|---|---|---|
| Incidence of CDAD (complete case)<br>Diarrhea as defined by authors + cytotoxin assay or culture<br>Follow-up: end of antibiotic treatment to 3 mo after antibiotic therapy was discontinued | **Study Population** | | 0.34 (0.24–0.49) | 3818 (20) | Moderate | Studies with low risk of bias (7/20) demonstrated a slightly more favorable protective effect than studies at high or unclear risk of bias (13/30). A test for subgroup differences did not find a statistically significant difference based on risk of bias ($P = 0.24$).<br>In 13 of 20 trials, data on CDAD were missing for 5% to 45% of participants. A sensitivity analysis using plausible and worst-plausible ratios of event rates in those with missing data compared with those who were successfully followed demonstrated that the CDAD results were robust to all assumptions (worst-plausible analysis RR, 0.50 [95% CI, 0.34 to 0.76]).<br>Effect sizes were consistent across all 20 studies ($I^2 = 0\%$; $P = 0.79$). The outcome assessed in all 20 studies was the outcome of interest for our health question.<br>Using standard $\alpha$ (0.05) and $\beta$ (0.20) values, for a RRR of 30%, the OIS (5676 persons) was greater than the total sample size (3818 persons), and the overall events totaled less than 150.<br>Funnel plot inspection, the Begg and Mazumdar rank correlation test ($P = 0.79$), and the Egger regression test ($P = 0.16$) did not suggest publication bias or other small-study effects. |
| | 59 per 1000 persons | 20 per 1000 persons (14–29) | | | | |
| | **Moderate‖** | | | | | |
| | 50 per 1000 persons | 17 per 1000 persons (12–25) | | | | |
| Adverse events (complete case), as reported by patients | **Study Population** | | 0.82 (0.65–1.05) | 3421 (17) | Moderate | Test for risk of bias in subgroup differences was not statistically significant ($P = 0.76$).<br>Minimal heterogeneity among trials ($I^2 = 17\%$; $P = 0.28$).<br>Outcome assessed in these 17 studies was the outcome of interest for our health question.<br>Using standard $\alpha$ (0.05) and $\beta$ (0.20) values, we calculated the OIS based on a relative risk increase of 30. The OIS (5686 persons) was greater than the total sample size (3421 participants). However, the number of events was relatively high (374), and the CI virtually excluded an increase in adverse events.<br>Funnel plot inspection, the Begg and Mazumdar rank correlation test ($P = 0.68$), and the Egger regression test ($P = 0.51$) did not suggest publication bias or other small-study effects. Included studies have risk of bias regarding documentation and reporting of adverse events.<br>Adverse events are an outcome of interest for all probiotics, yet only 17 of 20 included trials reported on adverse events, suggesting a selective reporting bias. The studies differed considerably in how adverse events were classified, and few studies stated their methods for classifying such events. Differences in classification may have overestimated the adverse events in the control groups. For example, in 3 studies, serious adverse events (all documented to be more common in the control group) were included that were not deemed to be related to the study product, whereas in other studies, symptoms (e.g., fever, abdominal cramping) classified as adverse events may have been due to conditions prevented by probiotics (e.g., *C. difficile* occurring in the control group rather than in the probiotic group). |
| | 129 per 1000 persons | 106 per 1000 persons (84–135) | | | | |
| | **Moderate‖** | | | | | |
| | 36 per 1000 persons | 30 per 1000 persons (23–37) | | | | |

CDAD = *Clostridium difficile*–associated diarrhea; OIS = optimal information size; RR = relative risk; RRR = relative risk reduction.
* The patient or population was adults and children given antibiotics; the settings were inpatients and outpatients; and the intervention was probiotics.
† Based on the mean control group risk from all included trials.
‡ Based on the assumed risk in the comparison group and the relative effect of the intervention (95% CI).
§ Grading of Recommendations Assessment, Development and Evaluation Working Group grades of evidence are as follows. High quality = further research is very unlikely to change our confidence in the estimate of effect; moderate quality = further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate; low quality = further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate; and very low quality = we are very uncertain about the estimate.
‖ Based on the median control group risk from all included trials.

