Title: *Lactobacillus* probiotics in the prevention of *Clostridium difficile* associated diarrhoea: A systematic review and meta-analysis

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Abstract (228 words)

Background: Recent meta-analyses of the efficacy of probiotics for prevention of *C. difficile* associated diarrhea have concluded that there is a large effect in favour of probiotics based on moderate quality evidence. We re-examine this evidence which contradicts the results of a more recent, large randomized controlled trial that found no benefit of lactobacillus probiotics.

Methods: We carried out an updated systematic review and meta-analysis using a Bayesian hierarchical model. We included only double-blinded studies of lactobacillus probiotics vs placebo in adults. In addition to pooling results across studies, we report on the between-study heterogeneity and the prediction interval which reflects the uncertainty in the efficacy estimate for a future study.

Findings: We identified 10 randomized controlled trials that met our inclusion criteria. The pooled risk ratio (95% credible interval) across these studies was 0.25 (0.08, 0.47). The corresponding prediction interval was (0.02, 1.34), reflecting high heterogeneity between studies. A meta-regression analysis suggested that the log risk ratio increased with the risk in the control group, though the association was not statistically significant.

Interpretation: Taking into account the between study heterogeneity reveals that there is considerable uncertainty regarding the efficacy estimate associated with lactobacillus probiotics. Most studies so far have been carried out in populations with a low risk of CDAD such that the evidence is inconclusive and inadequate to support a policy concerning routine use of probiotics.

Introduction

Clostridium difficile associated diarrhoea (CDAD) is associated with significant morbidity and mortality in hospitalized patients[1, 2], its manifestations ranging from debilitating diarrhoea, to toxic megacolon requiring surgical intervention, to death[3]. Nosocomial CDAD is strongly associated with antibiotic therapy[4], the mechanism of risk believed to be antibiotic-associated disruption of the intestinal flora. At an institutional level, prevention is by infection control measures. At a patient level, there has been great interest in re-establishing normal biota via fecal transfer in recurrent Clostridium difficile[5], and especially by prophylactic administration of purified microbial preparations ('probiotics'), due to their low cost and easy availability. Consistently, meta-analyses have estimated a protective effect of probiotics of various strains against CDAD[6-8], on the basis of a clinically heterogeneous group of small to medium sized studies of generally moderate quality. However, a recent large, well-run study failed to demonstrate statistically significant preventative effect of a mixture of *Lactobacillus acidophilus* and bifidobacteria in elderly hospitalized patients[9].

Such discrepancies between the results of early meta-analyses and subsequent, large randomized trials have been noted before[10], and attributed to publication bias in the form of selected publication of small randomized controlled trials reporting optimistic results. In the case of probiotics, we also believe that standard statistical meta-analysis methods may underestimate the statistical heterogeneity between the published studies. Meta-analyses published to date[6, 7, 11] have used the traditional DerSimonian-Laird approach for meta-analysis, which is known to underestimate heterogeneity[12]. In order to address the issue of between-study heterogeneity more thoroughly, we have updated our previous systematic review of probiotics in the prevention of CDAD in adults[13], and conducted a meta-analysis of *Lactobacillus* species probiotics, applying Bayesian hierarchical methods.

Methods

Our systematic literature search was first carried out for the period until 2005[13], and subsequently extended to include additional databases and updated[14, 15]. The search covered PubMed, EMBASE, AMED and CINAHL. Search terms included "probiotic*" (where * indicates a wildcard), "Lactobacill*", and terms specific for the organisms themselves, combined with "antibiotic associated diarrhea", "Clostridium" or "difficile" (see supplementary material for details). The search was last updated on March 20, 2014. In addition, we closely reviewed the included and excluded papers from published systematic reviews and meta-analyses for inclusion. Results were summarized using an adapted version of the PRISMA flow-diagram suitable for updates of systematic reviews[16].

To be included in the meta-analysis, a study had to have been described as a double-blind RCT recruiting predominately or exclusively adult in-patients who were receiving antibiotics of any kind and for any indication. Double-blinding of patients and study personnel responsible for measuring outcomes would reduce the risk of ascertainment bias of CDAD cases in studies with incomplete testing, and enabled us to use a missing at random assumption to adjust for missing

data (see below). The active treatment had to contain *Lactobacillus* species at any dose. We excluded yeast studies to reduce the heterogeneity of interventions, and studies in children and community-acquired CDAD because these represented a different population. CDAD had to be defined as diarrhea with a positive culture or toxin assay for *C difficile*, and the paper had to report sufficient information about numbers tested to allow for adjustment for incomplete testing for *C difficile.*

At least two authors (AS, XX and ND) participated in each of the literature searches, study design quality review or data extraction. From each study we extracted the number of patients in the intervention and placebo groups, the numbers of patients who had diarrhea in each group, the number who were tested for *C. difficile* and the number who were classified as having CDAD. In addition, we extracted descriptive information (study location, inclusion criteria, probiotic strain and dose, method of testing for *C difficile*, and source of funding; see Table 1) and patient demographics and covariates (mean age, proportion by sex, proportion receiving each group or class of antibiotics; see Table 1). We assessed the risk of four biases described by the Cochrane Collaboration (selection, treatment, attrition, and detection), as no, yes, and unclear (which included instances where we had insufficient information to assess risk of bias). We then classified studies as A (no major sources of bias), B (one possible source of bias), and C (two or more possible sources of bias). We used a funnel plot to assess the relationship between the standard error of the log risk ratio and its standard error reported in the individual studies that suggested publication bias.

