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Timely use of Probiotics in Hospitalized Adults Prevents *Clostridium difficile* Infection: a Systematic Review with Meta-Regression Analysis

Short title: Probiotics to prevent *C. difficile* Infection

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Abbreviations: CDI = *C. difficile* infection

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ABSTRACT

Background & Aims: Systematic reviews have provided evidence for the efficacy of probiotics in preventing *Clostridium difficile* infection (CDI), but guidelines do not recommend probiotic use for prevention of CDI. We performed an updated systematic review to help guide clinical practice.

Methods: We searched MEDLINE, EMBASE, International Journal of Probiotics and Prebiotics, and The Cochrane Library databases for randomized controlled trials evaluating use of probiotics and CDI in hospitalized adults taking antibiotics. Two reviewers independently extracted data and assessed risk of bias and overall quality of the evidence. Primary and secondary outcomes, respectively, were incidence of CDI and adverse events. Secondary analyses examined the effects of probiotic species, dose, timing, formulation, duration, and study quality.

Results: We analyzed data from 19 published studies, comprising 6261 subjects. The incidence of CDI in the probiotic cohort, 1.6% (54/3277), was lower than of controls, 3.9% (115/2984) (*P*<.001). The pooled relative risk of CDI in probiotic users was 0.42 (95% CI, 0.30-0.57; I^2=0.0%). Meta-regression analysis demonstrated that probiotics were significantly more effective if given closer to the first antibiotic dose, with a decrement in efficacy for every day of delay in starting probiotics (*P*=.04); probiotics given within 2 days of antibiotic initiation produced a greater reduction of risk for CDI (relative risk, 0.32, 95% CI, 0.22-0.48; I^2=0%) than later administration (relative risk 0.70, 95% CI, 0.40-1.23; I^2=0%) (*P*=.02). There was no increased risk for adverse events among patients given probiotics. The overall quality of the evidence was high.

Conclusions: In a systematic review with meta-regression analysis, we found evidence that administration of probiotics closer to the first dose of antibiotic

reduces the risk of CDI by more than 50% in hospitalized adults. Future research should focus on optimal probiotic dose, species, and formulation. Systematic Review Registration: PROSPERO CRD42015016395

Keywords: nosocomial infection; antibiotic associated diarrhea; meta-analysis; *lactobacillus*

INTRODUCTION

The incidence of *Clostridium difficile* infection (CDI) has more than doubled over the past ten years in the U.S., costing more than $4.8 billion dollars and causing significant morbidity and mortality, with more than 29,000 deaths in 2011.[1] Major independent risk factors for CDI include antibiotic use, hospitalization, increasing age, and co-morbidities.[2] Greater than 20% of patients with CDI experience initial treatment failure, and 40-60% have a second recurrence.[3] Recurrence rate and attributable mortality increase with advancing age,[1] and with more than 50% of hospitalized adults receiving antibiotics with increasing comorbidity burden, improved prevention of CDI would have substantial public health benefits.[4, 5]

It has been postulated that co-administration of probiotics with antibiotics can prevent CDI through non-specific and toxin-specific targets. Trillions of microbes compose the gastrointestinal microbiota working in a symbiotic relationship with the host's innate and adaptive immune response to prevent colonization of opportunistic bacteria. Antibiotic use disrupts this colonization resistance, creating intestinal dysbiosis that allows pathogens to cause intestinal infections.[6] Probiotics are oral preparations of live microorganisms that confer some health benefit to the host.[7, 8] Through direct and indirect actions, co-administration of probiotics could prevent CDI. *Lactobacillus* and *Bifidobacterium* species have been shown to colonize the intestine regardless of concurrent antibiotic use.[9] Evidence suggests that *Lactobacillus kefir* strains available in fermented milk produce S-layer proteins that antagonize *C. difficile* toxins.[10] In powder form, *Saccharomyces boulardii* makes a protease capable of digesting *C. difficile* toxins.[11] *Lactobacillus casei* improves mucosal immunity by increasing IgA levels.[12, 13]

Prior systematic reviews showed probiotic efficacy at preventing CDI in adults and children in hospitalized and community settings,[14-16] and in 2015, a modified Delphi panel of infectious disease experts unanimously recommended using *L. acidophilus* and *L. casei* concurrently with antibiotics to prevent CDI.[17] Yet current guidelines from the American College of Gastroenterology (ACG) and the Society for Healthcare Epidemiology of America (SHEA) do not recommend probiotics for primary prevention of CDI.[18, 19] Though the most recent systematic review included the recently-conducted, large, multi-center, randomized, United Kingdom trial,[16, 20] which was a negative study, that systematic review did not explicitly follow the PRISMA guidelines, include all eligible studies, evaluate for attrition bias, or interpret the results using the GRADE criteria. In addition, that review did not perform a meta-analysis on trials restricted to hospitalized adults taking antibiotics, which is the population at highest risk of CDI.

Given these contradictory recommendations for probiotic use, we conducted an updated systematic review with meta-analysis to evaluate probiotic efficacy in preventing CDI in the high-risk population of hospitalized adults taking antibiotics. Additionally, we performed meta-regression to identify reasons for the conflicting study results.

METHODS

This review is reported according to the recommendations from the Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) statement.[21]

Data Sources and Searches

Electronic searches

A search was performed to identify studies focused on the use of probiotics for the prevention of CDI in hospitalized adults receiving antibiotics. Potentially relevant articles were found by searching the biomedical electronic databases Ovid MEDLINE (1946 to April 22, 2016), the Cochrane Library (1992 to April 22, 2016), Ovid EMBASE (1974 to April 22, 2016), and the International Journal of Probiotics and Prebiotics (2006 to 2014). Relevant subject heading and free text terms were used. There was no restriction on publication language. Full details of the MEDLINE search strategy are presented in the supplementary material.

Searching for other resources

The International Journal of Probiotics and Prebiotics was searched for relevant articles using ProQuest Research Library database (2010-2014) and World Cat (2006-2009). References of included articles were also reviewed. In addition, we used the International Clinical Trials Registry Platform to identify unpublished and on-going trials. We attempted to contact the investigators of trials without published data, but we received no responses.

Study Selection

Types of studies

Randomized controlled trials (RCT) reporting CDI (diarrhea and either positive stool cytotoxin, culture, or polymerase chain reaction testing for *C. difficile*) were considered for inclusion.

Types of participants

Hospitalized adults (≥ 18 years) receiving antibiotic therapy (IV and/or oral) for any reason were included.

Types of interventions

Interventions using probiotics (any strain or dose) to prevent CDI. Studies investigating probiotics as a treatment for CDI were excluded. The control group intervention was either placebo or usual care (no probiotic).

Types of outcome measures

Primary outcome was incidence of CDI in each randomized group. Secondary outcomes were all other adverse events.

Data Extraction and Quality Assessment

Selection of studies

Two authors (NS, AM) independently reviewed all titles and abstracts. All journal articles chosen for full text review were evaluated independently by two authors (NS, AP) to assess inclusion criteria to consider for analysis. A third reviewer (AM) resolved differences.

Data extraction and management

Data were extracted independently by two authors (NS, KA) using a standardized form to record: numbers of experimental and control subjects, mean age, number of CDI events, probiotic species, dose, duration of therapy, timing relative to starting antibiotic, missing data, and follow-up. A third author (AM) resolved any discrepancies.

Risk of bias and quality assessment

All included trials were independently assessed for bias using the Cochrane Handbook for Systematic Review of Interventions by two authors (NS, KA), with disagreements resolved by a third author (AM).[22, 23] Bias was assessed on selection (randomization, allocation concealment), performance (blinding of participants and personnel), detection (blinding of outcome assessment), attrition (incomplete outcome data), reporting (selective reporting), and other (funding, etc). The overall quality of the evidence was evaluated using the GRADE system,[24] starting at high for RCTs and downgrading based on study limitations, inconsistency of results, indirectness of evidence, imprecision, and reporting bias. Results were also compared to the findings of prior meta-analyses.[14-16]

Data Synthesis and Analysis

Statistical analysis

Studies meeting the inclusion criteria were tabulated, and, if statistically and clinically appropriate, combined using the random effects meta-analysis method of DerSimonian and Laird to calculate a summary relative risk (RR) and 95% confidence interval (CI). For trials with more than one active treatment group, the higher potency group was combined with the lower potency group to form the treatment cohort. When conducting subgroup dose meta-regression, the control cohort was compared with each probiotic dose cohort. Pre-specified subgroup analyses were undertaken to evaluate whether the estimated effect was modified by probiotic species, dose, formulation, timing, and study quality.

Dealing with missing data

Some studies had missing data on the primary outcome. We assessed whether our results were sensitive to a range of assumptions about the missing data. For these sensitivity analyses, the incidence of CDI for missing data in the control group was assumed equal to the incidence in the non-missing control group data. We then assumed that the incidence of CDI in the missing experimental group data was higher—either two or five times higher—than the incidence in non-missing experimental group data.[25] We calculated the change in expected efficacy of probiotics (RR and 95% confidence interval) under these conservative assumptions about missing data. Data analysis was conducted using the statistical software Stata, version 14 (StataCorp LP, College Station, TX).

Assessment of heterogeneity

To assess heterogeneity, the I^2 statistic and the chi-squared test were computed.[22, 26] Pre-specified explanations for heterogeneity included: the risk of study bias (higher risk causing greater effect size); time of initiating probiotics (time closer to first antibiotic dose producing greater beneficial effect); probiotic dose (higher dose producing greater benefit); and probiotic species (greater effect with *S. boulardii* and *L. casei*).[15] The L'Abbé plot (control group incidence plotted on horizontal axis and intervention group incidence on vertical axis) allowed us to visually examine alternative measures of effect (relative risks, odds ratios, and risk differences) to determine which measure minimized statistical heterogeneity.

Assessment of publication bias

Funnel plots and Egger's regression were used to evaluate for publication bias.[27-29]

Meta-regression

We conducted a random-effects meta-regression in Stata to examine whether differences in treatment effect across studies could be explained by differences in study protocol (for example, maximum time allowed between starting antibiotics and starting probiotics).

RESULTS

Description of studies

Included studies

The systematic search identified 1647 studies, 19 of which (6,261 subjects) met inclusion criteria for meta-analysis (Figure 1). Among the 19 randomized trials, a total of 3,277 subjects were randomized to the probiotic intervention group and 2,984 subjects were randomized to placebo (except for two studies that used usual care—no probiotics—for their control group, rather than placebo).

