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Probiotics for the prevention of Clostridium difficile-associated diarrhea in adults and children (Review)

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[Intervention Review]

Probiotics for the prevention of Clostridium difficile-associated diarrhea in adults and children

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ABSTRACT

Background

Antibiotics can disturb gastrointestinal microbiota which may lead to reduced resistance to pathogens such as *Clostridium difficile* (*C. difficile)*. Probiotics are live microbial preparations that, when administered in adequate amounts, may confer a health benefit to the host, and are a potential *C. difficile* prevention strategy. Recent clinical practice guidelines do not recommend probiotic prophylaxis, even though probiotics have the highest quality evidence among cited prophylactic therapies.

Objectives

To assess the efficacy and safety of probiotics for preventing *C.difficile*-associated diarrhea (CDAD) in adults and children.

Search methods

We searched PubMed, EMBASE, CENTRAL, and the Cochrane IBD Group Specialized Register from inception to 21 March 2017. Additionally, we conducted an extensive grey literature search.

Selection criteria

Randomized controlled (placebo, alternative prophylaxis, or no treatment control) trials investigating probiotics (any strain, any dose) for prevention of CDAD, or *C. difficile* infection were considered for inclusion.

Data collection and analysis

Two authors (independently and in duplicate) extracted data and assessed risk of bias. The primary outcome was the incidence of CDAD. Secondary outcomes included detection of *C. difficile* infection in stool, adverse events, antibiotic-associated diarrhea (AAD) and length of hospital stay. Dichotomous outcomes (e.g. incidence of CDAD) were pooled using a random-effects model to calculate the risk ratio (RR) and corresponding 95% confidence interval (95% CI). We calculated the number needed to treat for an additional beneficial outcome (NNTB) where appropriate. Continuous outcomes (e.g. length of hospital stay) were pooled using a random-effects model to calculate the mean difference and corresponding 95% CI. Sensitivity analyses were conducted to explore the impact of missing data on efficacy and safety outcomes. For the sensitivity analyses, we assumed that the event rate for those participants in the control group who had missing data was the same as the event rate for those participants in the control group who were successfully followed. For the probiotic group, we

calculated effects using the following assumed ratios of event rates in those with missing data in comparison to those successfully followed: 1.5:1, 2:1, 3:1, and 5:1. To explore possible explanations for heterogeneity, a priori subgroup analyses were conducted on probiotic species, dose, adult versus pediatric population, and risk of bias as well as a post hoc subgroup analysis on baseline risk of CDAD (low 0% to 2%; moderate 3% to 5%; high > 5%). The overall quality of the evidence supporting each outcome was independently assessed using the GRADE criteria.

Main results

Thirty-nine studies (9955 participants) met the eligibility requirements for our review. Overall, 27 studies were rated as either high or unclear risk of bias. A complete case analysis (i.e. participants who completed the study) among trials investigating CDAD (31 trials, 8672 participants) suggests that probiotics reduce the risk of CDAD by 60%. The incidence of CDAD was 1.5% (70/4525) in the probiotic group compared to 4.0% (164/4147) in the placebo or no treatment control group (RR 0.40, 95% CI 0.30 to 0.52; GRADE = moderate). Twenty-two of 31 trials had missing CDAD data ranging from 2% to 45%. Our complete case CDAD results proved robust to sensitivity analyses of plausible and worst-plausible assumptions regarding missing outcome data and results were similar whether considering subgroups of trials in adults versus children, inpatients versus outpatients, different probiotic species, lower versus higher doses of probiotics, or studies at high versus low risk of bias. However, in a post hoc analysis, we did observe a subgroup effect with respect to baseline risk of developing CDAD. Trials with a baseline CDAD risk of 0% to 2% and 3% to 5% did not show any difference in risk but trials enrolling participants with a baseline risk of > 5% for developing CDAD demonstrated a large 70% risk reduction (interaction P value = 0.01). Among studies with a baseline risk > 5%, the incidence of CDAD in the probiotic group was 3.1% (43/1370) compared to 11.6% (126/1084) in the control group (13 trials, 2454 participants; RR 0.30, 95% CI 0.21 to 0.42; GRADE = moderate). With respect to detection of *C. difficile* in the stool pooled complete case results from 15 trials (1214 participants) did not show a reduction in infection rates. *C. difficile* infection was 15.5% (98/633) in the probiotics group compared to 17.0% (99/581) in the placebo or no treatment control group (RR 0.86, 95% CI 0.67 to 1.10; GRADE = moderate). Adverse events were assessed in 32 studies (8305 participants) and our pooled complete case analysis indicates probiotics reduce the risk of adverse events by 17% (RR 0.83, 95% CI 0.71 to 0.97; GRADE = very low). In both treatment and control groups the most common adverse events included abdominal cramping, nausea, fever, soft stools, flatulence, and taste disturbance.

Authors' conclusions

Based on this systematic review and meta-analysis of 31 randomized controlled trials including 8672 patients, moderate certainty evidence suggests that probiotics are effective for preventing CDAD (NNTB = 42 patients, 95% CI 32 to 58). Our post hoc subgroup analyses to explore heterogeneity indicated that probiotics are effective among trials with a CDAD baseline risk >5% (NNTB = 12; moderate certainty evidence), but not among trials with a baseline risk ≤5% (low to moderate certainty evidence). Although adverse effects were reported among 32 included trials, there were more adverse events among patients in the control groups. The short-term use of probiotics appears to be safe and effective when used along with antibiotics in patients who are not immunocompromised or severely debilitated. Despite the need for further research, hospitalized patients, particularly those at high risk of CDAD, should be informed of the potential benefits and harms of probiotics.

PLAIN LANGUAGE SUMMARY

The use of probiotics to prevent *Clostridium difficile* diarrhea associated with antibiotic use

What is *Clostridium difficile*-associated diarrhea?

Antibiotics are among the most prescribed medications worldwide. Antibiotic treatment may disturb the balance of organisms that normally populate the gut. This can result in a range of symptoms, most notably, diarrhea. *Clostridium difficile* (*C. difficile*) is a particularly dangerous organism that may colonize the gut if the normal healthy balance has been disturbed. *Clostridium difficile*-related disease varies from asymptomatic infection, diarrhea, colitis, and pseudo-membranous colitis to toxic megacolon and death. The cost of treatment is expensive and the financial burden on the medical system is substantial.

What are probiotics?

Probiotics are live organisms (bacteria or yeast). thought to improve the balance of organisms that populate the gut, counteracting potential disturbances to the gut microbial balance that are associated with antibiotic use, and reducing the risk of colonization by pathogenic bacteria. Probiotics can be found in dietary supplements or yogurts and are becoming increasingly available as capsules sold in health food stores and supermarkets. As 'functional food' or 'good bacteria', probiotics have been suggested as a means of both preventing and treating *C. difficile*-associated diarrhea (CDAD).

What did the researchers investigate?

The researchers investigated whether probiotics prevent CDAD in adults and children receiving antibiotic therapy and whether probiotics causes any harms (side effects). The researchers searched the medical literature extensively up to 21 March 2017.

What did the researchers find?

This review includes 39 randomized trials with a total of 9955 participants. Thirty-one studies (8672 participants) assessed the effectiveness of probiotics for preventing CDAD among participants taking antibiotics. Our results suggest that when probiotics are given with antibiotics the risk of developing CDAD is reduced by 60% on average. Among trials enrolling participants at high risk of developing CDAD (> 5%), the potential benefit of probiotics is more pronounced with a 70% risk reduction on average. Side effects were assessed in 32 studies (8305 participants) and our results suggest that taking probiotics does not increase the risk of developing side effects. The most common side effects reported in these studies include abdominal cramping, nausea, fever, soft stools, flatulence, and taste disturbance. The short-term use of probiotics appears to be safe and effective when used along with antibiotics in patients who are not immunocompromised or severely debilitated. Despite the need for further research, hospitalized patients, particularly those at high risk of CDAD, should be informed of the potential benefits and harms of probiotics.

Summary of findings for the main comparison. Probiotics compared to control for preventing *C. difficile* associated diarrhea

Probiotics compared to control for preventing *C. difficile* associated diarrhea

Patient or population: preventing *C. difficile* associated diarrhea
Setting: inpatient and outpatient
Intervention: probiotics
Comparison: control

Outcomes	Anticipated absolute effects* (95% CI)		Relative effect (95% CI)	№ of participants (studies)	Certainty of the evidence (GRADE)	Comments
	Risk with control	Risk with probiotics				
Incidence CDAD: complete case	Study population		RR 0.40 (0.30 to 0.52)	8672 (31 RCTs)	⊕⊕⊕⊖ MODERATE [1]	Note: Risk with control calculated by pooled event rate across control groups.
	40 per 1,000	16 per 1,000 (12 to 21)				
CDAD (baseline risk 0-2%)	Study population		RR 0.77 (0.45 to 1.32)	5845 (15 RCTs)	⊕⊕⊕⊖ MODERATE [2]	
	11 per 1,000	8 per 1,000 (5 to 14)				
CDAD (baseline risk 3-5%)	Study population		RR 0.53 (0.16 to 1.77)	373 (3 RCTs)	⊕⊕⊖⊖ LOW [3 4]	
	38 per 1,000	20 per 1,000 (6 to 67)				
CDAD (baseline risk >5%)	Study population		RR 0.30 (0.21 to 0.42)	2454 (13 RCTs)	⊕⊕⊕⊖ MODERATE [5]	
	116 per 1,000	35 per 1,000 (24 to 49)				
Incidence of *C. difficile* infection: complete case	Study population		RR 0.86 (0.67 to 1.10)	1214 (15 RCTs)	⊕⊕⊕⊖ MODERATE [6]	
	170 per 1,000	147 per 1,000 (114 to 187)				
Adverse Events: complete case	Study population		RR 0.83 (0.71 to 0.97)	8305 (32 RCTs)	⊕⊖⊖⊖ VERY LOW [7 8 9]	

	170 per 1,000	141 per 1,000 (121 to 165)			
Incidence AAD: complete case	Study population		RR 0.58 (0.48 to 0.70)	8870 (33 RCTs)	⊕⊕⊖⊖ LOW [10] [11]
	181 per 1,000	105 per 1,000 (87 to 127)			
AAD (Adults)	Study population		RR 0.62 (0.51 to 0.76)	7036 (23 RCTs)	⊕⊕⊖⊖ LOW [12] [13]
	174 per 1,000	108 per 1,000 (89 to 133)			
AAD (Children)	Study population		RR 0.38 (0.29 to 0.49)	1141 (6 RCTs)	⊕⊕⊕⊖ MODERATE [14]
	271 per 1,000	103 per 1,000 (79 to 133)			
Length of Hospital Stay: complete case	The mean length of Hospital Stay: complete case was 0 days	MD 0.17 days fewer (1.03 fewer to 0.68 more)	-	3484 (4 RCTs)	⊕⊕⊕⊖ MODERATE [15]

*__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; __OR:__ Odds ratio;

GRADE Working Group grades of evidence
High certainty: We are very confident that the true effect lies close to that of the estimate of the effect
Moderate certainty: We are moderately confident in the effect estimate: The true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different
Low certainty: Our confidence in the effect estimate is limited: The true effect may be substantially different from the estimate of the effect
Very low certainty: We have very little confidence in the effect estimate: The true effect is likely to be substantially different from the estimate of effect

[1] Much of the data for the overall effect estimate involves high baseline risk trials and therefore we have concerns of indirectness at the population level when applying to more moderate or lower baseline risk situations.
[2] Confidence intervals are wide and cross the line of no effect.
[3] Too few trials to conduct tests for publication bias.
[4] Confidence intervals are wide and cross the line of no effect.
[5] Since there is some residual uncertainty in our estimate of effect due to heterogeneity (i.e. post hoc subgroup) and imprecision (a modest sample size and a modest number of events), while minimal individually, when viewed together we have graded down one level.
[6] Total event rate of all 15 studies is very low (197) and the 95% confidence interval includes both no effect and a substantial effect size. We therefore rated down for imprecision.
[7] The improvement in AE seen in the primary analysis did not survive sensitivity analysis on missing outcome data and subgroup analysis suggests a trend (P = 0.06) of a subgroup difference along risk of bias with low risk of bias studies suggesting no difference in AE between probiotics and control.

[8] There was statistically significant heterogeneity (I^2 = 49%; P < 0.01)

[9] Visual inspection of the funnel plot and statistical analysis using the Harbord linear regression test were suggestive of small study effects (e.g. publication bias).

[10] Statistically significant heterogeneity was detected (I^2 = 61%; P < 0.01)

[11] Visual inspection of the funnel plot and statistical assessment via Harbord linear regression test were suggestive of small study effects (e.g. publication bias) (P = 0.03)

[12] Statistically significant heterogeneity noted between studies (I^2 = 59%; P < 0.01)

[13] Visual inspection of the funnel plot and statistical assessment via Harbord linear regression test were suggestive of small study effects (e.g. publication bias) (P = 0.02)

[14] With n=6 Harbord's linear regression test is underpowered to detect a significant interaction, however visual inspection of the funnel plot is suspicious for publication bias. Also, due to the review's inclusion criteria specific to CDAD not AAD we worry about the possibility of publication bias here.

[15] We suspect selective outcome reporting bias as only 4 of the identified trials, most of which occurred in hospitals, reported on length of hospital stay - a presumably important patient and hospital outcome. Of the four studies reporting on length of stay, one had an unclear risk of bias and three were rated as having a low risk of bias.

BACKGROUND

Description of the condition

Antibiotics are among the most prescribed medications worldwide. Antibiotic treatment may disturb the colonization resistance of gastrointestinal flora, resulting in a range of symptoms, most notably, diarrhea. Antibiotic use results in a depletion of the normal microflora, which allows *Clostridium difficile* (*C. difficile*) to more easily colonize and infect patients. The spectrum of *C. difficile*-related disease varies from asymptomatic intestinal colonization, diarrhea, colitis, and pseudo-membranous colitis to toxic megacolon and death (Evans 2015). In the United States, cost estimates of *C. difficile* infection for hospitalized patients range from USD 8911 to USD 30,049 (Nanwa 2015).