Statistical methods

Our primary outcome of interest was CDAD (Table 1). We analysed the data for all patients randomized in each study, assuming firstly that patients who had no recorded AAD were negative for AAD, and secondly that missing information on the results of testing for *C difficile* in patients with AAD was missing at random (MAR). The missing at random assumption was based on the expectation that, since double blinded studies were selected, the group to which a patient was randomized could not influence the decision to test for CDAD. Studies using different doses and formulations of lactobacillus probiotics were included, and where the study design included two probiotics groups (low and high dose), probiotic users were combined in a single group. There was considerable heterogeneity in the risk of CDAD in the placebo group of the selected studies. To examine the relation between this risk and the reported effect size we used a L'Abbé plot.

We used an exact Bayesian random effects meta-analysis model to pool the log risk ratios across studies while accounting for the heterogeneity within and between studies[17]. We used vague prior distributions over all parameters so as to let the observed data dominate the final results. From the meta-analysis, we extracted the pooled risk ratio together with its 95% credible interval. We also extracted the 95% prediction interval that reflects the uncertainty in the estimated risk ratio in an individual study in the future. If there is a high degree of between-study

heterogeneity, we would expect the prediction interval to be much wider than the credible interval for the pooled risk ratio.

We also carried out a Bayesian credibility analysis as an alternative approach to study the extent of uncertainty about the pooled result[18]. This approach measures the degree of scepticism needed in order to remain unconvinced by a statistically significant pooled risk ratio from a meta-analysis. If a reasonable degree of scepticism leads us to doubt the results of the meta-analysis, then we would draw a more nuanced conclusion despite the statistical significance. On the other hand, if an unreasonably high degree of scepticism is required, then we would be more convinced by the results of the meta-analysis. The credibility analysis approach requires the derivation of a 'critical' sceptical prior distribution for the pooled risk ratio, which is sufficiently influential so as to convert the significant result (based on the data alone) into a non-significant result. The critical sceptical prior distribution is a symmetric distribution centred over the risk ratio of 1. When the results of individual studies are consistent, i.e. when between-study heterogeneity is absent or low, the critical sceptical prior would be concentrated around 1 with a small variance (high scepticism). When results from individual studies are inconsistent, i.e. there is a high degree of between-study heterogeneity, the critical sceptical prior would be more spread out around 1 with a wider variance (low scepticism).

For comparison with standard meta-analytical techniques, we also used the widely applied approximate method described by DerSimonian and Laird[19]. For studies that had 0 cells in the cross-tabulation between probiotics and CDAD we added 0.5 to all cells. Here as well we adjusted the results in studies with incomplete testing. To do this we assumed the same proportion of positive CDAD tests among the missing as the non-missing.

Bayesian analyses were carried out using WinBUGS version 1.4.3 for Windows[20]. The frequentist analysis was implemented using the R statistical software version 3.0.1 [21] package rmeta version 1.6.2 [22]. Descriptive statistics and graphs were obtained using either the R 3.0.1 [21] or SAS 9.3[23] statistical software packages.

We examined possible sources of between-study heterogeneity using Bayesian meta-regression models[24] relating the pooled risk ratio to study quality (no major sources of bias [A] identified versus at least one source of bias[B/C]), population prevalence of CDAD (as estimated by the adjusted proportion of patients with CDAD in the placebo group, either as a continuous variable or dichotomized at the median value <6% versus ≥6%), composition of the probiotic (preparations containing only lactobacillus species vs. mixed preparations), dose of probiotic (dichotomized at the median value of <50 vs ≥50 million CFUs) and whether or not studies received industry funding (any support vs. none). By using the exact likelihood, the Bayesian approach addresses problems that have been previously identified with meta-regression models adjusting for the control group risk [25].

We conducted sensitivity analyses to study the robustness of our Bayesian analysis. We varied the prior distribution over the standard deviation of the between-study heterogeneity, as this is known to influence the results of the meta-analysis[24]. We conducted a meta-analysis including

the results of studies that did not report information on testing to see if they were systematically different from those in our primary analysis, assuming, as the authors did, that untested patients were negative for CDAD. We also performed a sensitivity analysis that excluded the study by Gao et al [26], reasoning that their results were not generalizable to other settings due to the extremely high risk of CDAD reported in the placebo group.

The PRISMA checklist[27] was completed to ensure proper reporting (Supplementary Table 2).