### Publication Bias

We found no graphical or statistical evidence of publication bias (figures available upon request).

## DISCUSSION

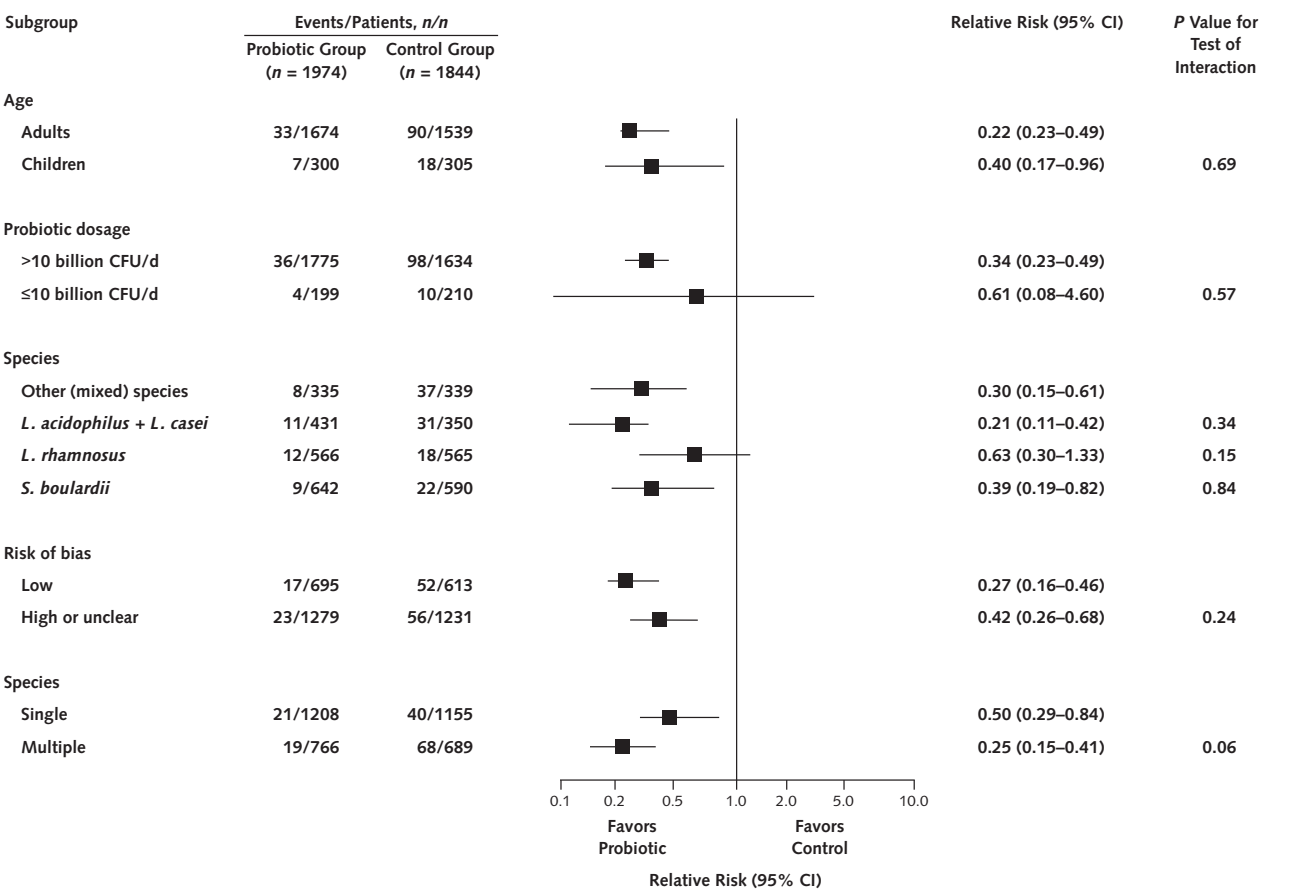
We found that 20 randomized trials testing the effect of probiotics (*Bifidobacterium*, *Lactobacillus*, *Saccharomyces*, or *Streptococcus* species) in patients receiving antibiotics showed a large relative risk reduction in the incidence of CDAD (relative risk, 0.34 [CI, 0.24 to 0.49]). Of the 20 trials, 19 were blinded (**Figure**), and results were robust to sensitivity analyses of worst-plausible-case assumptions regarding missing outcome data. Our judgment is that the evidence warrants moderate confidence in this large relative risk reduction (**Table 2**).