Description of the intervention

Probiotics are live organisms thought to improve the microbial balance of the host, counteracting potential disturbances in intestinal flora associated with antibiotic use, and reducing the risk of colonization by pathogenic bacteria (Sullivan 2002). Probiotics are becoming increasingly available as capsules and dairy-based food supplements sold in health food stores and supermarkets with use among consumers increasing four fold from 2007 to 2012 (Clarke 2015).

How the intervention might work

As 'functional food'' or 'good bacteria or yeast', probiotics have been suggested as a means of preventing *C. difficile*-associated diarrhea (CDAD). Proposed mechanisms of action include immune system stimulation as well as the ability to limit colonisation, adhesion, and invasion of the *Clostridium difficile* organism (Parkes 2009).

Why it is important to do this review

If effective, the low cost as well as the low incidence of adverse events may make probiotics an attractive intervention for preventing *Clostridium difficile*-related disease (Hempel 2012). This Cochrane systematic review is an update of a previously published systematic review (Goldenberg 2013).

OBJECTIVES

The primary objectives were to assess the efficacy and safety of probiotics for the prevention of *C. difficile*-associated diarrhea in adults and children.

METHODS

Criteria for considering studies for this review

Types of studies

Randomized controlled trials (RCT) reporting incidence outcomes for CDAD (diarrhea and detection of *C. difficile* toxin in stool) or detection of *C. difficile* (detection of *C. difficile* or toxin in stool) were considered for inclusion.

Types of participants

Participants included adult (> 18 years) and paediatric patients (0 to 18 years of age) receiving antibiotic therapy for any reason.

Types of interventions

The interventions of interest compared probiotics (any strain or dose) versus placebo, alternative prophylaxis, or no treatment for the prevention of *C. difficile*-associated diarrhea in adults and children receiving antibiotic therapy. Studies using probiotics for the treatment of *C. difficile* infection were excluded.

Types of outcome measures

Primary outcomes

The primary outcome was the incidence of *C. difficile*-associated diarrhea.

Secondary outcomes

Secondary outcomes included detection of *C. difficile* or toxin in stool, adverse events, antibiotic-associated diarrhea, and length of hospital stay.

Search methods for identification of studies

Electronic searches

On 21 March 2017, we performed a comprehensive search using the following electronic databases: PubMed (1966 to 2017), EMBASE (1966 to 2017), CENTRAL (inception to 2017), and the Cochrane IBD Group Specialized Register. Searches included both controlled vocabulary (e.g. Probiotics, Cultured Milk Products) and text words (e.g. 'fermented foods', gastroenteritis). No language, publication status, or date limits were applied. Each search strategy was adapted for the particular database. See Appendix 1 for the search strategies.

Searching other resources

In addition, reference lists for relevant studies and systematic reviews were checked to make sure all cited RCTs had been identified in the electronic searches. BIOSIS (Thomson Reuters; 1969 to 2016), and Scopus (1996 to 2016) were searched specifically for conference proceedings as well as the British Society of Gastroenterology Annual General Meeting abstracts (years: 2006 to 2016) and The American Gastroenterological Association's Digestive Disease Week (years: 2009 to 2016). Authors of pertinent presentations were contacted for further information. The following sources were also reviewed: Canadian Agency for Drugs and Technologies in Health; McGill University Health Centre, Technology Assessment Unit; trial registers, e.g. ISRCTN, WHO ICTRP, and ClinicalTrials.gov, dissertations abstracts (ProQuest's Dissertations and Theses); TRIP Database Highwire Press; and the Directory of Open Access Journals (DOAJ).

Data collection and analysis

Selection of studies

Using pre-specified eligibility criteria, two authors independently screened titles and abstracts for potential full text eligibility. If reviewers deemed any title or abstract as potentially eligible, the articles were retrieved for full-text eligibility assessment. Two authors independently assessed the eligibility of each full-text article. Disagreement was resolved by consensus.

Data extraction and management

Teams of two authors (JZG, LL, SM, BCJ) independently extracted data on patients, methods, interventions, and outcomes,

using a pre-constructed, standardized data extraction form. We extracted information on the number of patients allocated to each group, presence or absence of intention-to-treat analysis (whether patients for whom data were available were analyzed as randomized), and the number of participants with missing outcome data. If follow-up was incomplete, we extracted any reported reasons for missing data and information about methods of imputation. Disagreement was resolved by a third adjudicator. For articles published in abstract form only, further information was sought by contacting principal authors.

Assessment of risk of bias in included studies

Two authors (JZG, LL) independently assessed the risk of bias in the individual RCTs as described in the*Cochrane Handbook for Systematic Reviews of Interventions* (Higgins 2011). Risk of bias factors assessed were sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessors, incomplete outcome data, selective outcome reporting, and other sources of bias (e.g. distribution of baseline characteristics, industry initiation and funding, study stopped early).

Assessment of the quality of evidence

We rated the overall quality of evidence (i.e. certainty in effect-estimates) for each of the outcomes in the meta-analyses using the GRADE approach where randomised trials begin as high quality evidence, but may be rated down by one or more of five categories of limitations: (1) risk of bias, (2) inconsistency, (3) indirectness, (4) imprecision, and (5) reporting bias (Guyatt 2008). Rating of the overall quality of evidence for each outcome was done independently and in duplicate (BCJ, JZG) with disagreement resolved by consensus.

Statistical analysis

Statistical software used for data analysis included the RevMan Analyses statistical package in Review Manager (Review Manager 2014) and the statistical packages 'meta,' 'metafor,' 'rmeta' and 'extfunnel' within the statistical environment of R (R version 2.14.1) (Langan 2012; Lumley 2009; R Development Core Team 2010; Schwarzer 2010; Viechtbauer 2010).

Measures of treatment effect

Using a random-effects model, dichotomous data were reported as a risk ratio (RR) along with corresponding 95% confidence intervals (95% CI). The number needed to treat for an additional beneficial outcome (NNTB) or the number needed to treat for an additional harmful outcome (NNTH) were also calculated for each outcome as appropriate, as well as the absolute risk expressed as both a percentage and as natural units (See Summary of findings for the main comparison). For calculating natural units (risk per 1000 patients), the control group risk estimates come from the pooled estimate of the control arm of the meta-analysis.

Unit of analysis issues

If a trial had multiple intervention arms (such as low dose and high dose compared to placebo) we grouped the two probiotic arms together to avoid unit of analysis errors. However, to avoid losing important dose information for our subgroup meta-regression, we kept the probiotic arms intact and split the control group so that half served as comparator for each arm.

Dealing with missing data

For missing outcome data we followed recently published guidance for systematic reviewers on this topic and chose to use a complete case analysis (sometimes referred to as an available case analysis) for our primary analysis. For such an analysis, participants with missing outcome data are excluded from both the numerator and denominator when calculating risk ratios (or other dichotomous presentation methods). To investigate the potential bias from the exclusion of these participants, we then compared the complete case analysis to a series of sensitivity analyses to explore the impact of missing outcome data on efficacy results (Akl 2013).

For the purposes of this systematic review missing outcome data can be understood as incomplete ascertainment of the primary CDAD outcome for some participants. Patients for whom data were not available for the primary outcome were classified as 'missing'. If some trial participants who developed diarrhea were not tested for *C. difficile* (therefore not allowing for the determination of whether the outcome was AAD or CDAD), we also considered this missing outcome data for CDAD.

For the sensitivity analyses, we assumed that the event rate for those participants in the control group who had missing data was the same as the event rate for those participants in the control group who were successfully followed. For the probiotic group we calculated effects using the following assumed ratios of event rates in those with missing data in comparison to those successfully followed: 1.5:1, 2:1, 3:1, and 5:1 (Akl 2012). We then determined if the sensitivity results withstood the range of assumptions.

Assessment of heterogeneity

Heterogeneity in systematic reviews is generally described as clinical, methodological, and statistical (Higgins 2011). Clinical and methodological heterogeneity may or may not be reflected within formal tests for statistical heterogeneity but may still be present and important to investigate (Gagnier 2013). Our a priori clinical heterogeneity variables included: adult versus pediatric population, with a postulated larger effect in adults for CDAD and children for AAD (Hempel 2012); inpatients versus outpatients, with a postulated larger effect among inpatients; probiotic species, with a larger effect expected in trials of *Saccharomyces boulardii* (*S. boulardii*) or *Lactobacillus rhamnosus* (*L. rhamnosus*) (Johnston 2011); dosage of probiotic, with an expected larger effect in trials administering an increased dose (Johnston 2006; Johnston 2011); and, finally, the risk of bias, with an expected larger effect in trials at high or unclear risk of bias versus trials at low risk of bias (Higgins 2011). We also conducted a post hoc subgroup analysis on baseline risk for our primary outcome. We subjected all subgroups to the proposed checklist of subgroup credibility criteria (Sun 2014), including statistical tests such as the Chi^2 and I^2 statistics.

Assessment of reporting biases

To evaluate the potential for publication bias and other small study effects, we followed recently published guidelines and inspected the funnel plots for each outcome for visual evidence of asymmetry and then conducted Harbord's liner regression test to investigate the statistical evidence of small study effects (Harbord 2006; Sterne 2011).

Data synthesis

We conducted a meta-analysis with subgroup and sensitivity analyses as described in detail above.

Subgroup analysis and investigation of heterogeneity

Subgroup analyses were conducted in line with recently published guidance for systematic reviews as described in detail above (Gagnier 2013; Sun 2012).

Sensitivity analysis

We conducted a sensitivity analysis on missing outcome data assuming increasing ratios of event rates among those with missing data in comparison to those successfully followed (Akl 2012). We describe this in detail above. In the case of clear outliers we conducted a sensitivity analysis by removing the outlier trial and comparing the new meta-analytic result to the result before removal (Gagnier 2013).

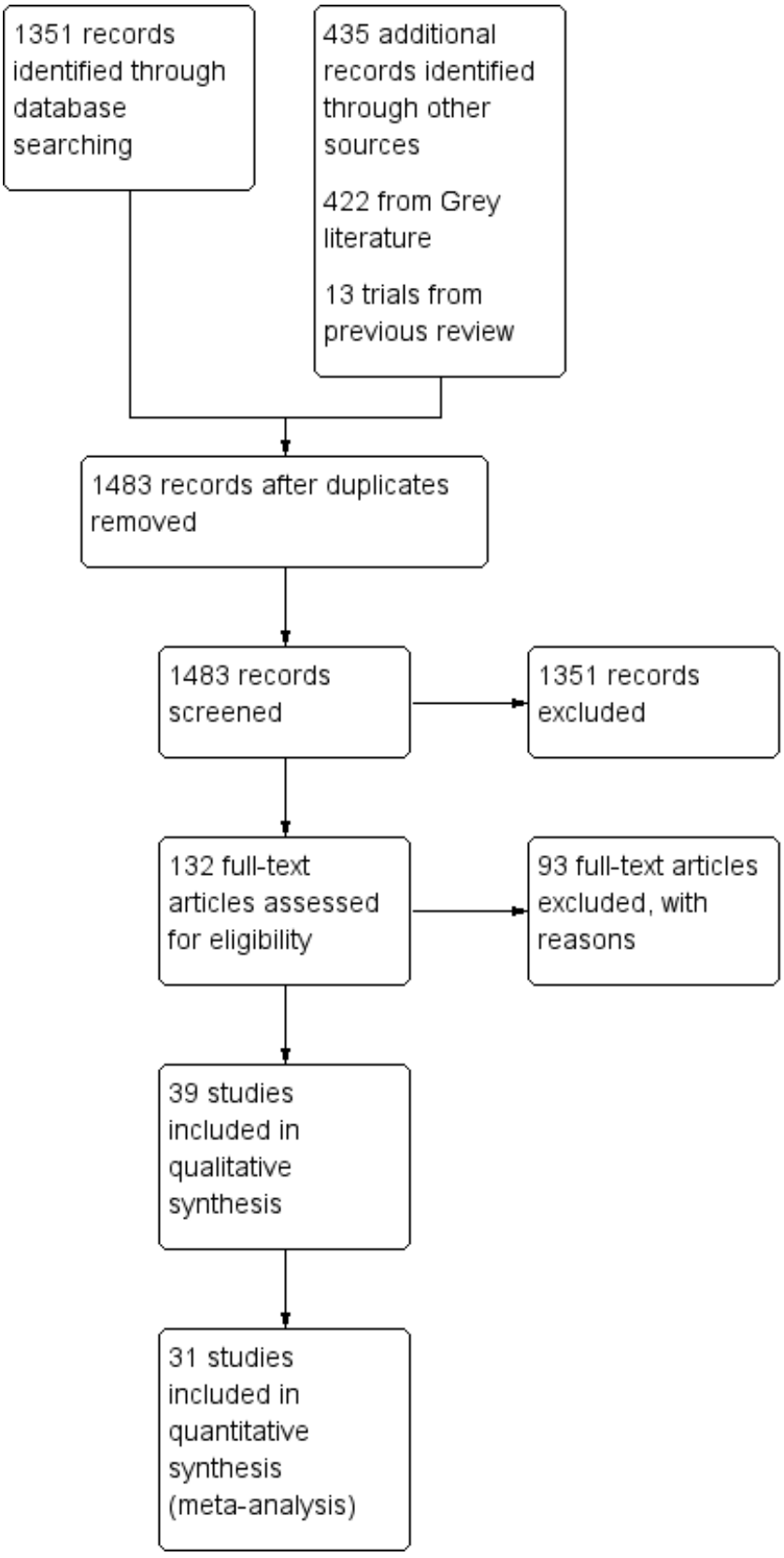
RESULTS

Description of studies

Results of the search

A total of 1351 studies were identified from the primary electronic databases with another 422 from grey literature sources and 13 trials from the previous version of this review. After removal of duplicates, a total of 1483 records remained for screening. One hundred and thirty-two studies were eligible for full text review and 93 studies were excluded with reasons (See Characteristics of excluded studies) yielding a total of 39 studies (9955 participants) that fit the inclusion criteria. Figure 1 summarizes the flow of studies.

Figure 1. Study flow diagram.