Results

Our search identified ten trials that qualified for inclusion[9, 26, 28-36], (Figure 1; Table 1). A further seven trials[37-42] were not included in the primary meta-analysis because we could not obtain information on the number tested for CDAD, but were included in a sensitivity analysis of unadjusted data.

Included trials ranged in size from 34 to 2981 patients, and were conducted in Canada, the US, the UK, and China. Patients received *Lactobacillus* species as single preparations or in combination, in doses ranging from <20 to 450 billion colony forming units (the latter figure was a total dose of a mixed preparation) given as yoghurt, capsules, or powder. A variety of definitions of diarrhoea were used, allowing for one to three liquid stools, over one to three days, with or without application of a formal scale. Details of the design in these studies are summarized in Table 1, including definition of diarrhoea, probiotic and dose, assay for *C difficile*, length of treatment and length of follow-up.

After adjustment for incomplete testing for *C difficile* in five studies, the proportion of patients with CDAD in the probiotics group ranged from 0 to 8%, and in the placebo group from 0 to 24% (Figure 2). In two studies, no cases of CDAD were identified. In total, CDAD was detected for 45 out of 2554 patients in the *Lactobacillus* group and 90 out of 2287 patients in the placebo group. The L'Abbé plot in Figure 2 shows that as the risk of CDAD in the placebo group increased, the apparent benefit of probiotics increased (i.e. the individual study risk ratio was more likely to be less than 1).

A forest plot summarizing the results of the Bayesian hierarchical meta-analysis is presented in Figure 3. The RRs of *Lactobacillus* versus placebo for individual studies ranged from 0.02 to 0.66. Using the Bayesian meta-analysis model, the median pooled RR was estimated to be 0.25 (95% credible interval [CrI]: 0.08, 0.47), indicating a statistically significant association on average between *Lactobacillus* and a lower risk of CDAD for inpatients, with a 75% risk reduction relative to placebo. The predicted RR in a new trial was estimated to be 0.25 (95% CrI: 0.02, 1.34), the wide CrI also reflecting the heterogeneity, and the resulting predictive uncertainty. Between-study standard deviation on the log risk ratio scale was 0.64 [95% CrI: 0.06, 1.75], which was relatively high, indicating considerable statistical heterogeneity between studies.

The critical sceptical prior over the pooled risk ratio was centred at 1 and ranged from (0.6, 1.7). This means a sceptic with a prior opinion that the true risk ratio ranges anywhere from 0.6 to 1.7 would remain unconvinced by the statistically significant pooled risk ratio that there is a favourable effect of probiotics as the posterior 95% credible interval would include a risk ratio of 1 (Figure 3). We considered this range to be quite wide and reflective of a reasonable degree of scepticism, as it is unusual for a pharmaceutical intervention to be associated with a risk ratio outside of this range. Thus the credibility analysis suggests that despite the statistically significant pooled risk ratio from the Bayesian meta-analysis, the evidence in favour of probiotics was not very robust and the final conclusion could be altered by use of a weakly informative prior distribution. The information in the critical sceptical prior distribution can also be expressed as being equivalent to information from a balanced, null randomized controlled trial with 30 CDAD events observed in each arm. In other words, despite the total number of cases of CDAD being 135 in the studies included in the meta-analysis, the combined information across the studies was relatively weak and could be displaced by information from a trial with only 60 observed cases of CDAD.

In comparison, when a random effects model with the DerSimonian-Laird estimator was applied to the adjusted data, the between study standard deviation is estimated to be 0.02 (Q-test p-value 0.4029) leading to the conclusion that there is no evidence of heterogeneity between studies. The resulting pooled estimate of the RR was 0.42 (95% CI 0.29, 0.59).

The funnel plot for the meta-analysis (Figure 4) suggests the potential for publication bias as we see an asymmetry in the scatter of points with studies with higher standard errors reporting smaller risk ratios favouring probiotics. However, it should be noted that this interpretation is limited by the small number of studies and the presence of heterogeneity between the studies[43].

The pooled risk ratio was not significantly associated with any of the covariates we examined, with the 95% credible intervals for the regression coefficient including zero in all cases (Table 3). None the less the probability of an association was high for some covariates. High study quality and high dose of probiotic were more likely to be associated with risk ratios approaching 1. Studies with a higher risk of CDAD in the placebo group or with industry funding were more likely to report a risk ratio well below 1.

Our sensitivity analyses showed that the pooled risk ratio is robust to changes in the prior distribution for the between-study variance (Supplementary Table 1). The width of the prediction interval increased but did not affect our inferences. The exclusion of the study by Gao et al. with the high risk of CDAD in the placebo group did not affect the results greatly. The inclusion of the 5 studies that did not have complete results on testing also did not change the pooled risk ratio, though the estimate of the between-study standard deviation decreased owing to ignoring the fact that the proportion of subjects tested was missing (Supplementary Table 1). Accordingly, the prediction interval was narrower, barely crossing 1.