The characteristics of the 19 included studies are described in Table 1 and Supplementary Table 1. Two studies listed twice in Table 1 compared different probiotic doses and were treated as different trials only when performing dose meta-regression; otherwise, they were analyzed as single trials.[30, 31] The weighted average age of subjects was 68 and 69 years in the experimental and control groups, respectively. Two trials, done separately by the same author, were treated as unique experiments.[32] Trials reported unique exclusion criteria. Commonly excluded subjects included those who were pregnant, were immunocompromised (neutropenia, HIV, malignancy undergoing chemotherapy or radiation, transplant patients on immunosuppression), required intensive care, had a prosthetic heart valve, or had a pre-existing gastrointestinal disorder (eg, inflammatory bowel disease, acute pancreatitis, or an ostomy). Not all trials specifically excluded daily probiotic users or those regularly eating yogurt. Some trials allowed inclusion of subjects with recent antibiotic use (within 2 weeks to 3 months of enrollment). Most trials accounted for comorbidities and proton pump inhibitor use. However, the

majority of trials did not account for narcotic, laxative, or anti-fungal use. Some trials allowed metronidazole to be the antibiotic administered.

Four probiotic species were studied (*Lactobacillus, Saccharomyces, Bifidobacterium,* and *Streptococcus*), either alone or in combination, for 12 unique probiotic formulations (Table 1). Five trials studied *S. boulardii* in capsule formulation,[33-37] three trials evaluated *L. rhamnosus GG* dispensed by capsule,[32, 38] one trial investigated *L. acidophilus* in capsule formulation,[39] and one trial used drink formulation of *L*. *casei* Shirota.[40] Two trials studied more than one strain of *Lactobacillus* administered by capsule.[30, 41] *Lactobacillus* was evaluated in combination with one or more species in 7 trials; specifically *Bifidobacterium* in four trials, three dispensed by capsule[20, 31, 42] and one by drink,[43] *Streptococcus* in one trial given as a drink,[44] and both *Bifidobacterium* and *Streptococcus* given by capsule[45] and by drink.[46] Doses ranged from 4-billion to 900-billion organisms per day. Median daily doses of *Saccharomyces, Lactobacillus,* and *Lactobacillus* in combination with another species, respectively, were 20 (range 10–36), 50 (range 7–120), and 30 (range 4–900) billion colony forming units (CFU).

Timing of administration of probiotics ranged from one to seven days after the first antibiotic dose. For *Saccharomyces, Lactobacillus,* and *Lactobacillus* in combination with another species, this ranged from 2 to 3 days, 1 to 3 days, and 1 to 7 days, respectively. Duration of probiotics was either fixed (14 to 21 days) or varied based on the antibiotic duration (extending 3 to 14 days after the last antibiotic dose). All *Saccharomyces* trials varied dosing in relationship to the antibiotic, 3 of the 7 *Lactobacillus* trials used fixed 14-day courses of probiotics,

and 3 of the 7 *Lactobacillus* in combination with another species used fixed courses ranging from 14 to 21 days.

Risk of bias in included studies:

The study characteristics related to study quality and risk of bias are described in Table 2. For seven studies, there was a high risk of bias because of missing data on the main outcome (attrition bias). There were five studies at high risk for other biases: lack of placebo[40, 45] and conflict of interest (e.g., relationship of the primary author with the probiotic manufacturer funding the study).[32, 42]

Effects of probiotic intervention

Incidence of CDI

The risk of CDI in the control group ranged from 0% to 40%, and the risk in the probiotic group ranged from 0% to 11%. The summary relative risk using a random effects model was 0.42 (95% CI: 0.30 to 0.57; *P*<.001) (Figure 2a). There was no evidence of significant heterogeneity of effect across the 19 studies (I^2=0.0%; *P*=.56). A meta-analysis updated sequentially with each published study (cumulative meta-analysis), shows the efficacy of probiotics (RR<0.5) becomes apparent beginning in 2007 (Figure 2b).

When alternative measures of effect were modeled (odds ratios or risk differences), the results were still statistically significant, although there was evidence for some heterogeneity, suggesting that relative risk is the preferred measure of effect (Supplementary Figure 1).

Because the median incidence of CDI in the control groups of the 19 studies was 4%, the relative risk for CDI of 0.42 can be translated into needing to treat 43 (95% CI 36 to 58) patients with probiotics to prevent one case of CDI. For CDI at the 25th percentile (1.2%), the number needed to treat to prevent one case of CDI was 144. For baseline incidence at the 75th percentile (7.4%), the number needed to treat was 23.

Sensitivity analysis for missing data

There were missing data on the main outcome in 10 of the 19 studies, ranging from 2% to 48% of subjects in the experimental groups and from 1% to 46% in the control groups. Among intervention subjects, even if we assumed that the incidence of CDI was five times higher in those with missing, compared to non-missing, data, the pooled relative risk remained significant, RR 0.60 (95% CI 0. 40 to 0.88; *P*=.009; I^2=36%) (Supplementary Figure 2b).

Subgroup analyses

Results of subgroup analyses are summarized in Table 3. The timing of probiotic administration was a significant predictor of CDI efficacy. Study protocols varied in the maximum time interval allowed from starting antibiotics to starting probiotics in the intervention group, from 1 day to 7 days. Eighteen of the 19 included studies initiated probiotics within 3 days of antibiotic use; only a single study (Allen et al)[20] allowed probiotics to be started 7 days after antibiotics. Not only was the Allen study unusual with respect to probiotic timing, it was also much larger than all other studies and its results were statistically insignificant. We therefore conducted a sensitivity analysis of the effect of timing of probiotics on efficacy by performing a

meta-regression with and without the Allen et al data. The meta-regression that included the Allen data demonstrated that probiotics were significantly more effective if given closer to the first antibiotic dose—the natural log of the odds ratio increased by 18% (a decrement in efficacy) for every day of delay in starting probiotics, *P*=.04) (Figure 3). When meta-regression was performed excluding data from Allen et al (which limited the range of probiotic timing from 1 to 3 days), the effect of a 1 day delay produced an even greater decrement in efficacy (coefficient increased from 0.18 to 0.54; *P*=.09). Thus, these analyses suggest that the decrement in efficacy with delay in starting probiotics is not sensitive to inclusion of a single large "outlier" study. In fact, inclusion only dampens the magnitude of the decrement in efficacy, although it is still clinically important and statistically significant.

Instead of assessing the timing of probiotic initiation as a continuous variable, we also simplified the analysis and dichotomized studies as those initiating probiotics within 2 days vs greater than 2 days (cutoff based on median of 2 days). This analysis showed a significant difference (*P*=.02) in RR for the two subgroups, with higher efficacy in studies that initiated probiotics within 2 days of starting antibiotics: RR 0.32 (95% CI: 0.22-0.48; I^2=0%) vs 0.70 (95% CI 0.40-1.23; I^2=0%) (Supplementary Figure 3).

Other subgroup analyses

Overall, there were no statistically significant differences in efficacy among studies evaluating unique probiotic formulations (*P*=.34) (Supplementary Figure 4a). However, those probiotic formulations with the most promising (but not convincing)

trends were *Lactobacillus* and *Lactobacillus* in combination with either *Streptococcus* or both *Streptococcus* and *Bifidobacterium* (Supplementary Figure 4b).[30, 41, 44, 45] A subgroup analysis of probiotic delivery via capsule or drink did not reach statistical significance (*P*=.28) (Supplementary Figure 5). A meta-regression to assess the effect of probiotic dose found no evidence for a difference in efficacy among the doses used in the 19 studies (*P*=.99) (Supplementary Figure 6). Probiotic efficacy also did not vary by study quality (*P*=.81) (Supplementary Figure 7).

Publication bias

Funnel plot and Egger's regression test showed no evidence of publication bias (Supplementary Figure 8).

Adverse effects

The incidence of adverse events in the 15 studies that collected these data were similar in the probiotic (14.2%; 433/3056) and control groups (15.9%; 439/2753), with a summary relative risk of 0.89 (95% CI: 0.69 to 1.14; *P*=.35; I^2=48%). The most common adverse events were cramping, nausea, fever, soft stools, flatulence, and taste disturbance (Supplementary Table 2). There were no cases of probiotic bacteremia or fungemia.

Quality of evidence

Using the GRADE system, the overall quality of evidence on the efficacy of probiotics for preventing CDI among hospitalized patients taking antibiotics was

high (Figure 4). The overall quality of evidence on the risk of adverse effects from probiotics was moderate (Figure 4).

CONCLUSIONS

The cumulative evidence from 19 randomized trials demonstrates the efficacy of probiotics in preventing CDI among hospitalized adults taking antibiotics, and we demonstrate for the first time, the importance of timely probiotic administration. Probiotics given within 2 days of the first antibiotic dose are more effective than if started later. However there was no convincing evidence of superior efficacy for any of the tested probiotic formulations, delivery methods (drink or capsule), or probiotic doses.

If a hospital's baseline incidence of CDI is in the range of 1.5% to 7.4% (the interquartile range among the 19 studies), then our results suggest that 1 case of CDI would be prevented for every 23 to 144 patients who are treated with probiotics when antibiotics are started. Given the evidence demonstrates a RR reduction greater than 50%, at an estimated annual rate of approximately 200 thousand infections among hospitalized adults in the U.S.,[1] probiotics might prevent over 100 thousand cases of CDI each year (95% CI: 86,000 to 140,000). Assuming that for each case of CDI the average cost will be at least $7,000,[47-49] the potential cost savings from using probiotics could approach over $500 million dollars annually.