Risk of bias in included studies

The risk of bias for each study was assessed for all outcomes as described in the *Cochrane Handbook* (Higgins 2011), and overall results are discussed with effects of interventions below. Figure 2 displays the risk of bias by domains and by study.

Figure 2. Risk of bias summary: review authors' judgements about each risk of bias item for each included study. Blanks cells indicate that this outcome was not assessed in the study.

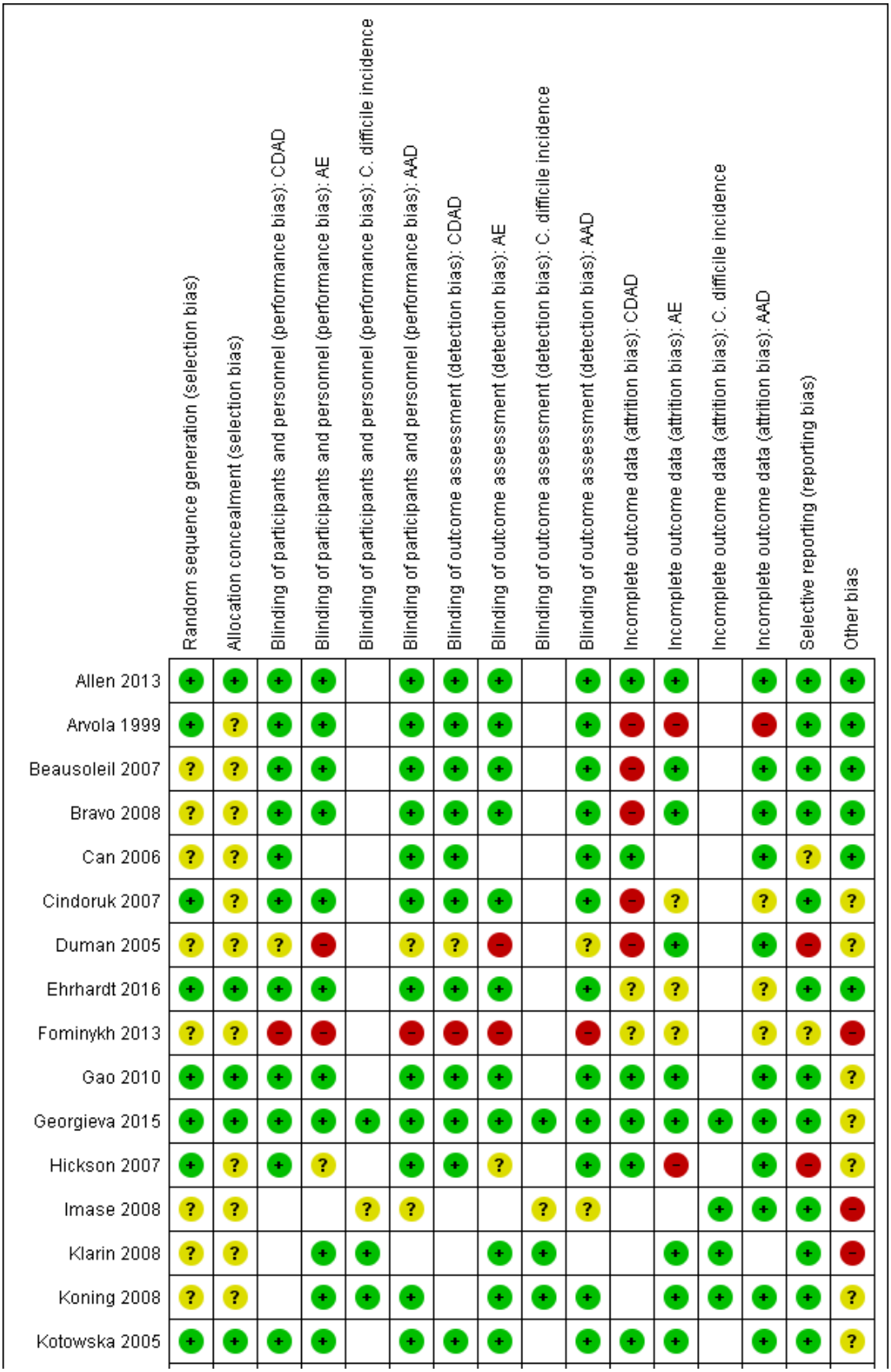
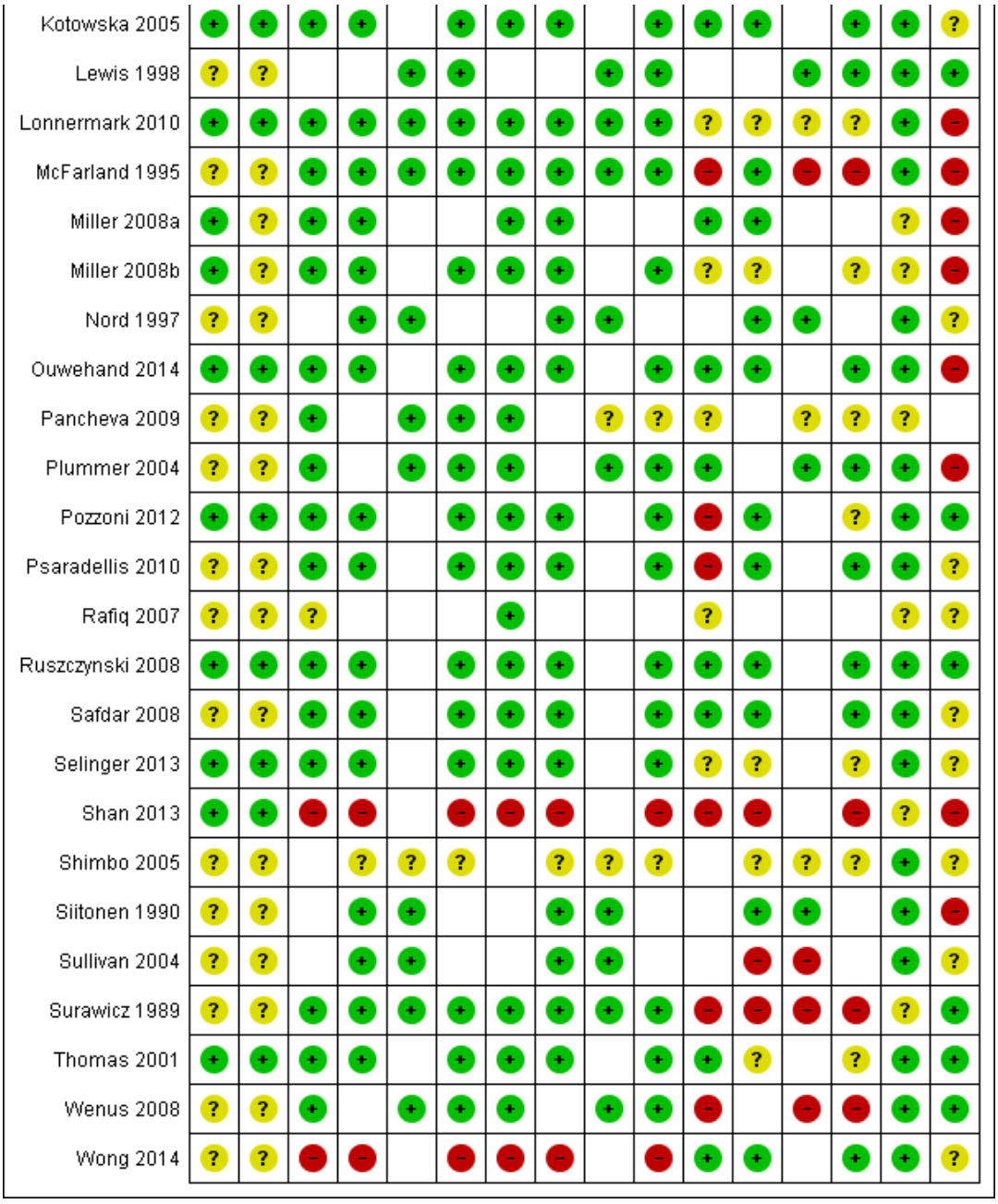


Figure 2. (Continued)



Effects of interventions

See: **Summary of findings for the main comparison** Probiotics compared to control for preventing *C. difficile* associated diarrhea

Outcomes

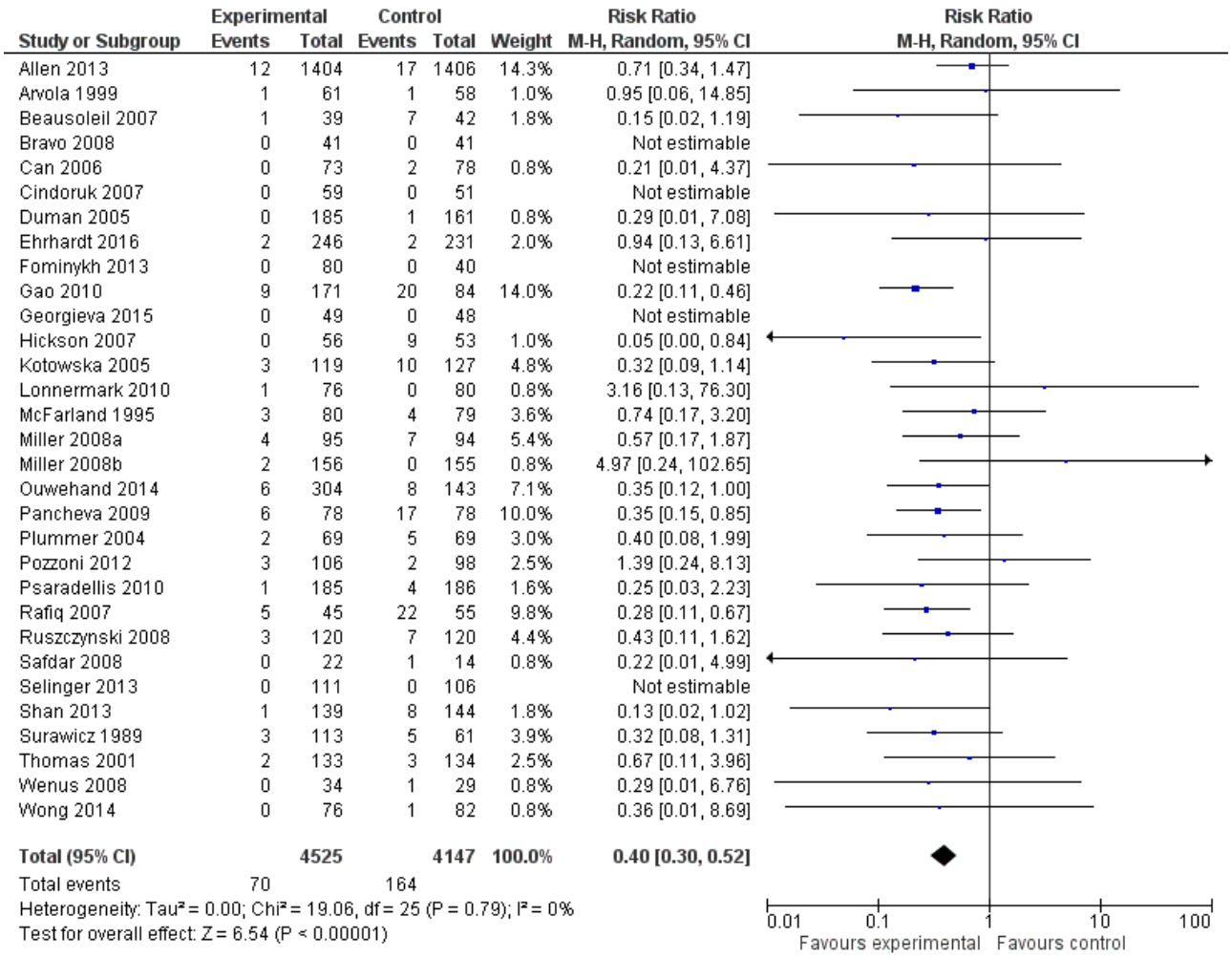
Incidence of C. difficile-associated diarrhea

To allow for a heterogeneous definition of CDAD, data (as a binary outcome) were included based on the primary authors' definition of the presence or absence of CDAD. If explicit CDAD counts were not given by the authors but could be deduced, we considered those participants with diarrhea and laboratory evidence of *C. difficile* to have CDAD. Thirty-one studies (n = 8672 per complete case) reported on the incidence of CDAD. Of these, 26 were placebo-controlled, four trials had a no treatment control group (Duman 2005; Fominykh 2013; Shan 2013; Wong 2014), and in one study, published in abstract form only, the control arm intervention was not reported (Rafiq 2007). Two trials had two probiotic arms of differing dose (Gao 2010; Ouwehand 2014). One trial had two probiotic arms with different timing of administration, either during antibiotic therapy or directly after cessation of antibiotic therapy (Fominykh 2013). To avoid a unit of analysis error we grouped the two probiotic arms together. To avoid losing important dose information for our subgroup meta-regression, we kept the

differing dose trials' probiotic arms intact and split the control group so that half served as comparator for each arm.

The overall pooled results using a complete case analysis favoured probiotics demonstrating a statistically significant reduction in the incidence of CDAD. The incidence of CDAD in the probiotic group was 1.5% (70/4525) compared to 4.0% (164/4147) in the placebo or no treatment control group (RR 0.40, 95% CI 0.30 to 0.52; random-effects) suggesting that 42 patients (95% CI 32 to 58) would need to be treated to prevent one case of CDAD (number needed to treat for an additional beneficial outcome). No statistically significant heterogeneity was detected for this comparison (P = 0.79; I^2 = 0%). The forest plot for this outcome can be found in Figure 3.

Figure 3. Forest plot of comparison: 1 C. difficile associated diarrhea, outcome: 1.1 Incidence CDAD: complete case.