Interpretation

Our meta-analysis suggests that probiotics containing *Lactobacillus* species have a preventative effect on CDAD with a pooled relative risk reduction of 75%. This effect size is consistent with other meta-analyses of various probiotics[6, 7, 11] using the standard DerSimonian and Laird method. However, the wide credible interval around the predicted benefit in a future study includes a risk ratio of 1 and reflects considerable heterogeneity in the included studies. Although on average there appears to be a beneficial effect of probiotics, it is uncertain whether this benefit would be observed in a future study that would meet our inclusion criteria. The observed heterogeneity could not be explained conclusively by study-level characteristics such as risk of bias, background risk of CDAD as measured by frequency in the placebo arm, higher versus lower dose of probiotics, or financial support from industry, due in part to the small number of studies we identified. However, there appears to be suggestive evidence that in order to observe a beneficial effect of CDAD in a single study, a high background risk of CDAD is needed. This leads us to conclude that it is premature to suggest that there is a beneficial effect of *Lactobacillus* probiotics in all settings.

Our conclusion is supported by a Bayesian credibility analysis which suggests that the statistically significant results of the meta-analysis could be altered by use of a weakly informative sceptical prior distribution.

Our inclusion criteria and our requirements for data for adjustment limited the number of studies included, although a sensitivity analysis with excluded studies did not change the results. The meta-regression for important covariates was therefore relatively insensitive on account of the small number of studies in each group. The total number of CDAD cases was small, with the largest number of cases contributed by a study[26] from China with the highest rate of CDAD in the placebo arm (24%, compared with 19% in a study conducted during an outbreak in Quebec hospitals[32]). Omitting that study, however, did not affect the effect estimate. The recent large RCT by Allen et al[9, 35] was planned during an outbreak, and the success of other preventative measures resulted in a lower incidence of CDAD than anticipated and a loss of power, while another trial was stopped early, having reached its co-primary endpoint for AAD before identifying any CDAD cases[29]. Thus we see that a challenge in conducting these RCTs is to attain a sufficient number of CDAD cases to allow estimation of the effect of probiotics. Other potential contributors to under-detection of cases were the insensitivity of the test used to detect CDAD[44] in earlier studies, and insufficiently long follow-up in some studies, since trials that followed patients after discharge identified additional CDAD [32, 33]. Neither of these conditions is expected to contribute to a differential misclassification and thereby introduce bias. We assumed that missing tests had the same probability of being positive as available tests, although, if testing depended upon severity of diarrhea, we may be overestimating the number of positive tests. Nevertheless, our adjustment produced only slight changes to the risk ratios.

Although the studies included in the meta-analysis met our inclusion criteria, they were clinically heterogeneous in factors relating to background risk of CDAD, and species, dose and duration of

probiotic. As is common with studies involving complementary medicines, there was wide variation in dose and formulation. We selected only studies where the probiotic was either a pure *Lactobacillus* preparation or a mixture of *Lactobacillus* and other species but did not restrict the dose, on the assumption that a minimum dose was required for colonization of the colon and that this minimum dose was achieved in all studies.

We did not assess safety in our analysis, since this has been done by others [11, 45, 46]. Probiotics appear to be safe in the population included in the randomized controlled trials, although these trials contained some important exclusions of patients at high risk of infection. More recent reviews have begun to address the safety of probiotics in these patients[47].

Conclusion

Statistical heterogeneity as well as clinical heterogeneity reduces our confidence in the evidence favouring probiotics containing *Lactobacillus* species for reducing the risk of *C difficile* associated infection in patients receiving antibiotics. This is consistent with the results of a recently released clinical trial of *Lactobacillus* in elderly patients, although for both the meta-analysis and the trial the number of cases was relatively few. Future research will need to take account of the changing epidemiology of *C difficile*, with reduced incidence in hospital settings where active prevention measures have been taken, and identification of risks in the community, and adjust trial sizes and settings accordingly.

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Table 1: Summary of design and risk of bias in randomized controlled trials of probiotics in the prevention of CDAD