Although probiotic efficacy was demonstrated in previous systematic reviews,[14-16] probiotic use has not been adopted as standard of care when prescribing antibiotics for hospitalized adults. One reason might be that the largest high-quality trial (PLACIDE)[20] concluded that there was "no evidence" that probiotics are effective in prevention of either antibiotic-associated diarrhea (RR 1.04; 95% CI

0.84 to 1.28; *P*=.71) or CDI (RR 0.71; 95% CI 0.34 to 1.47; *P*=.35). The study was initially powered to detect a 50% reduction in CDI, assuming an incidence of 4% in the control group. However, with an observed control group incidence of 1.2%, the post-hoc power was only 19%, consistent with the wide reported 95% confidence interval for the relative risk of CDI (0.34 to 1.47)[20], which not only included a RR of 0.53,[16] but also relative risks as small as 0.34.[14] The baseline incidence of CDI in PLACIDE was much lower than anticipated, in part due to not testing about 40% of cases of diarrhea for CDI. The PLACIDE study protocol specified starting the probiotic within seven days of starting antibiotics (the longest time interval among the 19 studies). This relaxed policy likely mitigated the therapeutic effect, since we demonstrate in the meta-regression that studies that required earlier administration of probiotics demonstrated significantly greater efficacy. The PLACIDE results are consistent with our summary RR and conclusion of efficacy—there was no statistical evidence for heterogeneity when PLACIDE was included with the other 18 trials (I^2 0.0%; *P*=.56), and there was substantial overlap between the PLACIDE confidence interval for RR (0.34 to 1.47) and the confidence interval for our random effects summary RR (0.30 to 0.57).

Limitations

Probiotic use was studied in a restricted patient population, limiting clinical applicability to non-pregnant, immune-competent hospitalized adults who do not have prosthetic heart valves and who are cared for outside of an intensive care unit**.** Although several studies excluded subjects with chronic gastrointestinal diseases because of possible confounding effects on stool output, other clinical trials have demonstrated the safety of using probiotics among patients with

inflammatory bowel disease.[50] There was significant clinical heterogeneity among studies in probiotic dose and species, but pooled estimates did not demonstrate significant statistical heterogeneity. Several studies had patients who were either lost to follow-up or had diarrhea not tested for CDI, and therefore had missing data on the main outcome variable. However, the finding of efficacy was robust, even when we assumed that in the missing data the incidence of CDI was much higher in the probiotic group than in the placebo group. For all studies with unclear or incomplete testing of diarrhea for CDI, patients and clinicians were blinded to group assignment, which should protect results from being biased. This is supported by the similar rates of testing for CDI among cases of diarrhea in both probiotic and placebo groups. If the sensitivity of detecting CDI is reduced because of missed testing, and if the reduction in sensitivity is similar between groups (because of design features such as blinding), then the incidence of CDI will be biased in each group, but the relative risk (ratio of CDI incidence in the two groups) will be unbiased and, therefore, remain unaffected. On the other hand, measures of risk difference will be biased (falsely too small), and the number-needed-to-treat will be falsely too large.

While half of the studies had moderate or high risk of bias, the magnitude of efficacy was preserved when only high quality trials were analyzed.

Strengths

A thorough literature search was conducted and prior systematic reviews with meta-analyses were critically reviewed to assure we incorporated all relevant studies, including those from the grey literature. There was no evidence of

publication bias, and there was little statistical heterogeneity in efficacy among the included studies.

Agreements and disagreements with other studies or reviews

The magnitude of effect found in our meta-analysis was similar to those reported in previous systematic reviews by Goldenberg et al in 2013 (RR 0.36; 95% CI 0.26 to 0.51; *P*<.001; I^2=0%), Johnston et al in 2012 (RR 0.34; 95% CI 0.24 to 0.49; *P*<.001; I^2=0%), and the review done by Lau et al in 2016 (RR 0.40; 95% CI 0.29 to 0.53; *P*<.001; I^2=0%).[14-16] Our estimated attrition bias was comparable to the 45% previously reported by Goldenberg and Johnston (Lau et al did not report attrition bias).[14-16] In our most conservative sensitivity analysis, in which the rate of CDI in subjects lost to follow up in the probiotics group was assumed to be five times the observed rate, probiotics were still effective, consistent with analogous assessments by Goldenberg (RR 0.57; 95% CI 0.38 to 0.85; I^2=33%) and Johnston (RR 0.50; 95% CI 0.34 to 0.76; I^2=28%) (sensitivity analysis was not performed by Lau et al).[14-16] Our analysis of adverse events demonstrated no significant difference between groups, which was comparable to the findings of Goldenberg (RR 0.80, 95% CI 0.68 to 0.95; I^2=37%) and Johnston (RR=0.82; 95% CI 0.65 to 1.05; I^2=17%) (meta-analysis of adverse events was not performed by Lau et al).[14-16] Prior systematic reviews rated the quality of evidence as moderate due to imprecision (Lau et al did not apply the GRADE criteria to interpret results).[14-16] In contrast, we rated the quality of evidence as high because the criterion for optimal information size (n=1,172 subjects in each group) was met with the inclusion of more recently published trials (Figure 4).[51]

Following the PRISMA guidelines and evaluating effects of attrition bias in sensitivity analysis, our analysis puts the results of PLACIDE—a large negative trial—into context, interpreting the results using the GRADE criteria for the first time. PLACIDE had little influence on the pooled magnitude of effect. Its unexpectedly low event rates and the allowance for probiotics to be started up to a week after the initiation of antibiotics, are likely responsible for the study's low power to detect a clinically important effect of probiotics.

We show for the first time the importance of probiotic timing. We focused our evaluation on the highest-risk population of hospitalized adults, while prior meta-analyses investigated the value of probiotics for different subgroups: inpatients versus outpatients (for combined adults and children) and for adults versus children (for combined inpatients and outpatients).

Implications for practice

Our study suggests that among hospitalized adults, probiotics reduce the risk of CDI by over 50% when taken concurrently with antibiotics within two days of the first antibiotic dose, with no evident increase in adverse events. Given the magnitude of benefit and the low cost of probiotics, the decision is likely to be highly cost effective. One cost-benefit analysis conducted in the United Kingdom evaluated the use of *L. paracasei* in hospitalized adults older than 65 years who were taking antibiotics and estimated a cost savings of over $500 per patient treated (£339).[52] Similarly, a recent study from Canada concluded that probiotic use would also be cost saving in the Canadian health system.[53]

Uncertainties about the optimal probiotic formulation, dose, duration, and commercial source prevent specific clear practical recommendations. Nevertheless, there is sufficient data to recommend higher doses of *Lactobacillus* or *Lactobacillus* in combination with another species, as either a drink or capsule, within 2 days of the first antibiotic dose for most hospitalized adults. To facilitate decision-making, we provide a table in the supplementary material that describes the different probiotics involved in the 19 studies, with their manufacturers (Supplementary Table 3). Clinicians should recall that these findings are restricted to non-pregnant, immune-competent hospitalized patients without prosthetic heart valves cared for outside of the intensive care unit. Clinical practice guidelines should be updated to reflect the evidence presented here.

Implications for research

Further research is necessary to clearly define the optimal probiotic species, dose, formulation, and duration of treatment. Cost-benefit analyses conducted in the United Kingdom and Canadian health care systems suggest that probiotic use is cost saving, but additional cost-benefit or cost-utility analyses should assess the value of probiotics for the range of incidence of CDI among hospitals in the United States.

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Author names in bold designate shared co-first authorship.

FIGURES:

Figure 1. Flow diagram. The flow diagram shows search results, studies screened, excluded, and reasons for exclusion or inclusion. There was 82% (36/44 selections) inter-rater agreement in selection of studies for full text review and 95% (18/19) in selection of included studies.

Figure 2a-b. Meta-analysis of the relative risk of Clostridium *difficile* infection (CDI) in hospitalized adults taking antibiotics and probiotics (experimental) vs no probiotics (control). Cumulative meta-analysis (b) suggests evidence of probiotic efficacy in 2007 with greater precision gained by the additional eight years and ten trials. Selinger et al[46] is not shown because no events occurred in either the probiotic or control cohort, making the risk ratio not estimable.

Figure 3: Subgroup meta-regression of timing of probiotic. Random-effects meta-regression of the study results (measured as the natural log of the study odds ratio) as a function of the maximum time allowed by study protocol from time antibiotics started to time of initiating probiotics (range among the 19 studies: 1–7 days). Each study was weighted by the standard error of its ln(OR). The results demonstrate a significant effect of the time variable: coefficient 0.18 (95% CI: 0.01 to 0.33; *P*=.04). This effect translates into an 18% increase in the OR (becoming closer to an OR=1) for each 1 day increase in the protocol allowed interval between starting antibiotics and starting probiotics. There was no evidence for heterogeneity across studies (tau2=0).

Figure 4: Assessment of each outcome of interest using the GRADE criteria. High quality of evidence was reported for the outcome of Clostridium *difficile* infection (CDI) and moderate quality of evidence for the outcome of adverse events.

Table 1. Characteristics of the 19 included randomized trials of probiotic use for preventing Clostridium *difficile* infection (CDI) among hospitalized adults taking antibiotics