Ten of the 31 studies were rated as having a low risk of bias, while 21 were rated as having a high or unclear risk of bias. On sensitivity analysis, the low risk of bias studies suggested a similar pooled protective effect of probiotics (RR 0.39, 95% CI 0.25 to 0.61) as the high risk of bias studies (RR 0.40, 95% CI 0.28 to 0.58) and a test of interaction between low and high or unclear risk of bias studies was not statistically significant (P = 0.93). Twenty-two of 31 trials had missing CDAD data ranging from 2% to 45%. Using the assumed plausible ratios of event rates in those with missing data in comparison to those successfully followed: 1.5:1, 2:1, 3:1, and 5:1 (Akl 2012), our results were robust to all assumptions: even assuming a 5 to 1 ratio of events in those with missing data versus those with complete data in the intervention group - the effect size was large and the confidence interval narrow (RR 0.54, 95% CI 0.39 to 0.74). Minimal heterogeneity was detected for this comparison (P = 0.09; I^2 = 28%).

Detection of C. difficile in stool

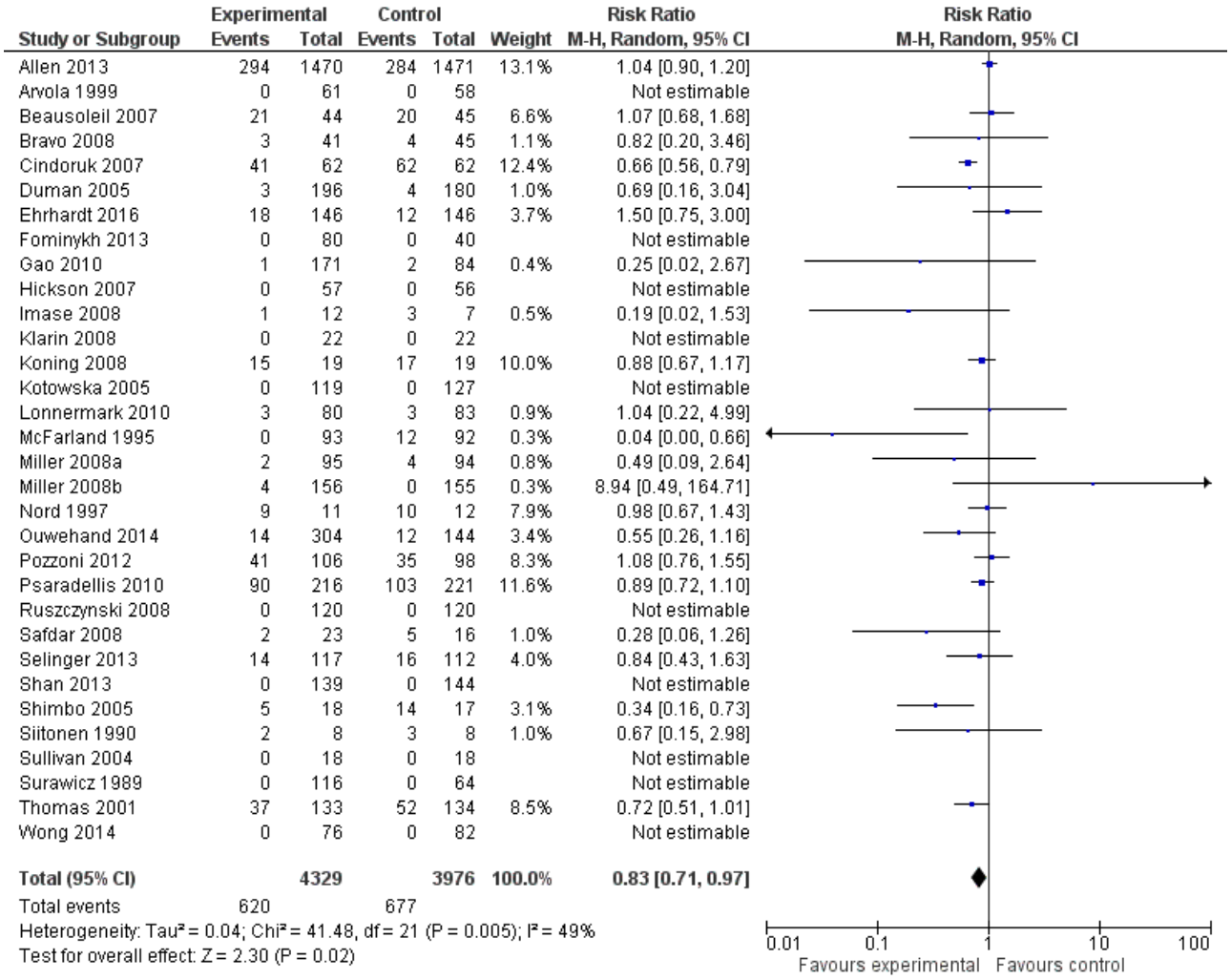
Fifteen studies (n = 1214) reported on the detection of *C. difficile*. Of these, 13 were placebo-controlled and two trials used a no treatment control arm (Imase 2008; Shimbo 2005). One trial had two probiotic arms with different doses (Imase 2008), and we grouped the two probiotics arms together (with the exception of our investigation of dose effects where we adjusted as discussed above). The overall pooled results using a complete case approach did not show a statistically significant reduction in detection of *C. difficile* in the stool. *C. difficile* detection was 15.5% (98/633) in the probiotics group compared to 17.0% (99/581) in the placebo or no treatment control group (RR 0.86, 95% CI 0.67 to 1.10; random-effects). Four of the 15 studies were rated as having a low risk of bias and 11 were rated as having a high or unclear risk of

bias. No statistically significant heterogeneity was detected for this comparison (P = 0.88; I^2 = 0%).

Incidence of adverse events

Thirty-two of the included studies (n = 8305) reported on adverse events (AEs), ten of which reported no AEs in either the treatment group or control group. Seven of the included studies reported serious AEs with none attributable to probiotic intervention (Allen 2013; Ehrhardt 2016; Miller 2008a; Miller 2008b; Pozzoni 2012; Ouwehand 2014; Psaradellis 2010). In both treatment and control groups the most common AEs included abdominal cramping, nausea, fever, soft stools, flatulence, and taste disturbance. The incidence of reported AEs in the probiotic group was 14.3% compared to 17.0% in the placebo or no treatment control group (RR 0.83, 95% CI 0.71 to 0.97), suggesting significantly fewer reported AEs in the probiotic group. Moderate heterogeneity was detected for this comparison; (P = 0.005; I^2 = 49%). Fourteen of the 32 studies were rated as having a low risk of bias and 18 were rated as having a high or unclear risk of bias. The forest plot for this outcome can be found in Figure 4.

Figure 4. Forest plot of comparison: 1 Probiotics versus control, outcome: 1.24 Adverse Events: complete case.



Nineteen of 32 trials had missing AE data ranging from 2% to 44%. Using the assumed plausible ratios of event rates in those with missing data in comparison to those successfully followed: 1.5:1, 2:1, 3:1, and 5:1 (Akl 2012), our results were not robust to all assumptions. Assuming a 1.5 to 1 ratio of events in those with missing data versus those with complete data in the intervention group - the effect was no longer statistically significant (RR 0.86, 95% CI 0.74 to 1.01). Moderate heterogeneity was detected for this comparison (P = 0.001; I^2 = 55%).

Based on visual inspection we identified an outlier trial for the outcome of AEs (McFarland 1995). We conducted a sensitivity analysis by removing this trial and comparing the results to those before removal. The meta-analytic results were not significantly changed.

Antibiotic-associated diarrhea

Thirty-three of the included studies (n = 8870) reported on antibiotic-associated diarrhea (AAD). Of these, 27 were placebo-controlled and six trials used a no treatment control arm (Duman 2005; Fominykh 2013; Imase 2008; Shimbo 2005; Shan 2013; Wong 2014). Three trials had two probiotic arms of differing dose that we grouped together as discussed above (Gao 2010;

Imase 2008; Ouwehand 2014). One trial had two probiotic arms of differing timing (Fominykh 2013). The overall pooled results using a complete case analysis favoured probiotics demonstrating a statistically significant reduction in the incidence of AAD. Twelve per cent (565/4618) of participants in the probiotics group developed AAD compared to 18% (771/4252) of the placebo or no treatment control group (RR 0.58, 95% CI 0.48 to 0.70). Statistically significant moderate heterogeneity was detected for this comparison (P < 0.001; I^2 = 61%). Of these 33 studies, 16 were rated as having a low risk of bias and 17 were rated as having an unclear or high risk of bias. A test of subgroup difference trended towards statistical significance when comparing the high/unclear risk of bias studies with low risk of bias studies (P = 0.06). Low risk of bias studies showed no statistically significant difference in reported AEs between the control and probiotic groups (RR 0.89, 95% CI 0.76 to 1.04, I^2 = 57%, n = 5281), while the high/unclear risk of bias studies suggested a statistically significant decrease in AEs (RR 0.56, 95% CI 0.36 to 0.88, I^2 = 23%, n = 3024).

Seventeen of 33 trials had missing AAD data ranging from 1% to 43%. Using the assumed plausible ratios of event rates in those with missing data in comparison to those successfully followed: 1.5:1, 2:1, 3:1, and 5:1 (Akl 2012), our results were robust to all assumptions. Even assuming a 5 to 1 ratio of events in those with missing data versus those with complete data in the intervention group - the effect was still statistically significant (RR 0.74, 95% CI 0.60 to 0.90). A substantial degree of heterogeneity was detected for this comparison (P < 0.001; I^2 = 65%).

Length of hospital stay

Four studies reported on length of hospital stay (Beausoleil 2007; Selinger 2013; Thomas 2001; Allen 2013). Standard deviations that had not been reported in the original articles (e.g. Allen 2013), were calculated using interquartile ranges (Wan 2014), or were provided by the author (Selinger 2013). The combined mean difference for length of hospital stay was -0.17 (95% CI -1.03 to 0.68) days and was not statistically significant. Three trials were rated as having a low risk of bias for this outcome (Beausoleil 2007; Thomas 2001; Allen 2013) and the other as unclear (Selinger 2013). No statistically significant heterogeneity was detected for this comparison (P = 0.49; I^2 = 0%).

Subgroup and heterogeneity variable analysis

In exploring clinical and methodologic heterogeneity, we considered dose, species, paediatric population versus adult, inpatient population versus outpatient, and baseline risk and subjected them to 5 published credibility criteria (Sun 2014):

- Can chance explain the apparent subgroup effect?
- Is the subgroup effect consistent across studies?
- Was the subgroup hypothesis one of a small number of hypotheses developed a priori with direction specified?
- Is there strong preexisting biological support?
- Is the evidence supporting the effect based on within- or between-study comparisons?

Dose

We conducted meta-regression against dose for the outcomes of CDAD, detection of *C. difficile*, AAD, and AE. With the exception of *C. difficile* detection (P = 0.04), none of the analyses showed a statistically significant dose dependent effect. The statistical significance of the *C. difficile* detection analysis was driven by a single outlier study (Klarin 2008). In our sensitivity analysis with the outlier study removed, the dose dependent effect was no longer statistically significant (P = 0.43). The dose effect did not appear to be particularly consistent across studies. While one could make a biological argument for a dose effect, not all outcomes had trials with different dose arms, but those that did, supported a dose dependent effect (e.g. Gao 2010). The criteria supporting a dose dependent effect are mixed and we are unable to clearly identify a credible dose dependent effect at this time.

Species (alone and in combination)

In evaluating the subgroup species, there were two examples of a statistically significant test for subgroup effects. For the CDAD outcome, comparing the *L. acidophilus* + *L. casei* subgroup (RR 0.21, 95% CI 0.11 to 0.42, I^2 = 0%, n = 781) to the *L. rhamnosus* subgroup (RR 0.63, 95% CI 0.30 to 1.33, I^2 = 88%, n = 1031) resulted in a statistically significant test of subgroup difference (P = 0.03). There was also a statistically significant test for subgroup difference when comparing probiotic species (P = 0.04) for the adverse event outcome. This subgroup effect is based on between study comparisons and is not consistent across studies. There were many comparisons of over 15 subgroup classes for single or combination species for both CDAD and AE outcomes making a statistically significant effect more likely to occur by chance alone. We are unaware of any biological or direct evidence that suggests that *L. acidophilus* + *L. casei* is superior to *L. rhamnosus* in terms of efficacy for CDAD nor that adverse events would be species specific. The criteria supporting a species specific effect are mixed and we are unable to clearly identify a credible subgroup effect.

Paediatric versus adult population

There was a statistically significant test for subgroup difference when comparing the adult subgroup (RR 0.62, 95% CI 0.51 to 0.76, I^2 = 59%, n = 7036) to the child subgroup (RR 0.38, 95% CI 0.29 to 0.49, I^2 = 0%, n = 1141) for the AAD outcome (P ≤ 0.01) but not for other outcomes. The subgroup is reasonably consistent across studies for the AAD outcome, there were a small number of a priori subgroups, and the subgroup and direction of effect were specified a priori. We consider it possible that this is a credible subgroup effect which may explain some of the statistically significant heterogeneity observed in the AAD outcome (P < 0.001; I^2= 61%).

Inpatient population versus outpatient

In exploring the subgroup of inpatient versus outpatient participants, no tests of interaction were statistically significant across outcomes tested. There was no within study evidence to support a hospitalization dependent effect, and the results were not consistent across studies. We do not consider this subgroup credible.

Baseline risk

The baseline risk (control event rate) for the studies investigating CDAD varied widely (0%-40%) and we were concerned that high baseline risk studies were impacting the overall effect leading to concerns of directness of evidence. We therefore conducted a post hoc subgroup analysis on baseline CDAD risk, our primary outcome, to investigate this further. We divided the studies into three groups based on baseline risk (0% to 2%; 3% to 5%; > 5%) to represent

low, moderate, and high (outbreak) clinical risk scenarios. The risk ratio for trials with a baseline risk of 0-2% was 0.77 (95% CI 0.45 to 1.32, I^2= 0%, n = 5845) and was not statistically significant (P = 0.34). Trials with baseline risks between 3% and 5% had a risk ratio of 0.53 (95% CI 0.16 to 1.77, I^2 = 0%, n = 373) and was not statistically significant (P = 0.70). Trials with baseline risk greater than 5% had a risk ratio of 0.30 (95% CI 0.21 to 0.42, I^2 = 0%, n = 2454) and this large risk reduction was statistically significant (P < 0.01). The risk reduction increased as baseline risk increased with only the high-risk subgroup (> 5% baseline risk) reaching statistical significance.