Results were similar in adults and children, with lower and higher doses, among trials administering similar probiotic species (for example, *S. boulardii* vs. *L. rhamnosus*), and in studies at higher and lower risk of bias (**Figure 4**). Trials using multiple species showed larger effects (relative risk, 0.25 [CI, 0.15 to 0.41]) than those using a single species (relative risk, 0.50 [CI, 0.29 to 0.84]). The subgroup effect was one of a small number of a priori hypotheses with a specified direction, and the test for interaction suggested a low likelihood that chance explains the apparent effect ($P = 0.06$) (**Figure 4**). However, the comparison is between-study rather than within-study; the interaction was not consistent across studies; and although it could be argued that multiple organisms may be more effective than single organisms, we are not aware of external evidence involving probiotics and CDAD to support this hypothesis. The hypothesis is sufficiently credible that it should be addressed in future studies (28).

Of 17 trials reporting adverse events, none reported a serious adverse event deemed attributable to probiotics, with the pooled estimate virtually excluding any increase in adverse events (relative risk, 0.82 [CI, 0.65 to 1.04]; $I^2 = 17\%$). We consider these results to warrant moderate confidence that short-term probiotic use in persons who are not immunodeficient or severely debilitated does not result in important side effects (20).

The most recent previous systematic review and meta-analysis of probiotics (52) addressed the treatment and prevention of antibiotic-associated diarrhea and included a

*Figure 4.* **Effect of probiotics on prevention of *Clostridium difficile*–associated diarrhea among subgroups.**




CFU = colony-forming units.

meta-analysis of probiotics for the prevention of CDAD. Among 14 included randomized, controlled trials, the pooled estimate of effect reported as a relative risk was 0.29, which is very close to ours. We conducted a comprehensive search particular to CDAD and identified an additional 6 randomized, controlled trials for inclusion, thus lowering the risk for publication bias while increasing the precision of the results and further increasing confidence in the estimate. We also systematically addressed the safety of probiotics in a meta-analysis of adverse events reported among 17 of 20 included trials.

Another systematic review (53) included 5 randomized trials (41, 42, 45, 46, 54). We included 4 of these in the previous review (41, 42, 45, 46) and excluded 1 study (54) that did not specify how many participants in each group developed CDAD. In addition to these 4 studies, we included 16 trials that were published after the previous review (53).

Dendukuri and colleagues (53) chose not to conduct a meta-analysis because of variability in the probiotic agent, probiotic dose, and criteria for diagnosing CDAD (53). We began with the hypothesis that probiotic agents had similar effects and that variation in diagnostic criteria would result in random error but would not bias estimates of treatment effect. We examined variability in results and found it to be completely consistent with chance ($I^2 = 0\%$) in the effect of probiotics for the prevention of CDAD. Despite this low variability, we conducted subgroup analyses and found that the age of the enrolled population, probiotic dose, probiotic species, and risk of bias failed to explain the clinical heterogeneity that did exist (all interaction $P > 0.05$) (55).

Three recent systematic reviews have addressed the safety of probiotics (20, 56, 57). The most comprehensive of these reviews (20) searched 12 electronic databases and included all study designs involving humans. For short-term probiotic use compared with control group participants, results were consistent with our findings: 208 randomized, controlled trials showed no statistically significantly difference in the overall number of adverse events (relative risk, 1.00 [CI, 0.93 to 1.07]), including serious adverse events (relative risk, 1.06 [CI, 0.97 to 1.16]; 66 randomized, controlled trials were primarily based on *Lactobacillus* species) (20).

Our review has limitations. First, the studies demonstrated some inconsistency in CDAD diagnostic methods. As mentioned, however, in blinded studies this inconsistency would result in random error that, if anything, would make detection of treatment effects less likely. Furthermore, the consistency of the results across trials suggests that differing diagnostic methods did not influence results. Although only 7 of 20 studies were at low risk of bias, results were similar between studies with lower and higher risk of bias.