Study	Place	Probiotic (formula)	Daily dose x 10^9 (CFU)	Trial follow-up (weeks)	Time from antibiotic to probiotic (days)	Probiotic duration (days)	Age, Experimental Group N	Age, Experimental Group Mean (SD or range)	Age, Control Group N	Age, Control Group Mean (SD or range)
Surawicz, 1989[33]	USA	*S. boulardii* (C)	20	2.5	2	AC + 14	116	49	64	45
McFarland, 1995[34]	USA	*S. boulardii* (C)	30	7[a]	3	AC + 3	97	41 (16)	96	42 (18)
Thomas, 2001[38]	USA	*L. rhamnosus GG* (C)	20	1[a]	1	14	133	57 (18)	134	54 (17)
Plummer, 2004[42]	UK	*L. acidophilus* + *B. bifidum* (C)	20	3	1.5	20	69	Elderly	69	Elderly
Can, 2006[35]	Turkey	*S. boulardii* (C)	20	4[a]	2	AC	73	(25-50)	78	(25-50)
Beausoleil, 2007[41]	Canada	*L. acidophilus* + *L. casei* (D)	50	3[a]	2	AC	44	69 (15)	45	73 (13)
Hickson, 2007[44]	UK	*L. casei* + *L. bulgaris* + *S. thermophilus* (D)	40	4[a]	2	AC + 7	57	74 (11)	56	74 (11)
Rafiq, 2007[45]	USA	*L. acidophilus* + *L. bulgaricus* + *B. bifidum* +*S. thermophilus* (C)	12	NS	1	NS	45	NS	55	NS
Wenus, 2008[43]	Norway	*L. rhamnosus GG* + *L. acidophilus* + *B. lactis* (D)	53	2	3	14	34	59 (17)	29	56 (19)
Safdar, 2008[39]	USA	*L. acidophilus* (C)	60	NS	1	AC + 14	23	67 (15)	17	73 (11)
Miller, 2008a[32]	Canada	*L. rhamnosus GG* (C)	40	NS	3	14	95	≥ 18	94	≥ 18
Miller, 2008b[32]	Canada	*L. rhamnosus GG* (C)	120	NS	3	14	157	≥ 18	159	≥ 18
Gao, 2010[30]	China	*L. acidophilus* + *L. casei* (C)	50	3[a]	1.5	AC + 5	85	60 (6)	84	60 (6)
Gao, 2010[30]	China	*L. acidophilus* + *L. casei* (C)	100	3[a]	1.5	AC + 5	86	60 (6)	84	60 (6)
Pozzoni, 2012[36]	Italy	*S. boulardii* (C)	10	12[a]	2	AC + 7	106	80 (10)	98	79 (10)
Selinger, 2013[46]	UK	VSL#3 (D)	900	3[a]	2	AC + 7	61	58	61	57
Allen, 2013[20]	UK	*L. acidophilus* + *B. bifidum* + *B. lactis* (C)	60	8	7	21	1470	77 (71-84)	1471	77 (71-84)
Ouwehand, 2014[31]	China	*L. acidophilus* + *L. paracasei* + *B. lactis* (C)	4	4[a]	1.5	AC + 7	144	49 (11)	146	50 (11)
Ouwehand, 2014[31]	China	*L. acidophilus* + *L. paracasei* + *B. lactis* (C)	17	4[a]	1.5	AC + 7	160	51 (11)	146	50 (11)
Wong, 2014[40]	UK	*L. casei* Shirota (D)	7	4[a]	1	AC + 7	76	53	82	51
Ehrhardt, 2016[37]	Germany	*S. boulardii* (C)	36	6[a]	2	AC + 7	146	60 (17)	146	57 (18)

CFU = Colony Forming Units; (C) = capsule; (D) = drink; AC = antibiotic course; USA = United States of America; UK = United Kingdom; VSL#3 = *B. breve* + *B. longum* + *B. infantis* + *L. acidophilus* + *L. plantarum* + *L. paracasei* + *L. bulgaricus* + *S. thermophilus*

[a]Follow-up started after last drug dose

Table 2. Risk of bias among the 19 randomized trials of probiotic use for preventing Clostridium *difficile* infection (CDI) among hospitalized adults taking antibiotics.

Study	Allocation concealment (selection bias)	Blinding of participants and personnel (performance bias)	Blinding of outcome assessment (detection bias)	Random sequence generation (selection bias)	Incomplete outcome data (attrition bias)	Selective reporting (reporting bias)	Other bias[a]
Surawicz, 1989[33]	L	L	L	L	H	L	L
McFarland, 1995[34]	L	L	L	L	H	L	H
Thomas, 2001[38]	L	L	L	L	L	L	L
Plummer, 2004[42]	L	L	L	L	L	L	H
Can, 2006[35]	L	L	L	L	L	L	L
Beausoleil, 2007[41]	L	L	L	L	H	L	L
Hickson, 2007[44]	L	L	L	L	L	L	U
Rafiq, 2007[45]	U	H	H	L	U	L	U
Wenus, 2008[43]	L	L	L	L	H	L	L
Safdar, 2008[39]	L	L	L	L	L	L	U
Miller, 2008a[32]	L	L	L	L	L	L	H
Miller, 2008b[32]	L	L	L	L	L	L	H
Gao, 2010[30]	L	L	L	L	L	L	U
Pozzoni, 2012[36]	L	L	L	L	H	L	L
Selinger, 2013[46]	L	L	L	L	H	L	L
Allen, 2013[20]	L	L	L	L	H	L	L
Ouwehand, 2014[31]	L	L	L	L	U	L	L
Wong, 2014[40]	L	H	H	L	L	L	L
Ehrhardt, 2016[37]	L	L	L	L	L	L	L

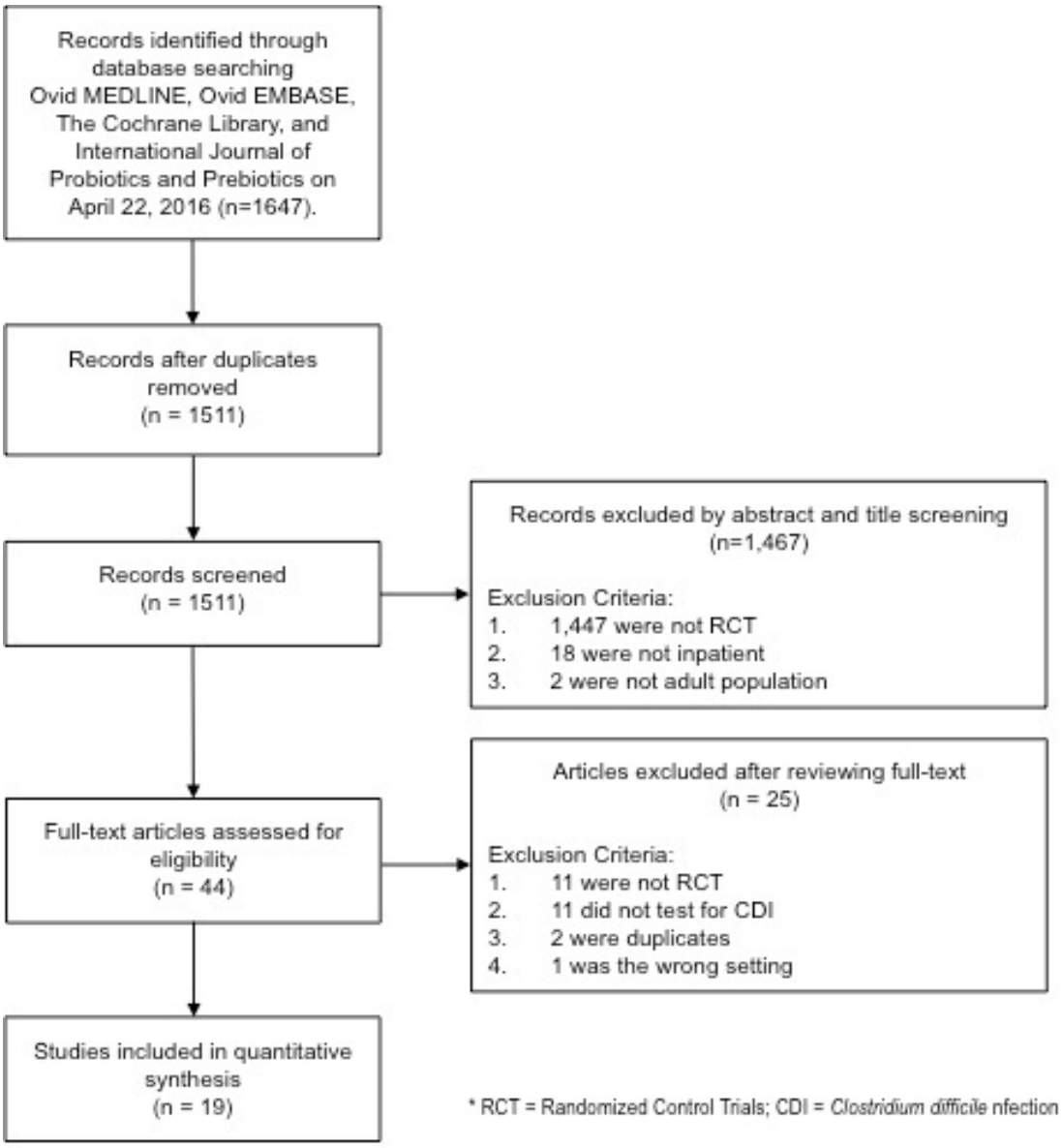
CDI = *Clostridium difficile* Infection; H = high risk of bias; L = low risk of bias; U = unclear risk of bias.

[a]Other bias includes: conflict of interest (relationship of the primary author with the probiotic manufacturer funding the study).

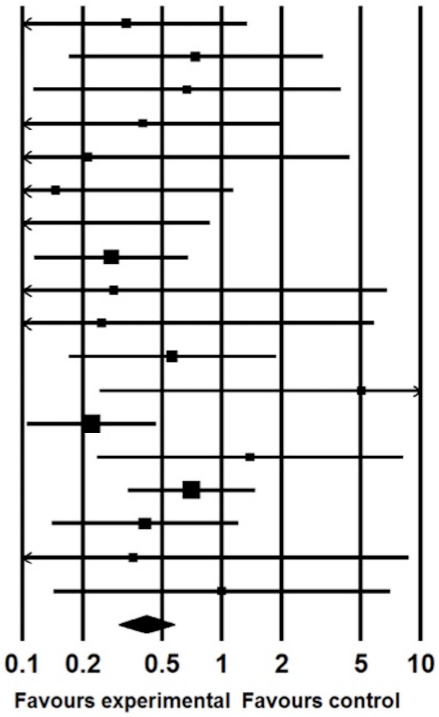
Table 3: Summary of subgroup analyses.