This subgroup had a statistically significant test for interaction (P = 0.01), is consistent across studies and while post hoc, was one of few subgroups tested. While we are unaware of any strong biological support, variable effects based on baseline risks are plausible (Sackett 2001). Finally, evidence for this subgroup effect is based on between study comparisons only. In sum, the evidence for the credibility of this subgroup effect is mixed. We think it is possible that this is a credible subgroup but readers should consider with caution until future research can investigate this finding further.

Small study effects

Visual inspection of the funnel plots and statistical analysis using the Harbord linear regression test were not suggestive of small study effects (e.g. publication bias) for the primary outcome CDAD (Funnel plot Figure 5). In investigating the post hoc subgroup of greater than 5% baseline risk within the CDAD outcome, visual inspection of the funnel plot was somewhat suggestive of small study effects but formal statistical testing was not (P = 0.95). The funnel plot and the Harbord linear regression test were suggestive of small study effects for adverse events (Funnel plot Figure 6; Harbord test P = 0.05) and AAD (Harbord test P = 0.03).

Figure 5. Funnel plot of comparison: 1 C. difficile associated diarrhea, outcome: 1.1 Incidence CDAD: complete case.

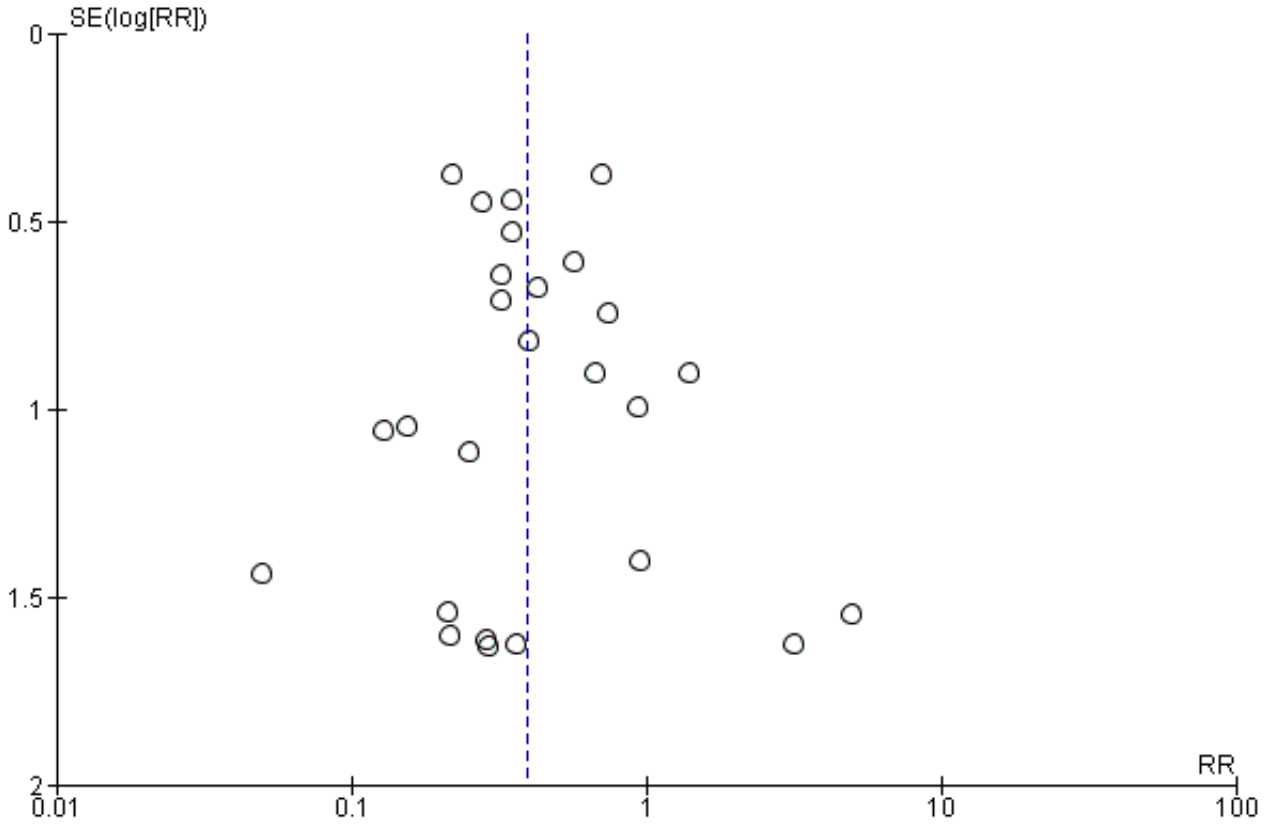
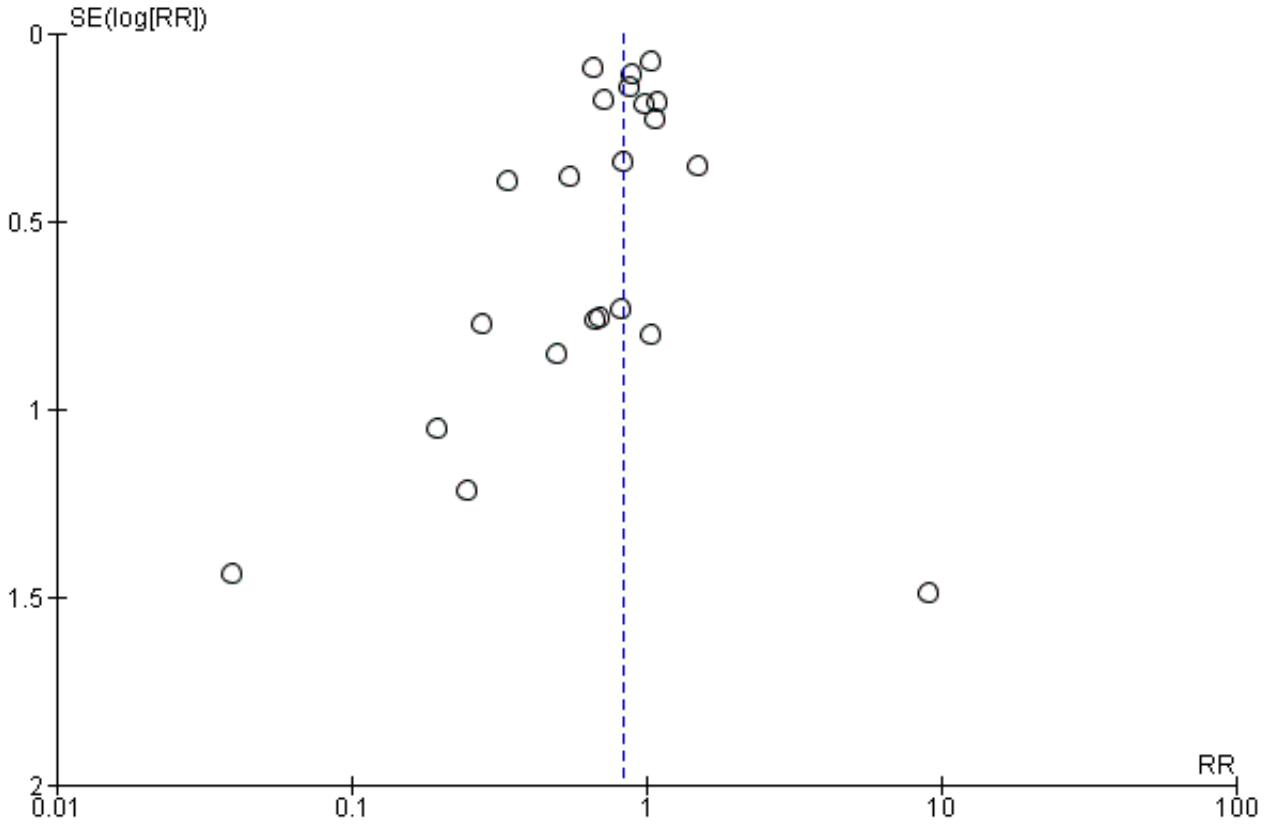


Figure 6. Funnel plot of comparison: 1 Probiotics versus control, outcome: 1.24 Adverse Events: complete case.



Overall quality (certainty) of evidence

We rated our results for the outcome of CDAD, *C. difficile* detection, and length of hospital stay as having a 'moderate' quality of evidence indicating "further research is likely to have an important impact on our certainty in the estimate of effect and may change the estimate." We rated our results for the outcome of AAD to be 'low' quality indicating "further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate" (Guyatt 2008). In considering the overall evidence for the short-term use of probiotics in patients that are not immune-compromised or severely debilitated, we rated our findings of a *decreased* adverse event rate with probiotics to be 'very low' quality indicating "we are very uncertain about the estimate" (Guyatt 2008).

We downgraded the overall certainty of evidence for CDAD to moderate because of indirectness of evidence on a population level. Much of the data for the overall effect estimate involves trials with high baseline risk (e.g. five trials had a baseline CDAD risk of > 15%) and therefore we conducted a subgroup analysis to address concerns of indirectness of evidence when applying our results to lower and more typical baseline risk estimates. Trials with a baseline CDAD risk of 0-2% and 3-5% did not show statistically significant effects but trials with a baseline risk of >5% demonstrated a large 70% risk reduction, a treatment effect that is statistically significant warranting high certainty in the estimate of effect. After applying a series of subgroup credibility assessments (Sun 2014), we believe it is plausible that baseline risk may be a trustworthy subgroup effect, but caution is warranted. Since there is some residual uncertainty in our estimate of effect due to heterogeneity (i.e. post hoc subgroup) and imprecision (a modest sample size and a modest number of events), while minimal individually, when viewed together we have graded the certainty in effect of the CDAD for the >5% subgroup down one level to moderate. Although 16 probiotic products were administered across the 39 eligible trials suggesting a potential issue with indirect evidence with respect the optimal probiotic intervention, we did not find credible evidence to suggest a related subgroup effect (see above).

We downgraded the overall certainty of evidence for detection of *C. difficile* in stool to moderate because of imprecision; the total event rate among all 15 studies was sparse (197) and the 95% confidence interval includes both no effect and a substantial effect size. We downgraded the certainty of evidence for length of hospital stay to moderate because we suspected selective outcome reporting bias as only four of the identified trials, most of which occurred in hospitals, reported on length of hospital stay - a presumably important patient and hospital outcome.

We downgraded the overall certainty of evidence for the incidence of AAD to low because of evidence suggesting the risk of publication bias; a visual inspection of the funnel plot and statistical assessment via Harbord linear regression test were suggestive of small study effects (P = 0.03) and there was statistically significant heterogeneity (I^2 = 61%; P < 0.01). We believe some of this heterogeneity may be explained by a credible adult versus child subgroup effect. We therefore also conducted GRADE analysis for

this subgroup. We downgraded the certainty of evidence of AAD in adults to low because statistically significant heterogeneity was still detected (I^2 = 59%; P < 0.01), and visual inspection of the funnel plot and statistical assessment via Harbord linear regression test were suggestive of small study effects (e.g. publication bias) (P = 0.02). We downgraded the certainty of evidence for the outcome AAD in children to moderate due to a risk of publication bias. While the Harbord's linear regression test was underpowered to detect a significant interaction in the studies investigating AAD in children (n=6), visual inspection of the funnel plot was somewhat suggestive for publication bias and the inclusion criteria for this systematic review was specific to CDAD and not AAD. Indeed, we recently published a Cochrane review on probiotics for the prevention of AAD in children that identified 23 RCTs totaling 3938 children, almost 3000 more children than we have identified in this review (Goldenberg 2015).

We downgraded the overall certainty of evidence for adverse events to very low because of risk of bias, heterogeneity and publication bias. First, the improvement in AEs seen in the primary analysis did not withstand our sensitivity analysis for missing outcome data and a subgroup analysis suggested a trend (P = 0.06) of a subgroup difference along risk of bias with low risk of bias studies suggesting no difference in AEs between probiotics and control. Second, there was statistically significant heterogeneity (I^2 = 49%; P < 0.01). Finally, visual inspection of the funnel plot and statistical analysis using the Harbord linear regression test were suggestive of small study effects (e.g. publication bias). These overall quality of evidence assessments can be found in Summary of findings for the main comparison.

DISCUSSION

Summary of main results

The primary objective of this study was to investigate the use of probiotics for preventing CDAD in patients taking antibiotics. We identified 31 randomised controlled trials (8672 participants) investigating this clinical question. A complete case analysis of these trials suggests that probiotics reduce the risk of CDAD by 60% (RR 0.40, 95% CI 0.30 to 0.52; random-effects). These results proved robust to sensitivity analyses of worst plausible assumptions regarding missing outcome data and were similar whether considering trials in adults or children, different probiotic species, lower versus higher doses of probiotics, inpatient versus outpatient status, or higher versus lower risk of bias. However, it is important to note that the largest study in this meta-analysis (Allen 2013; n = 2810 participants complete case) failed to find a statistically significant effect and the baseline risk for CDAD was very low (1.2%). Because our overall effect estimate was influenced by five trials with a CDAD baseline risk of greater than 15%, we are concerned that the effect estimate may not be directly applicable to lower baseline risk scenarios, such as was found in the Allen 2013 trial. Therefore, we have downgraded our level of confidence in the 60% risk reduction to moderate (Summary of findings for the main comparison), based on our subgroup analysis to address concerns of indirectness of evidence when applying our results to lower and more typical baseline risk estimates. Studies with baseline risks ranging from 0% to 2% demonstrated a small, non-statistically significant, risk reduction of 23% (RR 0.77, 95% CI 0.45 to 1.32; P = 0.34; moderate quality evidence). Similarly, studies with a moderate baseline risk (3% to 5%) were not statistically significant but did demonstrate a large risk reduction of 47% (RR 0.53, 95% CI 0.16 to 1.77; P = 0.30; moderate quality evidence), while studies at the highest baseline risk (> 5%) showed the largest risk reduction of 70% (RR 0.30, 95% CI 0.21 to 0.42), including a statistically significant treatment effect (P = 0.01). After applying a series of subgroup credibility assessments (Sun 2014), we believe it is plausible that baseline risk may be a trustworthy subgroup effect. Using the GRADE criteria, the quality of evidence for probiotic use in clinical scenarios with a baseline risk of greater than 5% was moderate (Summary of findings for the main comparison).