Author, year	Definition of diarrhea	Method for detecting *C difficile*	Sample size Pro; Pla	Probiotics[1] (cfu x 10^9)	Treatment duration (days)	Follow-up (days)	Risk of bias[2]
Ouwehand, 2014	≥3 stools Bristol scale 7	GDH and toxin A EIA; Toxin B by cell culture	Pro-2: 168 Pro-1: 168 Pla: 167	LA, LPl, B (Capsule; L Pro-1 2.08, Pro-2 8.5)	Abx[3] + 7	4 (post abx)	A
Allen, 2013	≥3 stools Bristol scale 5-7, or looser than normal by patient report) in 24 hours.	In-house tissue culture assay + EIA for toxins or ELFA for toxins or GDH and toxin A EIA	Pro: 1493 Pla: 1488	LA, B (Capsule; all 60)	21 days	8 weeks total	A
Selinger, 2013	≥3 stools Bristol scale 6-7	ELFA for toxins or GDH and toxin A EIA	112; 117	LA, LPl, LPa, LD, B, ST (Powder			B
Gao, 2010	≥3 liquid stools in 24 hours	Triage panel (unspecified) and Toxin B by cell culture	Pro-2: 86 Pro-1: 85 Pla: 84	LA and LC (Capsule Pro-1 L 50; Pro-2 L 100)	Abx3 + 5	21	A
Sampalis, 2010	≥1 episodes unformed or liquid stool in 24 hours	Toxin A and B (not specified)	216; 221	LA and LC (Milk; L 50)	Abx3 + 5	21	C
Safdar, 2008	Either watery or liquid stools for ≥2 consecutive days.	Toxin (not specified)	23; 17	LA (Capsule; L 60)	Pro: 22.8(9.4) Pla: 24.5(4.8)	0	B

[1] Dose of probiotics is given in the form of colony-forming units unless specified otherwise. Where two figures are given, the first represents the total dose of *Lactobacillus* species (L), and the second the dose of other species (O). Abbreviations: N, number; Pro, probiotics; Pla, placebo; comm., commercial; NA, not available; SD, standard deviation; B, *Bifidobacterium*; BC, *B. clausii*; LA; *Lactobacillus* acidophilus; LC, *Lactobacillus casei*; LB= *Lactobacillus bulgaricus*; LD, *Lactobacillus delbrueckii* subsp. *Bulgaricus*; LR, *Lactobacillus rhamnosus*; LPa, *Lactobacillus paracasei*, LPl=*Lactobacillus plantarum*; SB, *Saccharomyces boulardii*; ST, *Streptococcus thermophilus*; CB, *Clostridium butyricum*.

[2] We assessed risk of bias of RCTs included in the meta-analysis according to the Cochrane criteria, mainly focusing on selection bias, performance bias, and attrition bias [11]. RCT quality was categorized into 3 levels, A (low), B (moderate) and C (high).

[3] Probiotics were given for the duration of antibiotic therapy (plus an additional number of days, if indicated)

Author, year	Definition of diarrhea	Method for detecting *C difficile*	Sample size Pro; Pla	Probiotics[1] (cfu x 10^9)	Treatment duration (days)	Follow-up (days)	Risk of bias[2]
Beausoleil, 2007	≥3 liquid stools in 24 hours	Cytotoxin assay (not specified)	44; 45	LA and LC (Milk; L 50)	Abx3	21	C
Hickson, 2007	≥2 liquid stools a day in excess of normal for ≥2 days	Toxin (not specified)	69; 66	LC, LB, ST (Yogurt; L 22, O 20)	Abx3 + 7	28	A
Plummer, 2004	N.A.	Latex agglutinin or EIA for toxins	Elderly	LA and B (Capsule, total 20)	20	0	C
Heimburger, 1994	>200g stool in 24 hours	Culture and titres	16; 18	LA, LB (Granules, dose not given)	≥5	0	C

Table 2: Summary of efficacy and risk of bias in randomized controlled trials of probiotics on AAD and CDAD

Author (year)	AAD no./total no. (%)		P-value	CDAD [a] no./total no.[b] (%)		P-value	Risk of bias[c]	Industry funded[d]
	Pro	Pla		Pro	Pla			
Ouwehand, 2014	33/168 (19.6); 21/168 (12.5)	41/167 (24.6)	<0.05	3/33 (9.0); 3/21 (14.2)	8/41 (19.5)	<0.05	A	Yes
Allen, 2013	159/1493 (10.6)	153/1488 (10.3)	≥0.05	12/93 [e] (12.9)	17/88 [e] (19.3)	≥0.05	A	No
Selinger, 2012	5/117 (4.3)	10/112 (8.9)	<0.05	0/5 (0)	0/4 [e] (0)	-	B	Yes
Gao, 2010	13/86 (15.5); 24/85 (28.2)	37/84 (44.1)	<0.05	1/13 (7.7); 8/24 (33.3)	20/37 (54.1)	<0.05	A	Yes
Sampalis, 2010	47/216 (21.8)	65/221 (29.4)	≥0.05	1/16 [e] (6.3)	4/30 [e] (13.3)	≥0.05	C	Yes
Safdar, 2008	4/23 (17)	6/16 (37)	≥0.05	0/3 [e] (0)	1 /4 [e] (25)	≥0.05	B	Yes
Beausoleil, 2007	7/44 (16)	16/45 (36)	<0.05	1/2 [e] (50)	7/13 [e] (53.8)	≥0.05	C	Yes
Hickson, 2007	7/57 (12)	19/56 (34)	<0.05	0/56 (0)	9/53 (17)	<0.05	A	No
Plummer, 2004	15/69 (22)	15/69 (22)	≥0.05	2/15 (13)	5/15 (33)	≥0.05	C	No
Heimburger, 1994	5/16 (31)	2/18 (11)	≥0.05	0/5 (0)	0/2 (0)	-	C	No

[a] Definition of CDAD: Diarrhea was present and *C. difficile* test (however defined) was positive in stool sample

[b] Denominator is the number of patients with AAD who were tested for CDAD. Values are prior to adjustment for missing data.