	No. of Studies	Summary RR	95% CI	I^2 (%)	P value for interaction
OVERALL	19	0.42	0.30–0.57	0.0	
Timing: *Study protocol specifying maximum interval from starting antibiotics to starting probiotics*					
1–2 days	14	0.32	0.22–0.48	0.0	0.02
3–7 days	5	0.70	0.40–1.23	0.0	
Risk of study bias					
High	11	0.44	0.27–0.71	0.0	0.81
Low	8	0.40	0.24–0.67	14.7	
Probiotic species[a]					
L. acidophilus	1	0.25	0.01–5.79	0.0	
L. acidophilus, B. bifidum	1	0.40	0.08–1.99	0.0	
L. acidophilus, B. bifidum, B. lactis	1	0.70	0.34–1.47	0.0	
L. acidophilus, L. bulgaricus, B. bifidum, S. thermophilus	1	0.28	0.11–0.68	0.0	
L. acidophilus, L. casei	2	0.21	0.11–0.42	0.0	
L. acidophilus, L. paracasei, B. lactis	1	0.41	0.14–1.20	0.0	0.34
L. casei, L. bulgaris, S. thermophilus	1	0.05	0.00–0.87	0.0	
L. casei Shirota	1	0.36	0.02–8.69	0.0	
L. rhamnosus GG	3	0.73	0.29–1.88	0.0	
L. rhamnosus GG, L. acidophilus, B. lactis	1	0.29	0.01–6.76	0.0	
S. boulardii	5	0.63	0.29–1.37	0.0	
Probiotic formulation					
Capsule	14	0.44	0.32–0.62	0.0	0.28
Drink	5	0.15	0.04–0.58	0.0	

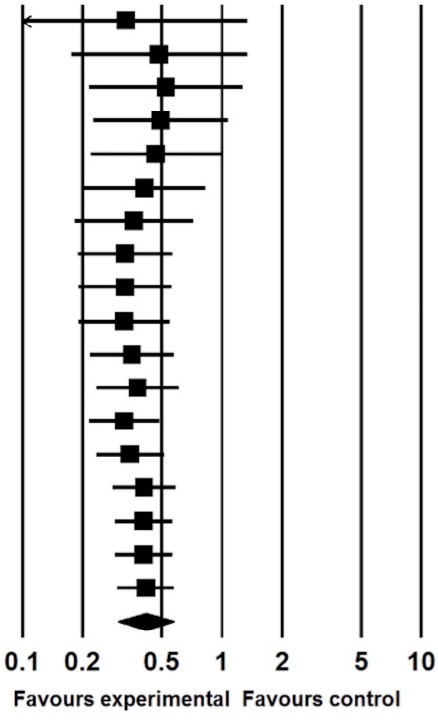
[a] VSL #3 studied by Selinger et al[46] results are not shown because of unestimable relative risk due to lack of events in both the probiotic and experimental cohorts.

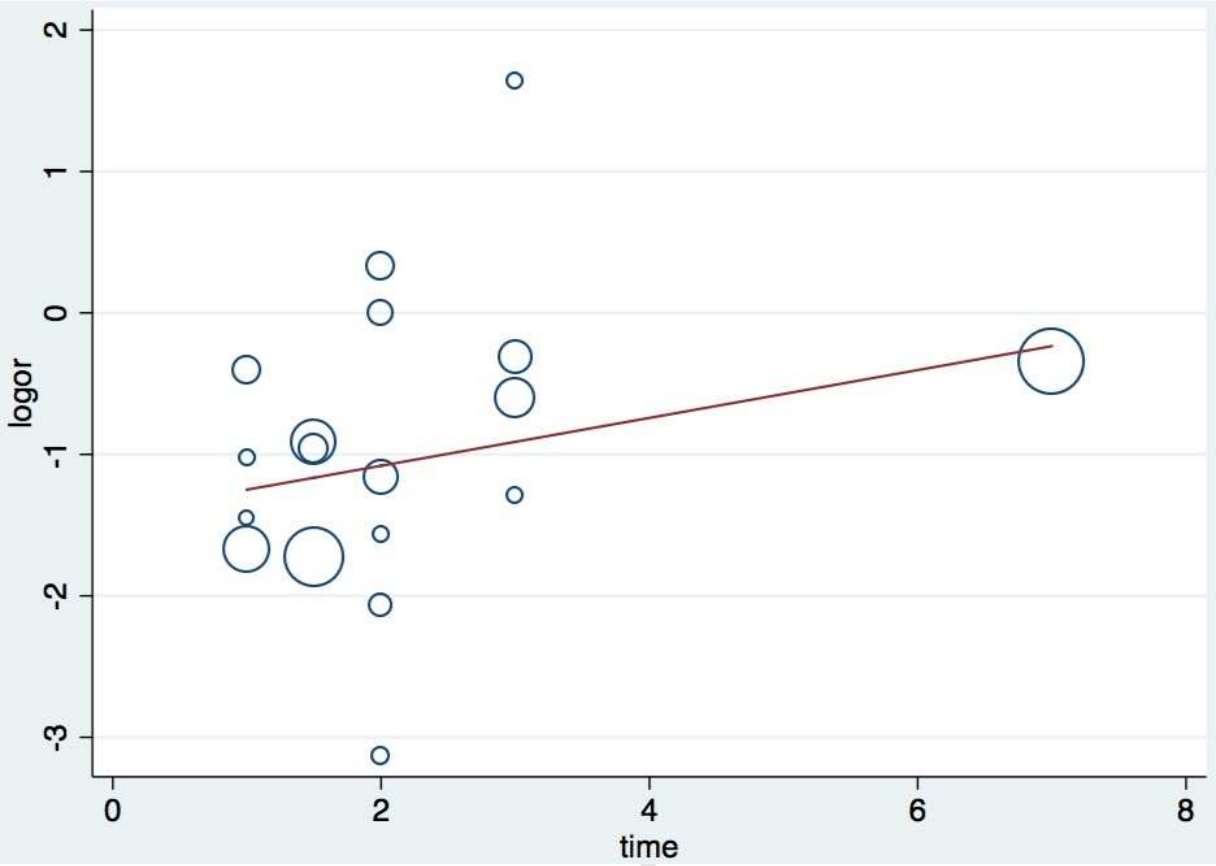

Records identified through database searching Ovid MEDLINE, Ovid EMBASE, The Cochrane Library, and International Journal of Probiotics and Prebiotics on April 22, 2016 (n=1647).
Records after duplicates removed (n = 1511)
Records screened (n = 1511)
Records excluded by abstract and title screening (n=1,467)
Exclusion Criteria:
1. 1,447 were not RCT
2. 18 were not inpatient
3. 2 were not adult population
Full-text articles assessed for eligibility (n = 44)
Articles excluded after reviewing full-text (n = 25)
Exclusion Criteria:
1. 11 were not RCT
2. 11 did not test for CDI
3. 2 were duplicates
4. 1 was the wrong setting
Studies included in quantitative synthesis (n = 19)
* RCT = Randomized Control Trials; CDI = Clostridium difficile nfection

Study name	Events / Total		Relative weight	Risk ratio
	probiotics	control		
Surawicz 1989	3 / 116	5 / 64	5.30	0.33
McFarland 1995	3 / 97	4 / 96	4.80	0.74
Thomas 2001	2 / 133	3 / 134	3.30	0.67
Plummer 2004	2 / 69	5 / 69	4.02	0.40
Can 2006	0 / 73	2 / 78	1.14	0.21
Beausoleil 2007	1 / 44	7 / 45	2.46	0.15
Hickson 2007	0 / 57	9 / 56	1.30	0.05
Rafiq 2007	5 / 45	22 / 55	13.16	0.28
Wenus 2008	0 / 34	1 / 29	1.04	0.29
Safdar 2008	0 / 23	1 / 17	1.05	0.25
Miller 2008a	4 / 95	7 / 94	7.26	0.57
Miller 2008b	2 / 157	0 / 159	1.13	5.06
Gao 2010	9 / 171	20 / 84	18.82	0.22
Pozzoni 2012	3 / 106	2 / 98	3.32	1.39
Allen 2013	12 / 1470	17 / 1471	19.16	0.71
Ouwehand 2014	6 / 304	7 / 146	9.01	0.41
Wong 2014	0 / 76	1 / 82	1.02	0.36
Ehrhardt 2016	2 / 146	2 / 146	2.74	1.00
Summary estimate				0.42



Study name	Cumulative statistics				
	Point	Lower limit	Upper limit	p-Value	Relative weight
Surawicz 1989	0.33	0.08	1.34	0.12	5.30
McFarland 1995	0.49	0.18	1.34	0.16	10.09
Thomas 2001	0.53	0.22	1.27	0.15	13.39
Plummer 2004	0.49	0.23	1.07	0.07	17.41
Can 2006	0.47	0.22	0.99	0.05	18.55
Beausoleil 2007	0.41	0.20	0.83	0.01	21.00
Hickson 2007	0.36	0.18	0.72	0.00	22.31
Rafiq 2007	0.33	0.19	0.56	0.00	35.46
Wenus 2008	0.33	0.19	0.56	0.00	36.50
Safdar 2008	0.32	0.19	0.55	0.00	37.55
Miller 2008a	0.36	0.22	0.57	0.00	44.81
Miller 2008b	0.38	0.24	0.61	0.00	45.94
Gao 2010	0.32	0.22	0.48	0.00	64.76
Pozzoni 2012	0.35	0.24	0.51	0.00	68.07
Allen 2013	0.41	0.28	0.59	0.00	87.23
Ouwehand 2014	0.41	0.29	0.57	0.00	96.24
Wong 2014	0.41	0.29	0.56	0.00	97.26
Ehrhardt 2016	0.42	0.30	0.57	0.00	100.00
Summary estimate	0.42	0.30	0.57	0.00	





Summary of findings:

Probiotics compared to No Probiotics for prevention of C. *difficile* infection (CDI) in hospitalized adults receiving antibiotics

Patient or population: prevention of C. *difficile* infection in hospitalized adults receiving antibiotics
Setting: Inpatient
Intervention: Probiotics
Comparison: No Probiotics

Outcomes	Anticipated absolute effects[a] (95% CI)		Relative effect (95% CI)	№ of participants (studies)	Quality of the evidence (GRADE)	Comments
	Risk with No Probiotics	Risk with Probiotics				
CDI	39 per 1,000	**16 per 1,000** (12 to 22)	**RR 0.42** (0.30 to 0.57)	6261 (19 RCTs)	⊕⊕⊕⊕ HIGH [b,c,d,e,f,g,h,i]	
Adverse Events (AE)	145 per 1,000	**141 per 1,000** (125 to 159)	**RR 0.97** (0.86 to 1.09)	6431 (15 RCTs)	⊕⊕⊕◯ MODERATE [e,j,k,kl,m,n]	

[a]**The risk in the intervention group** (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).
CI: Confidence interval; **RR:** Risk ratio

[b]The 8 low risk of bias studies did not demonstrate significantly more or less effect than the 11 high risk of bias studies.
[c]10 studies had loss to follow-up ranging from 2% to 48% (average 12%) in the experimental and 1% to 46% (average 12%) in the control (p=0.93). Sensitivity analysis of the worst-case-scenario showed probiotic efficacy with an upper 95% CI RR of 0.88.
[d]3 studies reported antibiotic associated diarrhea not tested for CDI that averaged 23% and 16% in the probiotic and control cohorts (p=0.62).
[e]Funnel plot and Egger's regression test did not demonstrate publication bias.
[f]Effect sizes were consistent across all studies.
[g]Test of heterogeneity was unremarkable. The I squared was equal to 0%.
[h]Outcome assessed in all studies was the outcome in question.
[i]For an alpha of 0.05 and power of 0.80, assuming an incidence of 3.9% in the control and RR of 0.50 in the probiotic, the optimal information size criterion of 1172 in each cohort was satisfied and the 95% CI excluded no effect.
[j]The 7 low risk of bias studies did not demonstrate significantly more effect than the 8 high risk of bias studies.
[k]Effect sizes were consistent across all studies.
[l]Test of heterogeneity was remarkable. The I squared was equal to 48%.
[m]The outcome assessed in all studies was the outcome of interest.
[n]For an alpha of 0.05 and power of 0.80, assuming an incidence of 20% in the control and RR of 0.30 in the probiotic, the optimal information size criterion of 90 in each cohort was satisfied and the 95% CI excluded no effect.