Interestingly, while we found evidence to suggest a large risk reduction in CDAD, the pooled results of the 15 trials (1214 participants) investigating the detection of *Clostridium difficile* in stool did not show a statistically significant effect (RR 0.86, 95% CI 0.67 to 1.10). In our assessment, the evidence warrants moderate confidence in this result (Summary of findings for the main comparison). The possibility therefore arises that while probiotics seem to reduce the risk of symptomatic *C. difficile* infection and CDAD, it may not necessarily prevent colonization. This question should be investigated further in future trials and may help elucidate the mechanisms by which probiotics prevent CDAD.

Thirty-two of the included studies (8305 participants) reported on adverse events. Compared to placebo or no treatment control, our pooled analysis indicates a statistically significant decrease in risk of adverse events among the probiotic group (RR 0.83, 95% CI 0.71 to 0.97), with the short-term use of probiotics in immunocompetent patients. However, concerns with risk of bias, heterogeneity, and publication bias lead us to conclude that the certainty in the evidence for this reduction in adverse events is very low (Summary of findings for the main comparison).

Thirty-three of the included trials (8870 participants) reported on AAD. Our pooled analysis indicates a statistically significant decrease in the risk of AAD (RR 0.58, 95% CI 0.48 to 0.70). However, there is evidence of publication bias as well as statistically significant heterogeneity across the 33 studies (P < 0.01; I^2 = 61%) and we rated the certainty of the evidence as low (Summary of findings for the main comparison). Exploring this heterogeneity using a priori defined subgroups revealed that an adult versus child subgroup effect may explain the observed heterogeneity (test of interaction: P < 0.01). Using five published criteria to evaluate subgroup effect credibility, we consider it possible that the adult versus pediatric subgroup represents a credible subgroup effect. We therefore also analysed the certainty of the subgroup effect estimates. Twenty-two of the included trials (4095 participants) reported on AAD in adults. Our pooled analysis indicates a statistically significant decrease in the risk of AAD in adults (RR 0.60, 95% CI 0.49 to 0.72). Further unexplained heterogeneity and publication bias lead us to consider the certainty in this effect estimate to be low (Summary of findings for the main comparison). Six of the included trials (1141 participants) reported on AAD in children. Our pooled analysis indicates a larger statistically significant decrease in the risk of AAD in children (RR 0.38, 95% CI 0.29 to 0.49). The potential for publication bias in this subgroup led us to consider the certainty in this effect estimate to be moderate (Summary of findings for the main comparison).

Only four trials investigated the length of hospital stay (3484 participants). We did not find a statistically significant difference

in length of hospital stay for those patients taking probiotics (MD -0.17, 95% CI -1.03 to 0.68) (moderate certainty in evidence).

Limitations

Other investigators chose not to pool trials using different species or strains of probiotics for the prevention of CDAD (Dendukuri 2005; Szajewska 2014). In contrast, we chose to do so as we started with the hypothesis that the mechanism of action of various probiotics was similar and that any variation in effect would be due to chance. In investigating the heterogeneity of effect size, we indeed found that the observed variability was consistent with that expected from chance (I^2 = 0%). However, non-significant tests of statistical heterogeneity do not necessarily preclude significant clinical heterogeneity (Thompson 1994), so we considered the possibility of species or strain differences in subgroup analysis (as well as a priori subgroups to explore the potential influence of population setting and age, probiotic dose and risk of bias). We applied five published criteria to investigate subgroup effects (Sun 2014), and did not find convincing evidence to suggest the presence of species or strain specific differences of the effect on CDAD. However, we did find a subgroup effect for AAD based on age (adults versus children). The subgroup hypothesis was sufficiently credible that it should be addressed in future studies.

There was significant missing data from multiple trials both in regards to patients lost to follow-up as well as the investigators' success in testing all fecal samples. To investigate the possible effect this might have had on our conclusions, we subjected this missing data to assumptions based on an extensive, but plausible sensitivity analysis (Akl 2012; Akl 2013). Our findings of reduced CDAD and AAD risk were robust to all sensitivity assumptions, while our findings of reduced risk of AEs were not.

The largest study in our meta-analysis (Allen 2013), did not find a statistically significant effect of probiotics for CDAD reduction, presumably because the study was underpowered due to the unexpected low baseline risk of developing CDAD (1.2%). Based on our post hoc subgroup analysis exploring the efficacy of probiotics among patients stratified by low, moderate and high baseline risk of CDAD, we downgraded the certainty in our overall effect estimate for indirectness of evidence from a population level baseline risk perspective. The subgroup hypothesis was sufficiently credible that it should be addressed in future studies.

Strengths

We conducted an extensive literature search and identified 39 trials (9955 participants) for analysis. For the most patient important CDAD outcome, we investigated statistical heterogeneity using the I^2 statistic (Higgins 2011), and found that the variation in effect sizes was compatible with that expected from chance (I^2 = 0%). We followed recently developed consensus guidelines on heterogeneity (Gagnier 2013; Sun 2014), and investigated the possibilities of subgroup effects including the risk of bias using five published criteria (Sun 2014). As suggested above, we also subjected missing participant data to a range of plausible assumptions, including worst plausible (assumed ratio of event rates 5:1) sensitivity analyses (Akl 2012). Because a correlation is sometimes observed between smaller trials and a more positive estimation of intervention effect, it is important to investigate possible 'small study effects' often referred to as publication bias (Begg 1989; Sterne 2000). In line with small study effect guidelines (Sterne 2011), we opted to use the Harbord method which, while conceptually similar to the more familiar Egger method (Egger 1997), utilizes the efficient score and its variance and therefore avoids certain mathematical concerns inherent in the Egger method (Harbord 2006). We independently applied GRADE criteria to determine the certainty in the estimate of effect for each of our outcomes and plausible subgroups (Guyatt 2008). Finally, CDAD incidence rates among many of the included trials were far above what would be expected in a hospital setting, which resulted in questions from clinicians and public health officials about the external validity of our previous meta-analyses (Goldenberg 2013). Optimally, we would have used cut-offs based on incidence densities (e.g. by 1000 patient days) as routinely used by infection prevention and control programs to guide decision making based on institutional baseline data, but the published studies consistently used incidence rates. There are no commonly accepted cut-offs for 'low', 'medium', and 'high' incidence of *C. difficile* infection, therefore, we used equal 3% increments to divide our data into three subgroups, an approach that had face validity as per our content expert (DM). These incremental subgroups will allow institutions to decide on the potential benefit of probiotic prophylaxis based on incidence data at their institution.

Agreements and disagreements with other studies or reviews

We previously reported a systematic review and meta-analysis to determine the efficacy and safety of probiotics for the prevention of CDAD (Goldenberg 2013). Among the 23 included randomized trials, the pooled effect estimate reported as a risk ratio was 0.36, which is very close to our pooled estimate. This updated Cochrane review identified an additional eight trials reporting on CDAD and six trials reporting on adverse events, thus increasing the precision of our earlier results and further increasing the overall certainty in the estimate of effect. This updated review has included additional outcomes of interest to decision-makers, including antibiotic-associated diarrhea, and the length of hospital stay.

Among five clinical practice guidelines produced by leading authoritative organizations, guidelines currently do not recommend probiotic prophylaxis, even though probiotics have the highest quality evidence among cited prophylactic therapies, including antibiotic stewardship, hypochlorite solutions, and bundle strategies (Lytvyn 2016). Guidelines cited concerns regarding insufficient evidence (Debast 2014; Surawicz 2013), safety concerns (Debast 2014; Dubberke 2014), and too much weight given to studies with high baseline CDAD (Dubberke 2014). Our subgroup analysis on baseline risk indicates that probiotics have moderate quality evidence if the risk of CDAD is greater than 5% and low to moderate quality evidence if the risk is less than or equal to 5%. Despite very low quality evidence for the observed decreased risk in adverse events when using probiotics, among 32 RCTs reporting on potential harms, no serious adverse events were attributed to probiotics. The most comprehensive systematic review to date on the safety of probiotics included all study designs involving humans and found no statistically significantly difference in the overall number of adverse events (RR 1.00, 95% CI 0.93 to 1.07), including serious adverse events (RR 1.06, 95% CI 0.97 to 1.16; 66 RCTs primarily based on *Lactobacillus spp*) (Hempel 2012).

AUTHORS' CONCLUSIONS

Implications for practice

Moderate quality evidence supports a large protective effect for probiotics (e.g. *S. boulardii* or *L. acidophilus* plus *L casei* at a dose of 10 to 50 billion CFUs per day) in preventing *Clostridium difficile*-associated diarrhea. A post hoc subgroup analysis suggests that probiotics may be more effective for people with a higher baseline risk of CDAD (> 5% risk; NNTB = 12; moderate quality evidence) than for people with a lower baseline risk of CDAD (≤ 5% risk). Stated in absolute terms, probiotic prophylaxis would prevent 85 *Clostridium difficile*-associated diarrhea episodes per 1000 patients at high risk of *C. difficile* diarrhea. Although adverse effects were reported among included trials, there were more adverse events among the patients in the control groups. The short-term use of probiotics appear to be safe and effective when used as an adjunct to antibiotics in immunocompetent patients.

Implications for research

Probiotics are superior to placebo or no treatment for preventing *Clostridium difficile*-associated diarrhea and, despite the need for further research, hospitalized patients, particularly those at high risk of CDAD, should be informed of the potential benefits and harms. Future head-to-head trials are warranted to distinguish optimal strains and dosages, including the assessment of cost effectiveness of probiotics compared to alternative drugs. These trials should be vigilant regarding minimizing losses to follow-up particularly for *Clostridium difficile* infection results among patients with an episode of diarrhea. Covariates of clinical interest such as age, baseline risk, length of hospitalization, length of antibiotic treatment, antibiotic class, and probiotic strain and dose, for example, need to be evaluated further. While further trials enrolling patients at high baseline risk for *C. difficile*-associated diarrhea may not be required, more trials among patients at low and moderate baseline risk are needed. To allow for an accurate assessment of the potential for adverse events, especially among immunocompromised individuals, standardized and clear adverse event reporting is essential for future trials.

ACKNOWLEDGEMENTS

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CHARACTERISTICS OF STUDIES

Characteristics of included studies *[ordered by study ID]*

Allen 2013

Methods	Placebo controlled parallel group randomized trial; multi-site; 12 weeks
Participants	65 years or older; inpatients; United Kingdom
Interventions	Lactobacillus acidophilus (CUL60, National Collection of Industrial, Food and Marine Bacteria [NCIMB] 30157; and CUL21, NCIMB 30156); Bifidobacterium bifidum CUL20, NCIMB 30153; and B lactis CUL34, NCIMB 30172) 6x10^10 CFU qd x 21 days
Outcomes	AAD, CDAD, length of hospital stay, and AE (no difference between groups reported but data for meta-analysis not published)

Allen 2013 *(Continued)*

Notes

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	“computer generated random sequence”
Allocation concealment (selection bias)	Low risk	Allocated sequentially. No one had access to allocation list until code broken
Blinding of participants and personnel (performance bias) CDAD	Low risk	Participants and providers blinded
Blinding of participants and personnel (performance bias) AE	Low risk	Participants and providers blinded
Blinding of participants and personnel (performance bias) AAD	Low risk	Participants and providers blinded
Blinding of outcome assessment (detection bias) CDAD	Low risk	CDAD was defined using assay. It is assumed laboratory scientists were blinded as well. Additionally the assays are objective outcomes less susceptible to bias from unblinding
Blinding of outcome assessment (detection bias) AE	Low risk	Participants and providers blinded
Blinding of outcome assessment (detection bias) AAD	Low risk	Participants and providers blinded
Incomplete outcome data (attrition bias) CDAD	Low risk	Extremely low MOD and analysed modified intention to treat
Incomplete outcome data (attrition bias) AE	Low risk	Extremely low MOD and analysed modified intention to treat
Incomplete outcome data (attrition bias) AAD	Low risk	Extremely low MOD and analysed modified intention to treat
Selective reporting (reporting bias)	Low risk	Protocol was published. Primary outcomes in protocol and in published final paper line up
Other bias	Low risk	No funding agency had access/control of study design, analysis or publication

Arvola 1999

Methods	Placebo controlled RCT, follow-up: 3 months post first antibiotic administration
Participants	Pediatric population, primarily outpatients (inpatients 5/119 outpatients 114/119), Finland, unclear if patients with recurrent *C. difficile* were included
Interventions	*L. rhamnosus GG 53103,* 40 x 10^9 cfu/day for duration of antibiotic treatment
Outcomes	CDAD, AAD and AE
Notes	

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	"The patients were randomized by means of a computer program"
Allocation concealment (selection bias)	Unclear risk	No pertinent information provided, therefore it is unclear if allocation was successfully concealed. Empirical data from an analysis of 1346 trials suggests that unclear and inadequately concealed allocation can bias trials with unpredictable magnitude. However, unclear or inadequately concealed allocation was associated with bias only with subjective outcomes. There is little evidence of such bias with objective outcomes (Wood 2008)
Blinding of participants and personnel (performance bias) CDAD	Low risk	"*Lactobacillus GG* and placebo capsules were indistinguishable in appearance and taste" "All patients received the same information and the follow-up was conducted in a similar manner" "*Lactobacillus GG* and placebo capsules also were indistinguishable in appearance and taste when opened"
Blinding of participants and personnel (performance bias) AE	Low risk	See above: Blinding of participants and personnel (performance bias) CDAD
Blinding of participants and personnel (performance bias) AAD	Low risk	See above: Blinding of participants and personnel (performance bias) CDAD
Blinding of outcome assessment (detection bias) CDAD	Low risk	There was no explicit statement about blinding of 'outcome assessors.' The outcomes of interest in our review relevant to this study are CDAD, AE and AAD. The outcome of diarrhea was assessed by the parents of the participants. As the parents were blinded we consider this outcome to be assessed blind. In cases of diarrhea, samples were analyzed for *C. difficile*. There is no mention of blinding of the cytotoxin assay personnel although this is a placebo controlled drug trial so we will consider the risk of bias to be low here. Additionally, we consider the cytotoxin assay to be an 'objective outcome' which is less susceptible to bias based on inadequate blinding (Wood 2008)
Blinding of outcome assessment (detection bias) AE	Low risk	"The parents reported no adverse effects of *Lactobacillus GG* or placebo." While not explicitly mentioned as an outcome in the 'methods' section AE were reported on in 'results.' It appears AE were assessed via report from the