Second, although the CI around the pooled estimate of effect on CDAD is narrow, the total sample size across studies did not meet the OIS (5676 persons). We therefore rated the overall confidence in effect estimates as moderate.

Third, there is considerable variability in the control group risk for CDAD across studies (**Table 1**). The control group risk was very high in some included studies (for example, 24% [37] and 40% [49]), but it is possible that these trials were conducted during *C. difficile* outbreaks. The absolute magnitude of benefit from use of probiotics will depend on the risk in patients who do not receive prophylaxis.

Finally, of the 20 included trials, 13 excluded patients who were immunodeficient or who were receiving immunosuppressive therapy (33–35, 37–40, 43, 45, 46–48). Although results suggested that no important adverse effects occurred in the studied population, the possibility of serious adverse effects in severely debilitated or immunocompromised populations remains (20).

Our systematic review also has several strengths. First, our search strategy was comprehensive, and we identified and included 5 studies from the gray-literature search (47–50). Second, we used 3 available approaches and found no suggestion of publication bias.

Third, we conducted a stringent assessment of plausible assumptions regarding missing outcome data (29), an aspect of rigor that has seldom been applied in meta-analyses to date. Akl and colleagues (29) reviewed the relevant literature regarding event rates in persons who are difficult to follow versus those who were followed and suggested a sensitivity analysis approach to explore vulnerability to missing participant data. The approach involves assuming that the event incidence among participants with missing data is higher by a specific ratio relative to the observed event incidence among followed up participants, referred to as the risk incidence in those lost to follow-up versus those followed up ($RI_{LTFU/FU}$). We chose the $RI_{LTFU/FU}$ values of 1:1, 1.5:1, 2:1, 3:1 and 5:1 in the intervention group versus the control group by following Akl and colleagues' recommendations. We chose an upper limit of 5 for $RI_{LTFU/FU}$ because it is the highest ratio reported in the literature of the incidence of bad outcomes in persons with missing data (58).

Fourth, we assessed variability in results and conducted subgroup exploration using optimal analytical and interpretational approaches (22). Finally, we applied GRADE criteria to interpret results and concluded that there is moderate confidence that probiotics reduce CDAD without resulting in important adverse effects. Given a total sample size of more than 3000 patients and narrow CIs that suggest a large effect on CDAD, our rating down of confidence in estimates on the basis of not meeting OIS criteria represents a conservative interpretation of these results. The OIS represents the sample size that would be required for a single optimally powered study using a modest estimate of treatment effect. It is a safeguard against premature conclusions regarding adequate precision that may, as in this case, arise when the apparent effect is very

large. If the OIS is met, it is reassuring; if it is not, it is probably wise to lower confidence in estimates of effect for imprecision.

Moderate-quality evidence supports a large protective effect of probiotics in preventing CDAD. Given the low cost of probiotics and the moderate-quality evidence suggesting the absence of important adverse effects, there seems little reason not to encourage the use of probiotics in patients receiving antibiotics who are at appreciable risk for CDAD.

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**Acknowledgment:** The authors thank Jane Saxton for conducting the literature search.

**Grant Support:** Dr. Johnston was supported in part by a postdoctoral fellowship from the SickKids Foundation. Mr. Goldenberg is supported by grants BUCSR-Y2-005 and BUCSR-Y2-019 from the Bastyr Center for Student Research.

**Potential Conflicts of Interest:** Dr. Johnston: *Grants/grants pending (money to institution):* Biocodex. Mr. Goldenberg: *Grant:* Bastyr Center for Student Research; *Support for travel to meetings for the study or other purposes:* Bastyr Center for Student Research. Disclosures can also be viewed at www.acponline.org/authors/icmje/ConflictOfInterestForms.do?msNum=M12-1183.