[c] We assessed risk of bias RCTs according to the Cochrane criteria, mainly focusing on selection bias, performance bias, and attrition bias. RCT quality was categorized into 3 levels, A (low), B (moderate) and C (high).

[d] Support from manufacturer included direct study sponsorship. We did not consider supply of investigational material without conditions or support for activities not associated with the study as support from manufacturer.

[e] Adjusted in the analysis for incomplete testing for CDAD.

Table 3 Results of meta-regression models

Potential source of heterogeneity	Number of studies	Regression coefficient (95% credible interval)	P(Regression coefficient > 0)[a]
Study quality	A: 4 B or C: 6	0.4 (-1.5, 2.5)	0.67
Type of probiotic	Lactobacillus only: 5 Mixture: 5	-0.4 (-2.3, 1.6)	0.30
Probiotic dose	≥50 10^6 CFU[b]: 3 <50 10^6 CFU: 6[c]	0.4 (-1.7, 2.4)	0.69
Support from manufacturer[d]	Yes: 6 No:4	-1.1 (-2.8, 0.7)	0.08
Proportion of CDAD in placebo group, dichotomous[e]	≥6%: 5 <6%: 5	-0.8 (-2.5, 0.8)	0.12
Proportion of CDAD in placebo group, continuous	-	-0.4 (-1.1, 0.6)	0.12

[a] High values of P(Regression coefficient>0) indicate a high probability of strong positive association between the covariate and the pooled risk ratio, whereas low values indicate a strong negative association between the covariate and the risk ratio.

[b] CFU: Colony forming units.

[c] Dose was missing for one study.

[d] Support from manufacturer included direct study sponsorship. We did not consider supply of investigational material without conditions or support for activities not associated with the study as support from manufacturer.

[e] Proportion of CDAD in placebo group was used to represent population risk (in hospitalized patients given antibiotics) of developing CDAD.

Figure 1: Flowchart of study selection

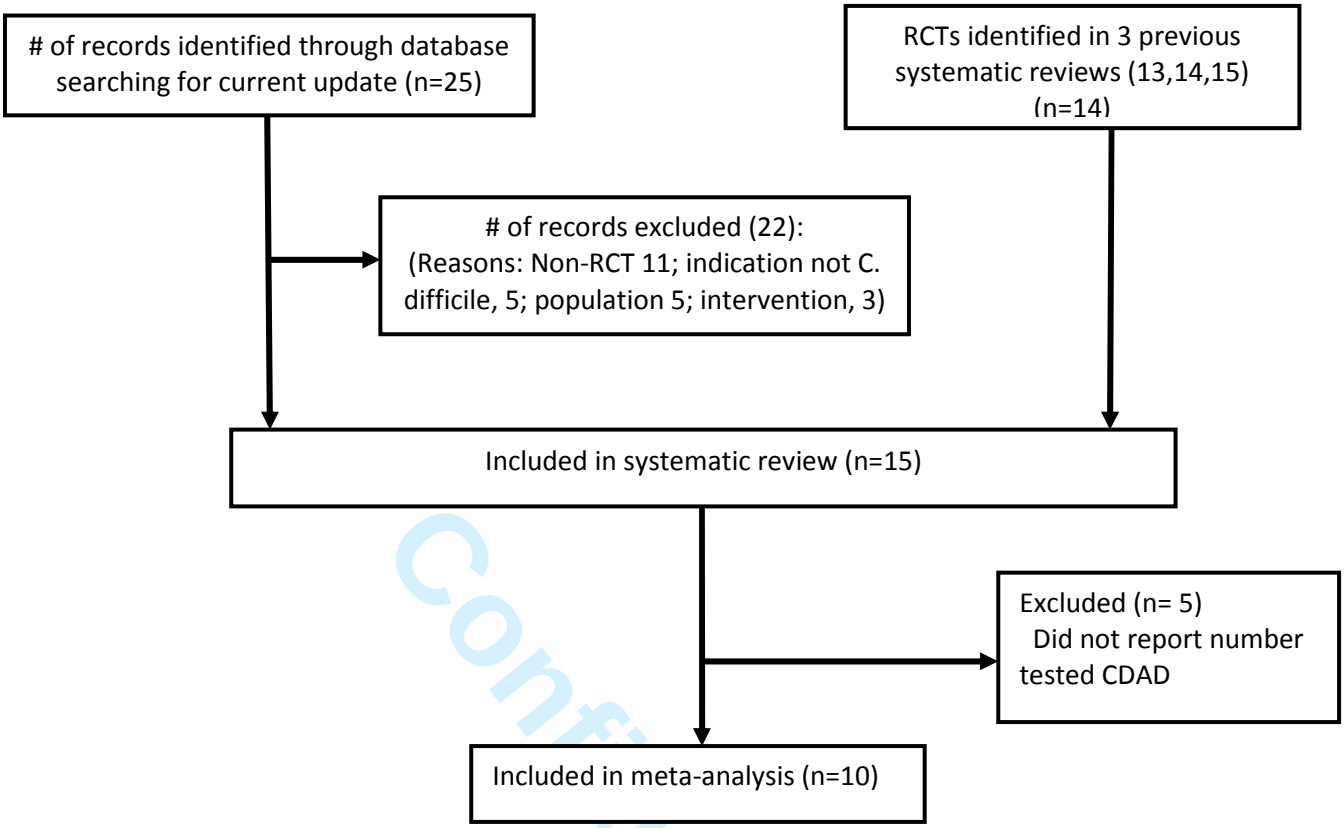
Figure 2: L'Abbé plot of proportion of patients with CDAD in Lactobacillus probiotics and placebo groups in the individual trials included in the meta-analysis.