Arvola 1999 *(Continued)*

		parents who were blinded therefore we consider this outcome to be assessed blinded as well
Blinding of outcome assessment (detection bias) AAD	Low risk	The outcomes of interest in our review relevant to this study are CDAD, AE and AAD. The outcome of diarrhea was assessed by the parents of the participants. As the parents were blinded we consider this outcome to be assessed blind
Incomplete outcome data (attrition bias) CDAD	High risk	29% dropout. No mention of intention-to-treat analysis. Unbalanced loss to follow-up (20 placebo, 28 active) with only two observed events of *C. difficile*. It seems a per protocol analysis was done. As the event rates were extremely low we consider this a high risk of attrition bias
Incomplete outcome data (attrition bias) AE	High risk	See above: Incomplete outcome data (attrition bias) CDAD
Incomplete outcome data (attrition bias) AAD	High risk	See above: Incomplete outcome data (attrition bias) CDAD
Selective reporting (reporting bias)	Low risk	No independent protocol was identified. All outcomes declared in 'methods' were reported on in 'results.' While not listed explicitly as outcomes, viral and bacterial analyses including *C. difficile* assay were described in 'methods' and reported on in 'results.' In addition, while not described in 'methods' AE were reported on in 'results' as well
Other bias	Low risk	Funding sources listed and did not include industry sponsors. Baseline characteristics of participants included in analysis appeared roughly equal and evenly distributed. No other risk of bias identified

Beausoleil 2007

Methods	Placebo controlled RCT, follow-up: 3 weeks after last drug dose
Participants	Adult population, inpatient, Canada, 2/44 patients in the treatment arm and 4/45 in the control arm had a history of *C. difficile* infection
Interventions	Fermented milk containing*L. acidophilus* CL1285 and L. casei 25 x 10^9 cfu/day for 2 days then 50 x 10^9 cfu/day for duration of antibiotic course or placebo fermented milk
Outcomes	CDAD, AAD, and AE
Notes	

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Unclear risk	The method of randomization was not adequately reported in this paper
Allocation concealment (selection bias)	Unclear risk	No pertinent information provided, therefore it is unclear if allocation was successfully concealed

Beausoleil 2007 *(Continued)*

Blinding of participants and personnel (performance bias) CDAD	Low risk	”Both preparations were provided in identically labelled containers; their taste and texture were similar”
Blinding of participants and personnel (performance bias) AE	Low risk	See above: Blinding of participants and personnel (performance bias) CDAD
Blinding of participants and personnel (performance bias) AAD	Low risk	See above: Blinding of participants and personnel (performance bias) CDAD
Blinding of outcome assessment (detection bias) CDAD	Low risk	No explicit statement about blinding of ‘outcome assessors.’ The primary outcome of AAD was defined by stool frequency and consistency. It appears as if this assessment was done by the participant. Secondary outcomes include adverse events (also reported by the participant) and cytotoxin assay. The participants were blinded so those outcomes involving participant assessment are assumed to be assessed blinded. There is no mention of blinding of the cytotoxin assay personnel although this is a placebo controlled drug trial so we will consider the risk of bias to be low here. Additionally, we consider the cytotoxin assay to be an ‘objective outcome’ which is less susceptible to bias based on inadequate blinding (Wood 2008)
Blinding of outcome assessment (detection bias) AE	Low risk	See above: Blinding of outcome assessment (detection bias) CDAD
Blinding of outcome assessment (detection bias) AAD	Low risk	See above: Blinding of outcome assessment (detection bias) CDAD
Incomplete outcome data (attrition bias) CDAD	High risk	The testing of samples for *C. difficile* differed between groups:. 7 patients in active arm developed AAD, of these 2 were tested for *C. difficile,* 1 of whom was positive. In the placebo arm 16 patients developed AAD, yet 13 were tested for *C. difficile* and 7 were positive. It is unclear why all diarrhea samples were not tested as this was part of protocol stated in the ‘methods.’ Therefore for the outcomes involving *C. difficile* there is substantial incomplete outcome data that could have resulted in ‘material’ bias of results for these outcomes
Incomplete outcome data (attrition bias) AE	Low risk	There were no patients lost to follow-up
Incomplete outcome data (attrition bias) AAD	Low risk	There were no patients lost to follow-up
Selective reporting (reporting bias)	Low risk	A protocol for this study could not be identified. All outcomes discussed in ‘methods’ were reported in ‘results’
Other bias	Low risk	“Product and placebo were provided by Bio-K+ International Inc, Laval, Quebec. A research grant was provided by Bio K+ International Inc to cover the pharmacy administration fees.” While a producer of the active treatment was a financial sponsor no author is from the sponsoring agency

Bravo 2008

Methods	Placebo controlled RCT, follow-up: 9 days after last study drug dose
Participants	Mixed population (15 to 81 years of age), outpatient, Chile, unclear if patients with recurrent *C. difficile* were included
Interventions	*S. boulardii* 10.2 x 10^9 cfu/day for 12 days (duration of antibiotic course 5 to 10 days) or placebo
Outcomes	CDAD, AAD and AE
Notes	

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	The method of randomization was not described in this paper
Allocation concealment (selection bias)	Unclear risk	Not enough information provided
Blinding of participants and personnel (performance bias) CDAD	Low risk	"In a controlled randomized, double blind trial..."
Blinding of participants and personnel (performance bias) AE	Low risk	See above: Blinding of participants and personnel (performance bias) CDAD
Blinding of participants and personnel (performance bias) AAD	Low risk	See above: Blinding of participants and personnel (performance bias) CDAD
Blinding of outcome assessment (detection bias) CDAD	Low risk	There is no explicit mention of outcome assessor blinding. There is no mention of blinding of the cytotoxin assay personnel although this is a placebo controlled drug trial so we consider the risk of bias to be low here. Additionally, we consider the cytotoxin assay to be an 'objective outcome' which is less susceptible to bias based on inadequate blinding (Wood 2008)
Blinding of outcome assessment (detection bias) AE	Low risk	It appears AE were assessed by participants reporting to study personal all of whom were blinded
Blinding of outcome assessment (detection bias) AAD	Low risk	See above: Blinding of outcome assessment (detection bias) AE
Incomplete outcome data (attrition bias) CDAD	High risk	There were four losses to follow-up reported in this paper, two from each group. Analysis was done intention-to-treat although sensitivity analysis was performed for efficacy. All 86 participants who were enrolled and not excluded from onset due to exclusion criteria were analyzed. However, not all diarrhea samples were tested for *C. difficile*. Three participants in the active arm developed AAD. Of these patients, 3 were tested for *C. difficile*, 0 of which were pos-

Bravo 2008 *(Continued)*

		itive for the toxin. In the placebo arm 5 participants developed AAD yet only 1 was tested for *C. difficile* and 0 were positive. Because 4 other placebo AAD cases were not evaluated for *C. difficile* we consider this a relatively high incomplete outcome rate for this outcome. For this reason we consider the CDAD outcome to have a high risk of 'material' bias
Incomplete outcome data (attrition bias) AE	Low risk	There were four losses to follow-up reported in this paper two from each group. Analysis was done intention-to-treat although sensitivity analysis was performed for efficacy. All 86 participants who were enrolled and not excluded from onset due to exclusion criteria were analyzed. We do not consider this small and balanced dropout rate to reasonably and 'materially' affect the AE reported event rate. For this reason we consider the outcome of AE to have a low risk of 'material' attrition bias
Incomplete outcome data (attrition bias) AAD	Low risk	See above: Incomplete outcome data (attrition bias) AE
Selective reporting (reporting bias)	Low risk	No protocol identified. All presumed outcomes from the 'methods' section were reported on in the 'results' section
Other bias	Low risk	"Funding: TUSCANY Laboratory." Funding was disclosed. It is unclear if TUSCANY produces the investigated product. No authors were associated with the funding organisation

Can 2006

Methods	Placebo controlled RCT, follow-up: 4 weeks after last antibiotic dose
Participants	Adult population, inpatient, Turkey, unclear if patients with recurrent *C. difficile* were included
Interventions	*S. boulardii* lyophilized 20 x 10^9 cfu/day ≤ 48 hours of antibiotic start dose (duration of study drug course not stated), additional information regarding length of probiotic treatment was unclear
Outcomes	CDAD and AAD
Notes	

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	The method of randomization was not adequately described
Allocation concealment (selection bias)	Unclear risk	No pertinent information provided and so it is unclear if allocation was successfully concealed
Blinding of participants and personnel (performance bias) CDAD	Low risk	"…a double-blind controlled study…"
Blinding of participants and personnel (performance bias)	Low risk	See above: Blinding of participants and personnel (performance bias) CDAD

Can 2006 *(Continued)*

AAD		
Blinding of outcome assessment (detection bias) CDAD	Low risk	No explicit statement about blinding of 'outcome assessors.' The outcome of diarrhea was assessed by the participants who were blinded. There is no mention of blinding of the cytotoxin assay personnel although this is a placebo controlled drug trial so in accordance with our a priori defined RoB criteria we will consider the risk of bias to be low here
Blinding of outcome assessment (detection bias) AAD	Low risk	The outcome of diarrhea was assessed by the participants who were blinded
Incomplete outcome data (attrition bias) CDAD	Low risk	No missing outcome data; number randomized is clearly stated and equal to number analysed. The risk of attrition bias is considered to be low for all outcomes
Incomplete outcome data (attrition bias) AAD	Low risk	See above: Incomplete outcome data (attrition bias) CDAD
Selective reporting (reporting bias)	Unclear risk	A protocol for this trial could not be identified. Outcomes were not explicitly mentioned as 'outcomes' in the 'methods' section although it seems they included AAD, *C. difficile*, microscopic and macroscopic stool examination, and type of antibiotic used. In the 'results' section all were reported with the exception of stool examination. This outcome is not particularly of interest in our review, however it is suggested that this domain be assessed at the study level not outcome level (Higgins 2011). It is also unclear how 'material' the bias to our review would be from this omission
Other bias	Low risk	"Source of support: Departmental sources." No other source of bias identified

Cindoruk 2007

Methods	Placebo controlled RCT, follow-up: 6 weeks after last antibiotic dose
Participants	Adults, not specified, Turkey, unclear if patients with recurrent *C. difficile* were included
Interventions	*S. boulardii* 500 mg twice daily for 2 weeks
Outcomes	CDAD, AAD and AE
Notes	

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	"Randomization was done using computer-based random numbers"
Allocation concealment (selection bias)	Unclear risk	Not specifically discussed.
Blinding of participants and personnel (performance bias)	Low risk	"Double blind"

Cindoruk 2007 *(Continued)*

CDAD		
Blinding of participants and personnel (performance bias) AE	Low risk	See above: Blinding of participants and personnel (performance bias) CDAD
Blinding of participants and personnel (performance bias) AAD	Low risk	See above: Blinding of participants and personnel (performance bias) CDAD
Blinding of outcome assessment (detection bias) CDAD	Low risk	CDAD assessment done by *C. difficile* toxin so risk of bias assumed to be low
Blinding of outcome assessment (detection bias) AE	Low risk	AE assessment filled out by participants who were blinded
Blinding of outcome assessment (detection bias) AAD	Low risk	No mention of how diarrhea was determined or assessed. Assumed to have been assessed by subjects who were blinded
Incomplete outcome data (attrition bias) CDAD	High risk	*C. difficile* was only measured in a subset of diarrhea patients
Incomplete outcome data (attrition bias) AE	Unclear risk	No mention of patients lost to follow-up after treatment period. Diarrhea is listed with other AE
Incomplete outcome data (attrition bias) AAD	Unclear risk	Incomplete outcome data (attrition bias) AE
Selective reporting (reporting bias)	Low risk	No mention of a clinical trial register in text. Trial not found on clinicaltrials.gov. Outcome measures discussed in methods section were AE and *H pylori*. These outcomes were reported in results section. There was no explicit mention of a *C. difficile* outcome in the methods section although it was reported on in the results section
Other bias	Unclear risk	Baseline differences not statistically significant. No mention of funding sources

Duman 2005

Methods	No treatment controlled RCT, follow-up: 4 weeks after last study drug dose
Participants	17 to 81 yrs of age, not specified, Turkey, unclear if patients with recurrent *C. difficile* were included
Interventions	*S. boulardii* 30 x 10^9 cfu/day for 14 days (i.e. for duration of antibiotic course) or no treatment
Outcomes	CDAD, AAD and AE
Notes	