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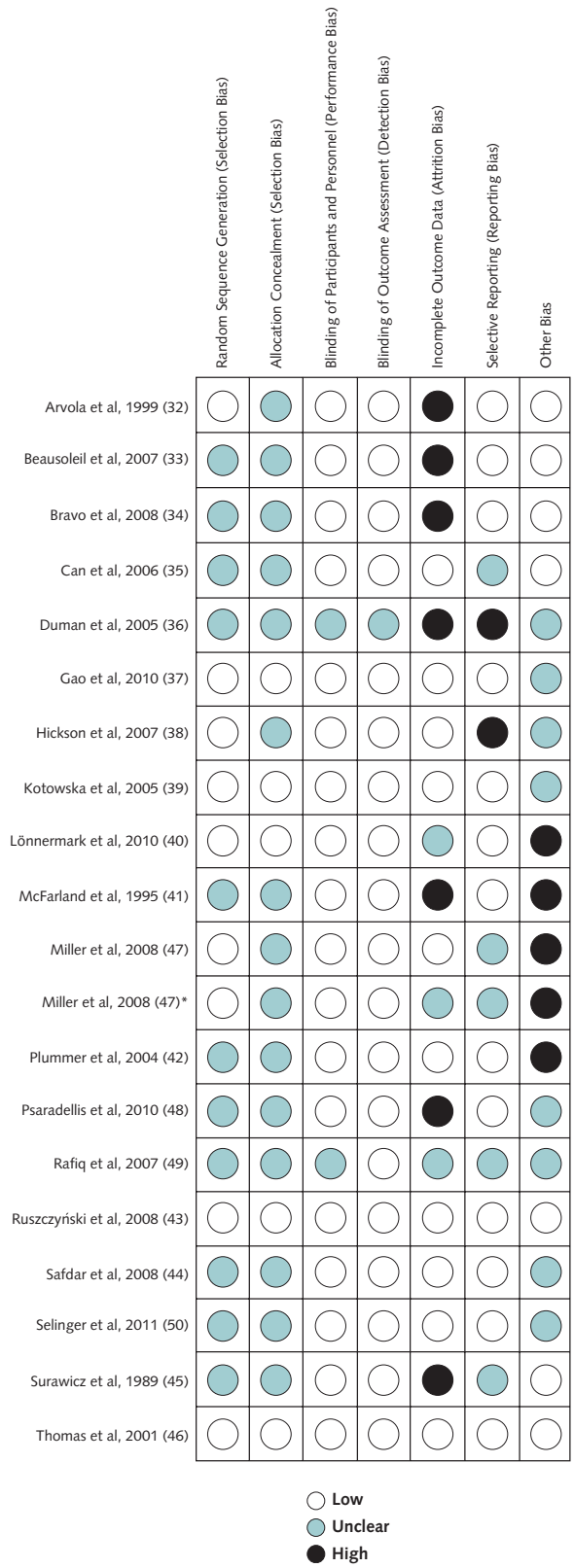
## *Appendix Table.* Antibiotics and *Clostridium difficile* History in Included Trials