Supplemental Materials

Methods: Details of literature search

The search conducted in MEDLINE used the terms:
((Clostridium difficile/ OR Clostridium difficile.mp. OR Clostridium Infections.mp. OR Clostridium Infections/ OR Diarrhea/ OR Diarrhea.mp. OR CDAD.mp. OR Antibiotic-associated diarrhea.mp. OR AAD.mp.) AND (Anti-Bacterial Agents.mp. OR Anti-Bacterial Agents/ OR Antibiotic*.mp.) AND ((Lactic Acid Bacteria.mp. OR 1Probiotics/ OR Probiotic*.mp. OR Bifidobacter*.mp. OR Bifidobacterium/ OR Bifidobacterium longum.mp. OR Bifidobacterium longum bb536.mp. OR Bifidobacterium lactis.mp. OR exp Lactobacillus/ OR Lactobacillus bulgaricus.mp. OR Lactobacillus casei.mp. OR Lactobacillus plantarum.mp. OR Lactobacillus delbrueckii.mp. OR Lactobacillus brevis.mp. OR Lactobacillus leichmannii.mp. OR Lactobacillus helveticus.mp. OR Lactobacillus reuteri.mp. OR Lactobacillus rhamnosus.mp. OR Lactobacillus casei L39.mp. OR Lactobacillus rhamnosus L34.mp.) OR (Saccharomyces/ OR Saccharomyces.mp. OR Streptococcus/ OR Streptococcus.mp. OR Enterococcus/ OR Enterococcus.mp. OR Bacillus.mp. OR Bacillus/) OR (Active probiotic microorganisms.mp. OR Yogurt.mp. OR Yogurt/ OR Yoghurt.mp. OR Yogurt beverage.mp. OR Yoghurt beverage.mp. OR Live probiotic bacteria.mp. OR Lyophilized probiotic microorganisms.mp. OR Freeze-dried probiotics microorganisms.mp. OR Freeze-dried probiotics bacteria.mp. OR Lyophilized probiotic bacteria.mp. OR Probiotic preparations.mp. OR Symbiotic preparations.mp.)) AND (Diarrhea prevention.mp. OR Diarrhea/pc [Prevention & Control] OR Primary Prevention/ OR Primary Prevention.mp. OR Secondary Prevention.mp. OR Secondary Prevention/ OR Reinfection.mp. OR Recurrence/ OR Recurrence.mp. OR Relapse.mp.)

Supplemental Table 1: Study description of potential confounders and effect modifiers and whether used as exclusion criteria.

	STUDY NUMBER, in consecutive order																		
	1[33]	**2**[34]	**3**[38]	**4**[42]	**5**[35]	**6**[41]	**7**[44]	**8**[45]	**9**[43]	**10**[39]	**11**[32]	**12**[32]	**13**[30]	**14**[36]	**15**[46]	**16**[20]	**17**[31]	**18**[30]	**19**[49]
Potential confounder or effect modifier:																			
History of:																			
Immune compromise	–	–	–	?	–	–	–	?	–	+	?	?	–	?	–	–	–	–	–
Daily probiotic use	?	?	?	?	?	+	?	?	–	?	?	?	–	+	–	–	–	–	–
Recent antibiotic use	?	+	–	?	?	?	–	?	–	?	?	?	–	+	+	?	–	?	–
Previous CDI	?	?	–	?	?	?	?	?	?	–	?	?	–	?	?	–	–	?	?
Serious comorbidity	?	+	+	?	–	+	+	+	+	+	?	?	+	+	+	+	?	+	?
During hospitalization, use of:																			
Systemic anti-fungal agent	–	–	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	–
Proton pump inhibitor	+	?	?	?	–	+	?	+	?	+	?	?	?	+	+	+	?	+	?
Laxatives	+	+	?	?	?	+	?	?	?	–	?	?	?	?	?	+	?	?	?
Narcotics	+	?	?	?	?	+	?	?	?	–	?	?	?	?	?	?	?	?	?
Metronidazole, as part of antibiotic regimen	–	–	+	?	–	–	?	?	?	?	?	?	?	–	+	+	?	+	+

CDI = *Clostridium difficile* Infection; – = excluded; + = included; ? = not specified.

Table 2: Frequency of adverse events by study

Study	Experimental		Control	
	Events	Total	Events	Total
Surawicz[33]	0	116	0	64
McFarland[34]	0	97	12	96
Thomas[38]	37	133	52	134
Beausoleil[41]	21	44	20	45
Hickson[44]	0	57	0	56
Safdar[39]	2	23	5	17
Miller[32]	2	95	4	94
Miller[32]	4	157	0	159
Gao[30]	1	171	2	84
Pozzoni[36]	41	106	35	98
Selinger[46]	8	61	10	61
Allen[20]	294	1470	284	1471
Ouwehand[31]	14	304	12	146
Wong[40]	0	76	0	82
Ehrhardt[37]	9	146	3	146

Table 3: Probiotic Manufacturing

Probiotic Species	Trials	Study Manufacturer	Contact
L. acidophilus	1	Florajen® American Lifeline, Inc., WI, USA	800-257-5433
L. acidophilus, B. bifidum	1	Cultech, Swansea, UK	01639 825100
L. acidophilus, B. bifidum, B. lactis	1	NCIMB, UK	+44 (0) 1224 711100
L. acidophilus, L. bulgaricus, B. bifidum, S. thermophilus	1	NS	
L. acidophilus, L. casei	2	Bio-K+International Inc., Quebec, Canada	800-593-BIOK
L. acidophilus, L. paracasei, B. lactis	1	HOWARU® Restore, Danisco USA, Inc., WI, USA	danisco.com
L. casei, L. bulgaris, S. thermophilus	1	Actimel, Danone, France	0 800 125 125
L. casei Shirota	1	Yakult Light® Yakult Honsha Co., Ltd., CA, USA	714-434-6500
L. rhamnosus GG	3	CAG Functional Foods, ConAgra Foods, Inc., NE, USA	402-595-5117
L. rhamnosus GG, L. acidophilus, B. lactis	1	Biola® TINE, Oslo, Norway	+47 513 71 513
S. boulardii	5	Biocodex (head office in France), USA and Italy distributed	877-356-7787
		Parenterol® forte, Germany	+49 (0) 2371 937-0

NS = not specified; NCIMB = National Collection of Industrial, Food and Marine Bacteria;

FIGURES

Figure 1. L'Abbé plot of incidence of *Clostridium difficile* infection (CDI) in the probiotics group (experimental) vs no probiotics group (control) for the 19 included randomized trials, with a line representing the summary relative risk. The line demonstrates that the absolute risk reduction between the two groups (absolute benefit) increases as the baseline (control group) incidence of CDI increases.

Figure 2. Subgroup sensitivity analysis accounting for loss to follow-up. Meta-analyses assigning *Clostridium difficile (CDI)* infection event rates to subjects lost to follow-up in a (a) 2:1 and (b) 5:1 multiple of the observed incidence of CDI in experimental subjects with no missing data.

Figure 3: Subgroup timing of probiotic. Meta-analysis by subgroup of studies with protocols specifying that probiotics be given within 1–2 days vs within 3–7 days of the first antibiotic dose. Results demonstrate that requiring that probiotics be given earlier increases efficacy. Results were also statistically significant if the maximum time interval was dichotomized as 1 day vs 2–7 days.

Figure 4: Subgroup species and strains analysis. Meta-analysis grouped by (a) species and strains of the different probiotics and by (b) probiotic species. Results suggest maintained efficacy (95% CI that do not cross the null) in particular probiotic formulations containing multiple *Lactobacillus* strains or

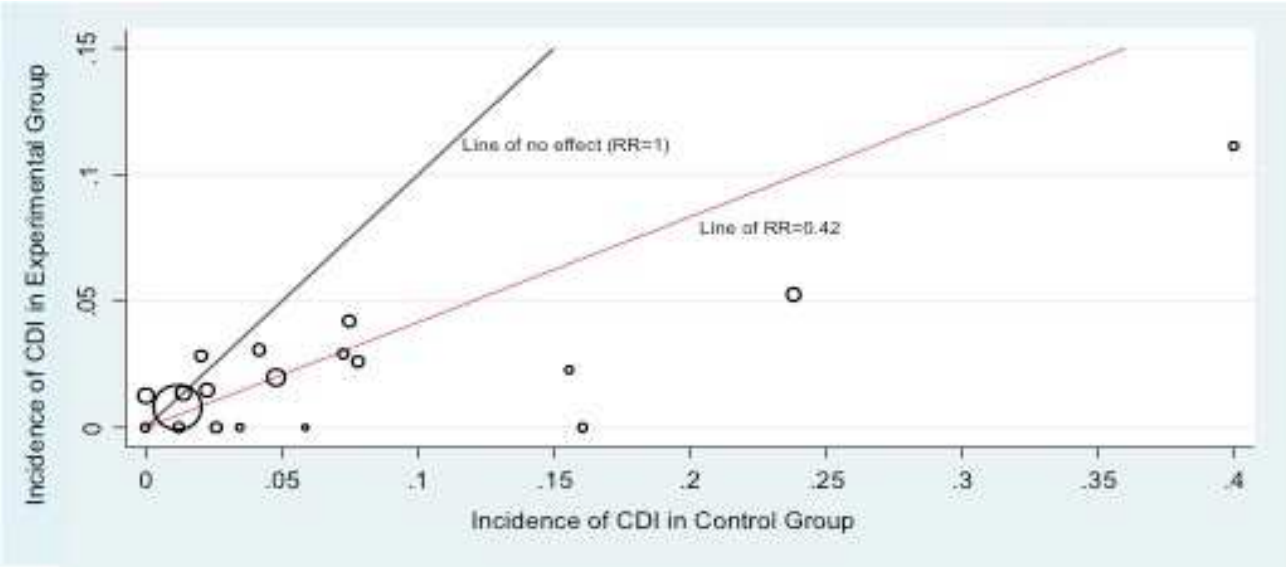
Lactobacillus in combination with another species. LA = *L. acidophilus*; LA, Bb = *L. acidophilus* + *B. bifidum*; LA, Bb, Bl = *L. acidophilus* + *B. bifidum* + *B. lactis*; LA, LB, Bb, ST = *L. acidophilus* + *L. bulgaricus* + *B. bifidum* + *S. thermophiles*; LA, LC = *L. acidophilus* + *L. casei*; LA, LC, Bl = *L. acidophilus* + *L. paracasei* + *B. lactis*; LC, ST, LB = *L. casei* + *S. thermophiles* + *L. bulgaris*; LcS = *L. casei* Shirota; LG = *L. rhamnosus GG*; LG, LA, Bl = *L. rhamnosus GG* + *L. acidophilus* + *B. lactis*; SB = *S. boulardii.*

Figure 5: Subgroup formulation. Meta-analysis grouped by capsule and milk formulation. The overlapping CI that do not cross the null suggests maintained efficacy regardless of if a capsule or drink is administered.