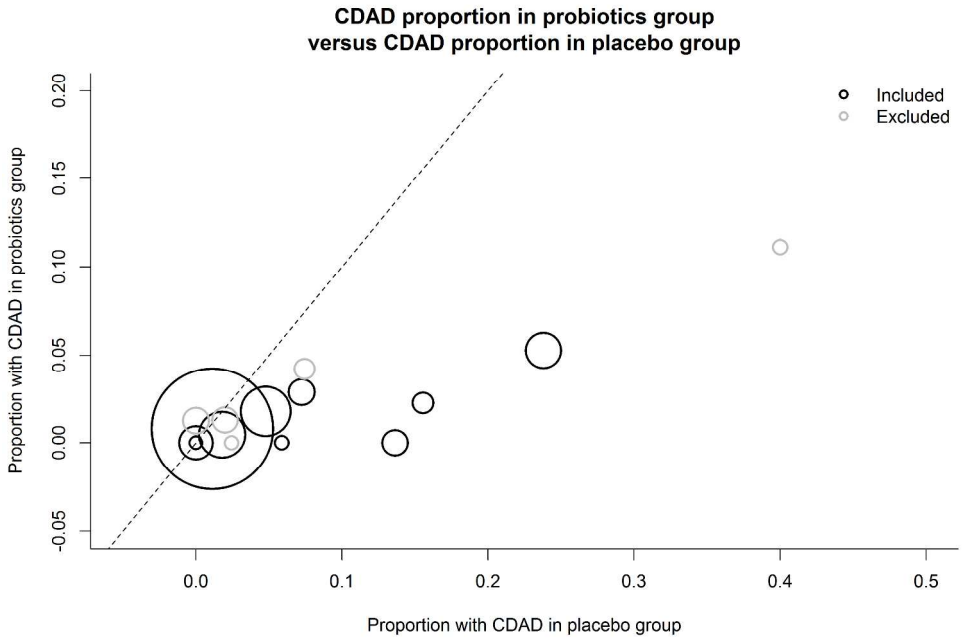
The area of the circles is proportional to the number of patients randomized in the group. Black circles indicate studies included in the primary analysis, in which adjustment was used to estimate missing data. Grey circles indicate studies added for the sensitivity analysis, in which the data for adjustment was not available and patients with missing data were assumed to be CDAD negative.

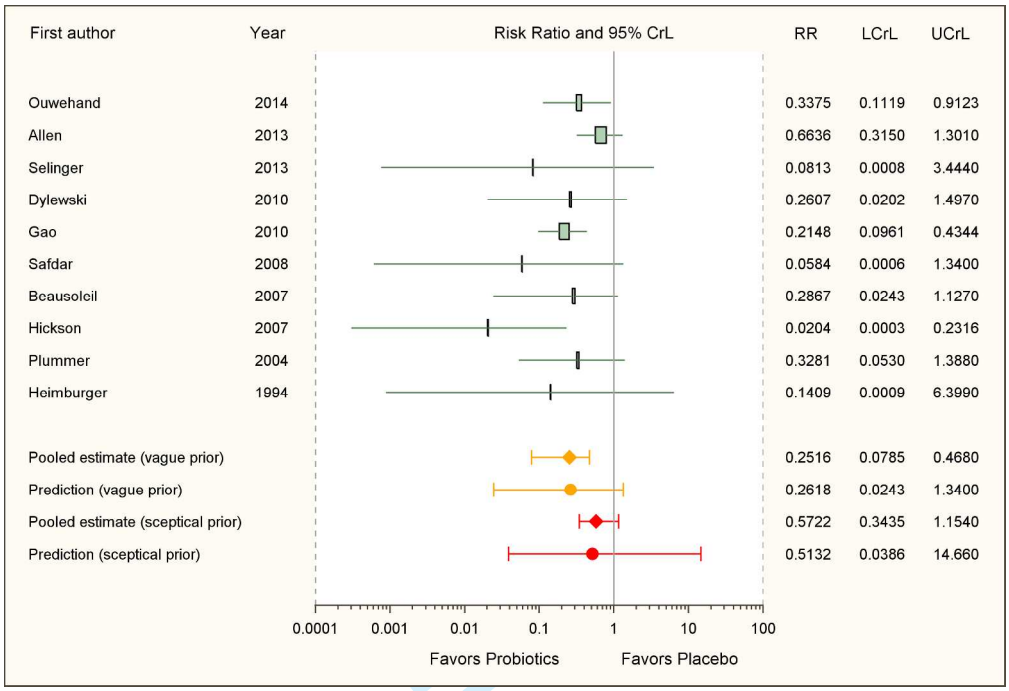
Figure 3: Forest plot of effect of Lactobacillus on prevention of CDAD (Bayesian analysis)