| Study, Year (Reference) | Indications for Antibiotics | Antibiotics Administered | History of CDAD/CDI |
|---|---|---|---|
| Arvola et al, 1999 (32) | Otitis, tonsillitis, pneumonia, bronchitis, sinusitis | Penicillins, amoxicillin, cephalosporins, erythromycin, trimethoprim–sulfamethoxazole | NS |
| Beausoleil et al, 2007 (33) | Respiratory infection, other (urinary tract, skin, and soft-tissue infections) | β-lactams (includes penicillins and cephalosporins), macrolides, quinolones, other | Treatment group, 2/44 patients; control group, 4/45 patients (history of CDAD or AAD)<br>Excluded patients having CDI within the past 3 mo |
| Bravo et al, 2008 (34) | Respiratory infection, dental infection | Amoxicillin | NS |
| Can et al, 2006 (35) | NS | Combination including β-lactam versus not receiving β-lactam antibiotic | NS |
| Duman et al, 2005 (36) | *Helicobacter pylori* eradication | Clarithromycin + amoxicillin | History of AAD (treatment group, 18 cases; control group, 10 cases) |
| Gao et al, 2010 (37) | Various types of infections (NS) | Cephalosporins, penicillins, clindamycin | Excluded patients having CDI within the past 3 mo |
| Hickson et al, 2007 (38) | Respiratory infection, prophylaxis before/after surgery, urinary tract infection, other (cellulitis, ulcer, pressure sore, sepsis, sore throat, cholecystitis) | Metronidazole, aminoglycosides (gentamicin), tetracyclines (oxytetracycline), sulfonamides (trimethoprim), macrolides (azithromycin, clarithromycin, erythromycin), quinolones (ciprofloxacin), aminopenicillin (amoxicillin, benzylpenicillin, coamoxiclav, flucloxacillin), cephalosporins (cefalexin, ceftazidime, cefuroxime) | NS |
| Kotowska et al, 2005 (39) | Bronchitis, otitis media, pneumonia, tonsillitis, other | Cefuroxime axetil, amoxicillin + clavulanate, amoxicillin, intravenous cefuroxime, penicillin, clarithromycin, roxithromycin, other | NS |
| Lönnermark et al, 2010 (40) | Respiratory infection, skin or soft-tissue infection, urinary tract infection, septicemia/meningitis, viral infection, fever of unknown origin, other | Various types of antibiotics (including cephalosporins, clindamycin, ampicillin derivatives, quinolones) | NS |
| McFarland et al, 1995 (41) | NS | Single or multiple β-lactams (medium- to broad-spectrum penicillins, combination penicillins [penicillins with a β-lactamase inhibitor, or any cephalosporin]) with or without another antibiotic | NS |
| Miller et al, 2008 (47)* | NS | Cephalosporin, fluoroquinolone, β-lactam or carbapenem, intravenous vancomycin, metronidazole, macrolide, sulfa, aminoglycoside, nitrofurantoin, clindamycin | Excluded patients having CDI within the past 3 mo |
| Miller et al, 2008 (47)*† | NS | Cephalosporin, fluoroquinolone, β-lactam or carbapenem, intravenous vancomycin, metronidazole, macrolide, sulfa, aminoglycoside, nitrofurantoin, clindamycin | Excluded patients having CDI within the past 3 mo |
| Plummer et al, 2004 (42) | Acute emergencies (NS) requiring treatment with antibiotics | NS | NS |
| Psaradellis et al, 2010 (48)† | NS | NS | Treatment group, 3/216 patients; control group, 5/221 patients (history of CDAD or AAD)<br>Excluded patients having CDI within the past 3 mo |
| Rafiq et al, 2007 (49)* | NS | NS | NS |
| Ruszczyński et al, 2008 (43) | Respiratory infection, otitis media, urinary tract infection, other (lymphadenitis, osteomyelitis, skin infection, stomatitis, scarlet fever, erythema nodosum) | Penicillins, broad-spectrum penicillins (ampicillin, amoxicillin, amoxicillin plus clavulanate), cephalosporins, macrolides (clarithromycin, roxithromycin), clindamycin | NS |
| Safdar et al, 2008 (44) | Pulmonary infection, urinary tract infection, bacteremia, skin/soft-tissue infection, cholangitis | β-lactams, fluoroquinolones, macrolides | Excluded patients having CDI within the past 3 mo |
| Selinger et al, 2011 (50)* | NS | NS | NS |
| Surawicz et al, 1989 (45) | NS | Penicillins, clindamycin, trimethoprim–sulfamethoxazole, cephalosporins, others | NS |
| Thomas et al, 2001 (46) | Cellulitis, pneumonia, urinary tract infection, pyelonephritis, other (NS) | Penicillins, cephalosporins, carbapenems, fluoroquinolones, macrolides, aminoglycosides, glycopeptides, tetracycline, azoles | Excluded patients having CDI within the past 3 mo |

AAD = antibiotic-associated diarrhea; CDAD = *Clostridium difficile*–associated diarrhea; CDI = *Clostridium difficile* infection; NS = not specified.
* Abstract.
† Miller and colleagues reported on 2 studies; the dosage of probiotic used was 3 times greater than in the study above.

*Appendix Figure.* **Risk of bias of included trials.**




* Miller and colleagues reported on 2 studies; the dosage of probiotic used was 3 times greater than in the study above.