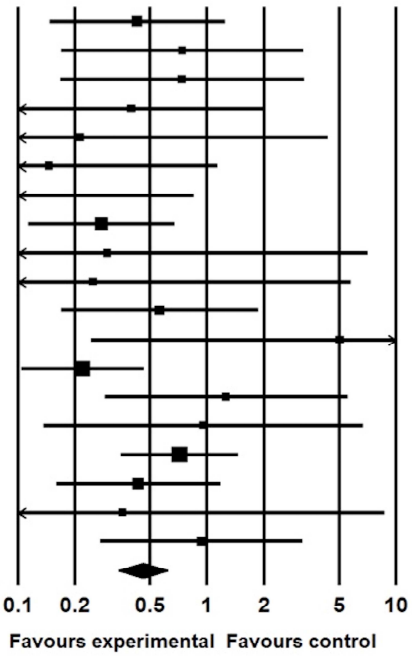
Figure 6: Dose meta-regression. Random-effects meta-regression of the study results (measured as the natural log of the study odds ratio) as a function of the maximum probiotic dose used by all trials (range 4 to 900 billion colony forming units (CFU). Each study was weighted by the standard error of its ln(OR). The results demonstrate an insignificant effect.

Figure 7: Subgroup bias analysis. Meta-analysis of studies grouped as high risk of bias and low risk of bias showing no variance in probiotic efficacy by study quality.

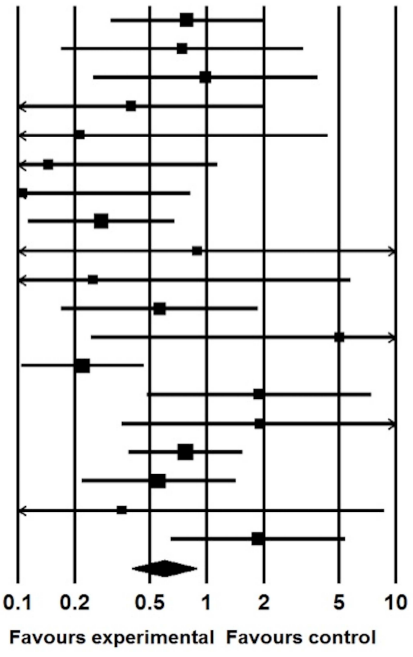
Figure 8: Funnel plot. Visual inspection of the funnel plot was not suggestive of publication bias.



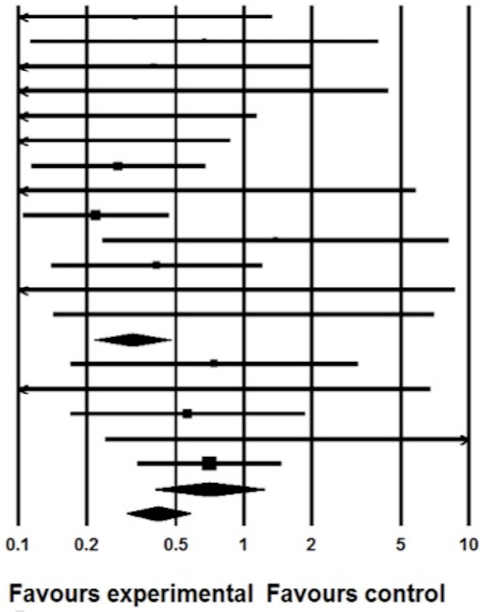
Study name	Events / Total		Relative weight	Risk ratio	Lower limit	Upper limit
	probiotics	control				
Surawicz 1989	6 / 212	7 / 106	7.85	0.43	0.15	1.24
McFarland 1995	3 / 97	4 / 96	4.12	0.74	0.17	3.23
Thomas 2001	3 / 152	4 / 150	4.07	0.74	0.17	3.25
Plummer 2004	2 / 69	5 / 69	3.45	0.40	0.08	1.99
Can 2006	0 / 73	2 / 78	0.98	0.21	0.01	4.37
Beausoleil 2007	1 / 44	7 / 45	2.11	0.15	0.02	1.14
Hickson 2007	0 / 69	9 / 66	1.12	0.05	0.00	0.85
Rafiq 2007	5 / 45	22 / 55	11.30	0.28	0.11	0.67
Wenus 2008	0 / 46	1 / 41	0.88	0.30	0.01	7.12
Safdar 2008	0 / 23	1 / 17	0.90	0.25	0.01	5.79
Miller 2008a	4 / 95	7 / 94	6.24	0.57	0.17	1.87
Miller 2008b	2 / 157	0 / 159	0.97	5.06	0.25	104.63
Gao 2010	9 / 171	20 / 84	16.17	0.22	0.11	0.46
Pozzoni 2012	4 / 141	3 / 134	4.07	1.27	0.29	5.56
Selinger 2013	2 / 117	2 / 112	2.36	0.96	0.14	6.68
Allen 2013	13 / 1493	18 / 1488	17.67	0.72	0.35	1.46
Ouwehand 2014	7 / 336	8 / 167	8.95	0.43	0.16	1.18
Wong 2014	0 / 79	1 / 85	0.88	0.36	0.01	8.67
Ehrhardt 2016	5 / 246	5 / 231	5.92	0.94	0.28	3.20
Summary estimate				0.46	0.34	0.62

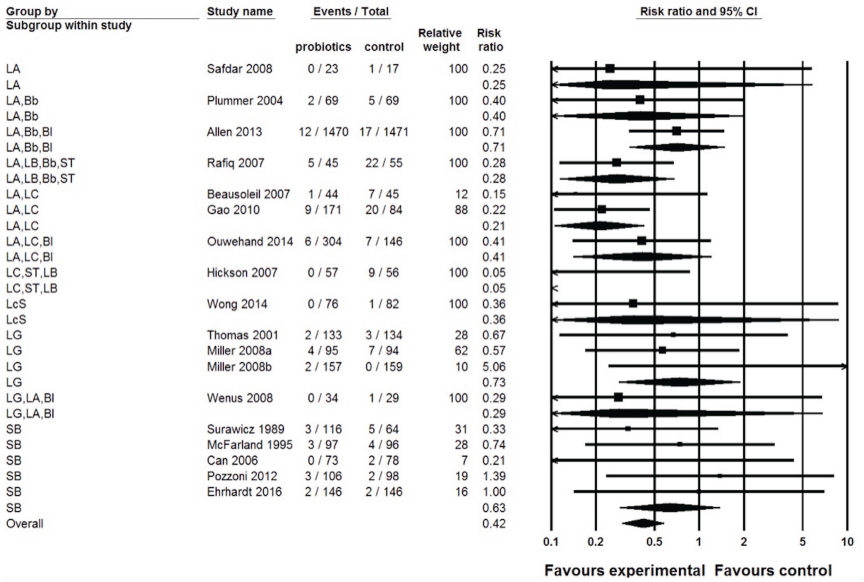


Study name	Events / Total		Relative weight	Risk ratio	Lower limit	Upper limit
	probiotics	control				
Surawicz 1989	11 / 212	7 / 106	8.74	0.79	0.31	1.97
McFarland 1995	3 / 97	4 / 96	4.96	0.74	0.17	3.23
Thomas 2001	4 / 152	4 / 150	5.49	0.99	0.25	3.87
Plummer 2004	2 / 69	5 / 69	4.37	0.40	0.08	1.99
Can 2006	0 / 73	2 / 78	1.51	0.21	0.01	4.37
Beausoleil 2007	1 / 44	7 / 45	2.96	0.15	0.02	1.14
Hickson 2007	1 / 69	9 / 66	3.00	0.11	0.01	0.82
Rafiq 2007	5 / 45	22 / 55	9.04	0.28	0.11	0.67
Wenus 2008	1 / 46	1 / 41	1.80	0.89	0.06	13.80
Safdar 2008	0 / 23	1 / 17	1.41	0.25	0.01	5.79
Miller 2008a	4 / 95	7 / 94	6.54	0.57	0.17	1.87
Miller 2008b	2 / 157	0 / 159	1.50	5.06	0.25	104.63
Gao 2010	9 / 171	20 / 84	10.53	0.22	0.11	0.46
Pozzoni 2012	6 / 141	3 / 134	5.50	1.90	0.49	7.45
Selinger 2013	4 / 117	2 / 112	4.09	1.91	0.36	10.25
Allen 2013	14 / 1493	18 / 1488	11.05	0.78	0.39	1.55
Ouwehand 2014	9 / 336	8 / 167	8.60	0.56	0.22	1.42
Wong 2014	0 / 79	1 / 85	1.37	0.36	0.01	8.67
Ehrhardt 2016	10 / 246	5 / 231	7.54	1.88	0.65	5.41
Summary estimate				0.60	0.40	0.88