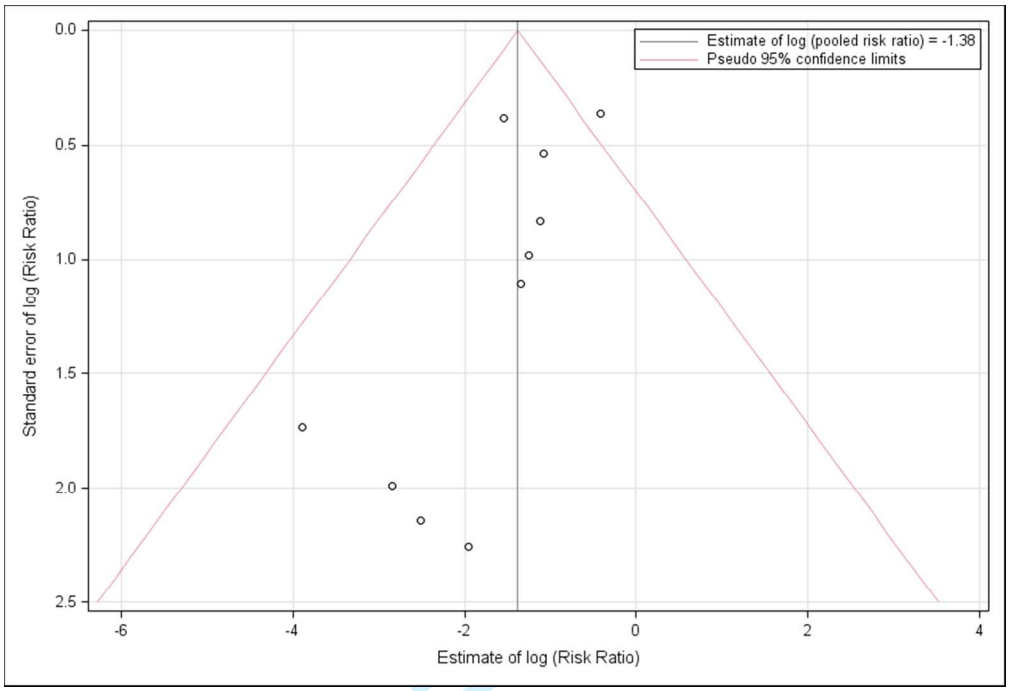
In order from the top, the plot shows the median RRs and 95% credible intervals estimated using the individual studies, the pooled RR and 95% credible interval and the 95% prediction interval estimated using a Bayesian meta-analysis with a vague (non-informative prior). The final two lines are the pooled RR together with the 95% credible and prediction intervals from a Bayesian meta-analysis with the critical sceptical prior.

Figure 4: Funnel plot of studies included in meta-analysis of Lactobacillus in prevention of CDAD









Supplementary Table 1: Results of sensitivity analyses

Analysis	Pooled risk ratio Median (95% credible interval)	95% Prediction interval	Between-study standard deviation in log risk ratio Median (95% credible interval)
Results reported in the main text*	0.25 (0.08, 0.47)	(0.02, 1.34)	0.64 (0.06, 1.75)
Alternative prior over variance I (Standard Deviation** ~ Uniform (0,3))	0.24 (0.07, 0.47)	(0.02, 1.55)	0.65 (0.09, 2.17)
Alternative prior over variance II (Standard Deviation** ~ Cauchy (scale=25))	0.24 (0.07, 0.48)	(0.02, 1.77)	0.66 (0.06, 2.53)
Including studies that did not report results of testing	0.28 (0.15, 0.45)	(0.06, 1.01)	0.44 (0.05, 1.33)
Excluding study by Gao et al.[24]	0.24 (0.06, 0.54)	(0.02, 1.77)	0.80 (0.09, 1.87)

* Between-study standard deviation in log risk ratio ~ U(0,2); ** Between study standard-deviation in log risk ratio

Supplementary Table 2: PRISMA checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	3
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	5
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	5
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	No
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	5,6
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	5
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	5
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	5,6
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	6

Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	6
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	6
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	6
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	6,7