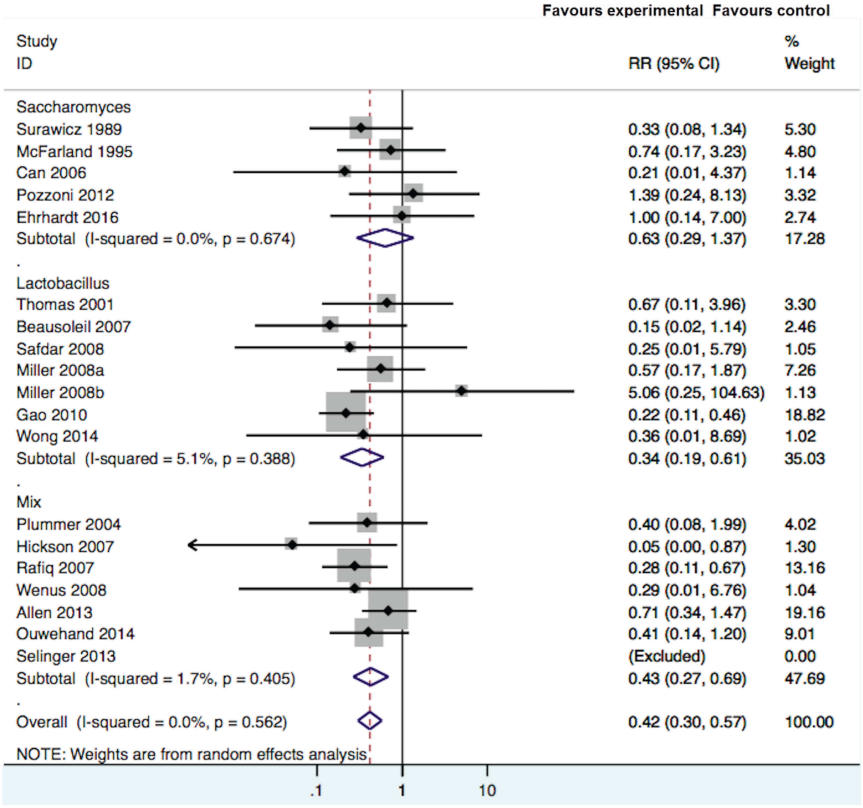


Time to dose	Study name	Events / Total		Relative weight	Risk ratio	p-Value
		probiotics	control			
2.000	Surawicz 1989	3 / 116	5 / 64	8	0.33	0.12
2.000	Thomas 2001	2 / 133	3 / 134	5	0.67	0.66
2.000	Plummer 2004	2 / 69	5 / 69	6	0.40	0.26
2.000	Can 2006	0 / 73	2 / 78	2	0.21	0.32
2.000	Beausoleil 2007	1 / 44	7 / 45	4	0.15	0.07
2.000	Hickson 2007	0 / 57	9 / 56	2	0.05	0.04
2.000	Rafiq 2007	5 / 45	22 / 55	20	0.28	0.00
2.000	Safdar 2008	0 / 23	1 / 17	2	0.25	0.39
2.000	Gao 2010	9 / 171	20 / 84	28	0.22	0.00
2.000	Pozzoni 2012	3 / 106	2 / 98	5	1.39	0.72
2.000	Ouwehand 2014	6 / 304	7 / 146	14	0.41	0.10
2.000	Wong 2014	0 / 76	1 / 82	2	0.36	0.53
2.000	Ehrhardt 2016	2 / 146	2 / 146	4	1.00	1.00
Summary estimate for 2.000					0.32	0.00
3.000	McFarland 1995	3 / 97	4 / 96	14	0.74	0.69
3.000	Wenus 2008	0 / 34	1 / 29	3	0.29	0.44
3.000	Miller 2008a	4 / 95	7 / 94	22	0.57	0.35
3.000	Miller 2008b	2 / 157	0 / 159	3	5.06	0.29
3.000	Allen 2013	12 / 1470	17 / 1471	57	0.71	0.35
Summary estimate for 3.000					0.70	0.22
Overall					0.42	0.00

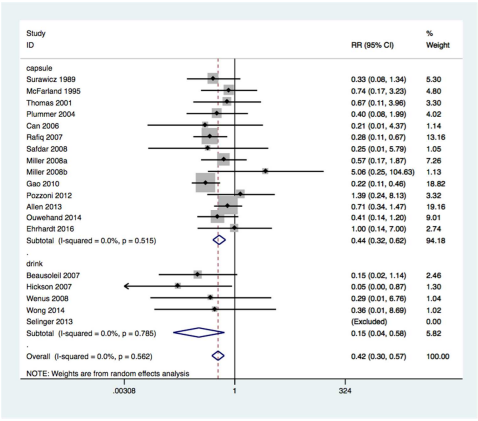


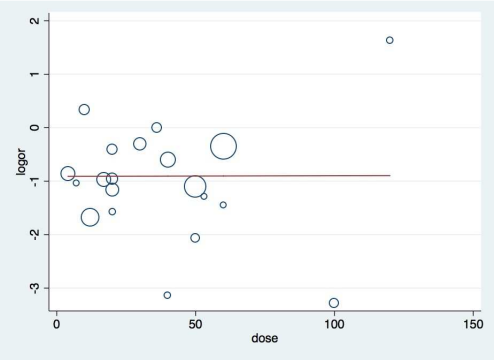


Group by Subgroup within study	Study name	Events / Total probiotics	Events / Total control	Relative weight	Risk ratio
LA	Safdar 2008	0 / 23	1 / 17	100	0.25
LA					0.25
LA,Bb	Plummer 2004	2 / 69	5 / 69	100	0.40
LA,Bb					0.40
LA,Bb,Bl	Allen 2013	12 / 1470	17 / 1471	100	0.71
LA,Bb,Bl					0.71
LA,LB,Bb,ST	Rafiq 2007	5 / 45	22 / 55	100	0.28
LA,LB,Bb,ST					0.28
LA,LC	Beausoleil 2007	1 / 44	7 / 45	12	0.15
LA,LC	Gao 2010	9 / 171	20 / 84	88	0.22
LA,LC					0.21
LA,LC,Bl	Ouwehand 2014	6 / 304	7 / 146	100	0.41
LA,LC,Bl					0.41
LC,ST,LB	Hickson 2007	0 / 57	9 / 56	100	0.05
LC,ST,LB					0.05
LcS	Wong 2014	0 / 76	1 / 82	100	0.36
LcS					0.36
LG	Thomas 2001	2 / 133	3 / 134	28	0.67
LG	Miller 2008a	4 / 95	7 / 94	62	0.57
LG	Miller 2008b	2 / 157	0 / 159	10	5.06
LG					0.73
LG,LA,Bl	Wenus 2008	0 / 34	1 / 29	100	0.29
LG,LA,Bl					0.29
SB	Surawicz 1989	3 / 116	5 / 64	31	0.33
SB	McFarland 1995	3 / 97	4 / 96	28	0.74
SB	Can 2006	0 / 73	2 / 78	7	0.21
SB	Pozzoni 2012	3 / 106	2 / 98	19	1.39
SB	Ehrhardt 2016	2 / 146	2 / 146	16	1.00
SB					0.63
Overall					0.42



Study ID	RR (95% CI)	% Weight
Saccharomyces		
Surawicz 1989	0.33 (0.08, 1.34)	5.30
McFarland 1995	0.74 (0.17, 3.23)	4.80
Can 2006	0.21 (0.01, 4.37)	1.14
Pozzoni 2012	1.39 (0.24, 8.13)	3.32
Ehrhardt 2016	1.00 (0.14, 7.00)	2.74
Subtotal (I-squared = 0.0%, p = 0.674)	0.63 (0.29, 1.37)	17.28
.		
Lactobacillus		
Thomas 2001	0.67 (0.11, 3.96)	3.30
Beausoleil 2007	0.15 (0.02, 1.14)	2.46
Safdar 2008	0.25 (0.01, 5.79)	1.05
Miller 2008a	0.57 (0.17, 1.87)	7.26
Miller 2008b	5.06 (0.25, 104.63)	1.13
Gao 2010	0.22 (0.11, 0.46)	18.82
Wong 2014	0.36 (0.01, 8.69)	1.02
Subtotal (I-squared = 5.1%, p = 0.388)	0.34 (0.19, 0.61)	35.03
.		
Mix		
Plummer 2004	0.40 (0.08, 1.99)	4.02
Hickson 2007	0.05 (0.00, 0.87)	1.30
Rafiq 2007	0.28 (0.11, 0.67)	13.16
Wenus 2008	0.29 (0.01, 6.76)	1.04
Allen 2013	0.71 (0.34, 1.47)	19.16
Ouwehand 2014	0.41 (0.14, 1.20)	9.01
Selinger 2013	(Excluded)	0.00
Subtotal (I-squared = 1.7%, p = 0.405)	0.43 (0.27, 0.69)	47.69
.		
Overall (I-squared = 0.0%, p = 0.562)	0.42 (0.30, 0.57)	100.00





Group by Subgroup within study	Study name	Events / Total probiotics	Events / Total control	Relative weight	Risk ratio
high	Surawicz 1989	3 / 116	5 / 64	12.19	0.33
high	McFarland 1995	3 / 97	4 / 96	11.03	0.74
high	Plummer 2004	2 / 69	5 / 69	9.24	0.40
high	Beausoleil 2007	1 / 44	7 / 45	5.65	0.15
high	Rafiq 2007	5 / 45	22 / 55	30.25	0.28
high	Wenus 2008	0 / 34	1 / 29	2.38	0.29
high	Miller 2008a	4 / 95	7 / 94	16.69	0.57
high	Miller 2008b	2 / 157	0 / 159	2.60	5.06
high	Pozzoni 2012	3 / 106	2 / 98	7.62	1.39
high	Wong 2014	0 / 76	1 / 82	2.35	0.36
high					0.44
low	Thomas 2001	2 / 133	3 / 134	7.41	0.67
low	Can 2006	0 / 73	2 / 78	2.71	0.21
low	Hickson 2007	0 / 57	9 / 56	3.09	0.05
low	Safdar 2008	0 / 23	1 / 17	2.51	0.25
low	Gao 2010	9 / 171	20 / 84	30.04	0.22
low	Allen 2013	12 / 1470	17 / 1471	30.40	0.71
low	Ouwehand 2014	6 / 304	7 / 146	17.61	0.41
low	Ehrhardt 2016	2 / 146	2 / 146	6.24	1.00
low					0.40
Overall					0.42