Duman 2005 *(Continued)*

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	The method of randomization was not described
Allocation concealment (selection bias)	Unclear risk	No pertinent information provided so it is unclear if allocation was successfully concealed
Blinding of participants and personnel (performance bias) CDAD	Unclear risk	"This is a multicenter, prospective, open label and randomized study." This is an open label study and therefore there was knowledge of the allocated intervention and we consider this to have a high risk of performance bias. However, the magnitude of the bias may differ depending on the outcome in question. Additionally, there is little empirical evidence that objective outcomes are subject to bias due to lack of blinding (Wood 2008)
Blinding of participants and personnel (performance bias) AE	High risk	"This is a multicenter, prospective, open label and randomized study"
Blinding of participants and personnel (performance bias) AAD	Unclear risk	See above: Blinding of participants and personnel (performance bias) CDAD
Blinding of outcome assessment (detection bias) CDAD	Unclear risk	This is an open label study and therefore there was knowledge of the allocated intervention and we consider this to have a high risk of performance bias. Participants were not blinded and they self-reported diarrhea. Therefore these outcome assessments were definitely not blinded. There is no mention of blinding for microscopic and macroscopic investigation, nor for cytotoxin ELISA. However, we assume the assessors were not blinded as this was an open label trial. The magnitude of the bias may differ depending on the outcome in question. Additionally, there is little empirical evidence that objective outcomes are subject to bias due to lack of blinding (Wood 2008)
Blinding of outcome assessment (detection bias) AE	High risk	This is an open label study and therefore there was knowledge of the allocated intervention and we consider this to have a high risk of performance bias. Participants were not blinded and they self-reported adverse events. Therefore these outcome assessments were definitely not blinded
Blinding of outcome assessment (detection bias) AAD	Unclear risk	See above: Blinding of outcome assessment (detection bias) CDAD
Incomplete outcome data (attrition bias) CDAD	High risk	"The C. difficile toxin test was tested in the stool in 16 patients with diarrhea (11 in the control group and five in the treatment group) and it was positive only in one patient in the control group." Total diarrhea cases included 28 participants in control group and 14 in treatment group. It appears that only one third of diarrhea cases in each group were assessed for *C. difficile*. We are very concerned with the risk of this missing outcome data especially considering the low event rate for the *C. difficile* and CDAD outcomes
Incomplete outcome data (attrition bias) AE	Low risk	It appears from the presentation of results that the analysis was done with intention-to-treat. All 389 patients randomized were analysed in their groups as randomized. Missing outcome data is balanced in numbers across intervention groups, with similar reasons for missing data across groups

Duman 2005 *(Continued)*

Incomplete outcome data (attrition bias) AAD	Low risk	See above: Incomplete outcome data (attrition bias) AE
Selective reporting (reporting bias)	High risk	A protocol for this study was not identified. All outcomes discussed in 'methods' were reported in 'results.' However an additional outcome was reported in 'results' (cumulative diarrhea rate). Therefore the primary outcome of rate of diarrhea was "reported using measurements, analysis methods or subsets of the data (e.g. subscales) that were not pre-specified." This classifies as a high risk of bias (Higgins 2011)
Other bias	Unclear risk	No mention of funding source. According to our a priori criteria for RoB assessment we will assess this as an unclear risk of bias

Ehrhardt 2016

Methods	Placebo controlled parallel group randomized trial; multi site; 7 weeks post antibiotic treatment
Participants	Adults; Inpatients; Germany
Interventions	Saccharomyces boulardii (Perenterol® Forte) 9 x 10^9 CFU qd for length of antibiotics and 7 additional days
Outcomes	AAD, CDAD and AE
Notes	

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	"computer assisted"
Allocation concealment (selection bias)	Low risk	"Allocation concealment was achieved by an internet-based central treatment allocation"
Blinding of participants and personnel (performance bias) CDAD	Low risk	"double masked"
Blinding of participants and personnel (performance bias) AE	Low risk	"double masked"
Blinding of participants and personnel (performance bias) AAD	Low risk	"double masked"
Blinding of outcome assessment (detection bias) CDAD	Low risk	"participants, study staff, and data analysts were masked to treatment assignment"

Ehrhardt 2016 *(Continued)*

Blinding of outcome assessment (detection bias) AE	Low risk	"participants, study staff, and data analysts were masked to treatment assignment"
Blinding of outcome assessment (detection bias) AAD	Low risk	"participants, study staff, and data analysts were masked to treatment assignment"
Incomplete outcome data (attrition bias) CDAD	Unclear risk	They used ITT. All randomized patients were used in denominator. There were significant patients with missing outcome data however, the results were null
Incomplete outcome data (attrition bias) AE	Unclear risk	They used ITT. All randomized patients were used in denominator. There were significant patients with missing outcome data however. The results did not favor the intervention
Incomplete outcome data (attrition bias) AAD	Unclear risk	They used ITT. All randomized patients were used in denominator. There were significant patients with missing outcome data however, the results were null
Selective reporting (reporting bias)	Low risk	Clinicaltrials.gov NCT01143272 Original outcomes listed in clinicaltrials.gov line up with reported outcomes in paper
Other bias	Low risk	No funding bias. Stopped early but not for benefit

Fominykh 2013

Methods	Three armed randomized trial; unknown follow-up time; Group 1 probiotics concurrent to antibiotics; Group 2 probiotics after antibiotics; Group 3 no treatment control
Participants	Adults; unknown hospitalization; unknown country
Interventions	Lactococcus lactis, Lactobacillus, Bifidobacterium and Streptococcus thermophilus(RioFlora^{TM} Balance); 1x10^{10} CFU qd; 14 days
Outcomes	CDAD; AAD; AE
Notes	Abstract only; 2 active arms: first arm probiotics in parallel with antibiotics and second arm after antibiotics

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Not described
Allocation concealment (selection bias)	Unclear risk	Not described
Blinding of participants and personnel (performance bias) CDAD	High risk	2 active arms and one no treatment control

Fominykh 2013 *(Continued)*

Blinding of participants and personnel (performance bias) AE	High risk	2 active arms and one no treatment control
Blinding of participants and personnel (performance bias) AAD	High risk	2 active arms and one no treatment control
Blinding of outcome assessment (detection bias) CDAD	High risk	CDAD, AAD, and AE based on patient report or physician report none of whom were blinded
Blinding of outcome assessment (detection bias) AE	High risk	ibid
Blinding of outcome assessment (detection bias) AAD	High risk	ibid
Incomplete outcome data (attrition bias) CDAD	Unclear risk	It says 120 people completed the study but it does not report how many were randomized
Incomplete outcome data (attrition bias) AE	Unclear risk	ibid
Incomplete outcome data (attrition bias) AAD	Unclear risk	ibid
Selective reporting (reporting bias)	Unclear risk	This is an abstract. No enough information to determine and there does not appear to be a pre-published protocol
Other bias	High risk	It appears some of the authors are employed by a probiotic company

Gao 2010

Methods	Placebo controlled RCT with 2 actives arms (differing dose), follow-up: 3 weeks after last study drug dose
Participants	Adult population, inpatients, China, unclear if patients with recurrent *C. difficile* were included
Interventions	Probiotic arm 1: *L. acidophilus* CL1285 and *L. casei* LBC80R 50 x 10^9 cfu/day Probiotic arm 2: *L. acidophilus* CL1285 and *L. casei* LBC80R 100 x 10^9 cfu/day within 36 hours of antibiotic commencement until 5 days after discontinuation Placebo
Outcomes	CDAD, AAD and AE
Notes	

Gao 2010 *(Continued)*

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	"The randomization sequence used in this trial was generated by a computerized random-number generator (SAS, release 9.2; SAS Institute, Cary, NC) using a permuted block design that randomized among the three study groups while stratifying for age (50 – 59 vs. 60 – 70 years) and number of days on antibiotics (3 – 8 and 9 – 14 days)"
Allocation concealment (selection bias)	Low risk	"Study products were delivered to the investigative site in identical containers labelled only with the lot number and a sequentially numbered patient identification code"
Blinding of participants and personnel (performance bias) CDAD	Low risk	"This study was conducted using triple-blinding procedures. First, patients were blinded to the treatment received throughout the trial. Each patient received two pills each day, which were identical in shape, size, taste, smell, and color regardless of the assigned treatment group. Second, investigators and all involved clinicians were blinded to the treatment allocation throughout the course of the study. Finally, all study coordinators, clinical monitors, and biostatisticians were blinded to treatment allocation throughout the entire clinical study and until after all analyses were completed"
Blinding of participants and personnel (performance bias) AE	Low risk	See above: Blinding of participants and personnel (performance bias) CDAD
Blinding of participants and personnel (performance bias) AAD	Low risk	See above: Blinding of participants and personnel (performance bias) CDAD
Blinding of outcome assessment (detection bias) CDAD	Low risk	"This study was conducted using triple-blinding procedures. First, patients were blinded to the treatment received throughout the trial. Each patient received two pills each day, which were identical in shape, size, taste, smell, and color regardless of the assigned treatment group. Second, investigators and all involved clinicians were blinded to the treatment allocation throughout the course of the study. Finally, all study coordinators, clinical monitors, and biostatisticians were blinded to treatment allocation throughout the entire clinical study and until after all analyses were completed"
Blinding of outcome assessment (detection bias) AE	Low risk	See above: Blinding of outcome assessment (detection bias) CDAD
Blinding of outcome assessment (detection bias) AAD	Low risk	See above: Blinding of outcome assessment (detection bias) CDAD
Incomplete outcome data (attrition bias) CDAD	Low risk	No missing outcome data; number randomized is clearly stated and equal to number analysed. We consider the risk of attrition bias to be low for all outcomes
Incomplete outcome data (attrition bias) AE	Low risk	See above: Incomplete outcome data (attrition bias) CDAD

Gao 2010 *(Continued)*

Incomplete outcome data (attrition bias) AAD	Low risk	See above: Incomplete outcome data (attrition bias) CDAD
Selective reporting (reporting bias)	Low risk	Protocol was listed with clinicaltrials.gov (NCT00958308). All primary and secondary outcomes listed in protocol were reported in the 'results'
Other bias	Unclear risk	"Bio-K + International (Laval, Quebec, Canada) provided financial support for this clinical trial. Sprim Advanced Life Sciences helped with study planning, conduct, and analysis and with paper development." Three paper authors work for Sprim which is a CRO which we assume was funded by Bio-K+ since they were the sponsor of the study. So while no sponsoring employees were authors the sponsoring agency contracted the organization that planned and analyzed the study

Georgieva 2015

Methods	Placebo controlled parallel group randomized trial; single site; 3 weeks post antibiotic prescription
Participants	children (3 to 12 years of age); inpatients; Bulgaria
Interventions	Lactobacillus reuteri DSM 17938; 1x10^8 CFU qd; Length of abx tx plus 7 days
Outcomes	CDAD, AAD, C. difficile detection, AE
Notes	

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	"Eligible patients were randomized to either the treatment or placebo group using a computer generated randomization list of case numbers. Participants entered consecutively starting with the lowest case number in each stratum. Randomisation and labelling of the test-samples were made by an independent physician"
Allocation concealment (selection bias)	Low risk	"Randomisation and labelling of the test-samples were made by an independent physician"
Blinding of participants and personnel (performance bias) CDAD	Low risk	"The unblinding was done when all data were analysed by an independent statistician"
Blinding of participants and personnel (performance bias) AE	Low risk	ibid
Blinding of participants and personnel (performance bias) C. difficile incidence	Low risk	ibid

Georgieva 2015 *(Continued)*

Blinding of participants and personnel (performance bias) AAD	Low risk	ibid
Blinding of outcome assessment (detection bias) CDAD	Low risk	ibid
Blinding of outcome assessment (detection bias) AE	Low risk	ibid
Blinding of outcome assessment (detection bias) C. difficile incidence	Low risk	ibid
Blinding of outcome assessment (detection bias) AAD	Low risk	ibid
Incomplete outcome data (attrition bias) CDAD	Low risk	Less than 10% of patients missing outcome data
Incomplete outcome data (attrition bias) AE	Low risk	ibid
Incomplete outcome data (attrition bias) C. difficile incidence	Low risk	ibid
Incomplete outcome data (attrition bias) AAD	Low risk	ibid
Selective reporting (reporting bias)	Low risk	Registered on clinicaltrials.gov The original protocol outcomes are in line with the published result outcomes
Other bias	Unclear risk	Funded by industry. However all authors are not employed by the company. There is no clear statement if industry had any role in design or analysis although the study results null effect so the direction of bias less concerning

Hickson 2007

Methods	Placebo controlled RCT, follow-up: 4 weeks after last antibiotic or study drug dose
Participants	Adult population, inpatient, England, unclear if patients with recurrent *C. difficile* were included
Interventions	*L. casei imunitass* DN-114 001 19 x 10^9 cfu/day and *L. bulgaris* 1.9 x 10^9 cfu/day and *S. thermophilus* 19 x 10^9 cfu/day or placebo for length of course of antibiotics and for 1 week afterwards
Outcomes	CDAD, AAD and AE

Hickson 2007 *(Continued)*

Notes

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	"An independent statistician generated the random allocation sequence, which was stratified for hospital, sex, and two age groups (50-69 and ≥70). The sequence was given to the pharmacy on each site." While no explicit mechanism of randomization was mentioned we will consider the involvement of an independent statistician to have led to an adequate randomization
Allocation concealment (selection bias)	Unclear risk	"The sequence was given to the pharmacy on each site" "The pharmacies removed the commercial labels, then applied study labels to identify the patient…" There is no explicit mention of allocation concealment. It appears the randomization sequence was delivered to the pharmacy and that the pharmacy assigned bottles directly to patients. While this suggests that the allocation was concealed we cannot be certain from this description and must consider as unclear
Blinding of participants and personnel (performance bias) CDAD	Low risk	"Actimel is sold in 100 g white plastic bottles with removable labels; Yazoo is packaged similarly but in 200 ml bottles. We chose Yazoo as placebo because it looks identical in colour and consistency to Actimel... The pharmacies removed the commercial labels, then applied study labels to identify the patient, the drink's "use by" date, and storage instructions. We could not find a placebo in an identical bottle to Actimel. Patients and researchers were blind to the study drink as they did not see the bottle the drink came in. Nursing staff dispensed the drinks and were instructed to pour 100 ml into a cup for the patient; they were not told which bottle contained which drink. Older people in the UK are not generally familiar with these products, but it is possible some patients might have recognized the taste. However, we had excluded people who regularly took this or other probiotic products from the study. Potential bias through unblinding was possible but unlikely" While there is potential for unblinding here the risk of 'material' bias is unclear and would depend on how many participants could identify based on taste and/or the interactions of nursing staff who may have recognized the bottles with the researchers and participants. Outcomes from this study which are pertinent to our review include AE (which we consider to be a subjective outcome) and CDAD (which we consider to be an objective outcome). Because the blinding is unclear we will assess the risk of 'material' performance bias in AE (subjective outcome) to be unclear while we consider the risk of 'material' performance bias in CDAD (objective outcome) to be low
Blinding of participants and personnel (performance bias) AE	Unclear risk	See above: Blinding of participants and personnel (performance bias) CDAD
Blinding of participants and personnel (performance bias) AAD	Low risk	See above: Blinding of participants and personnel (performance bias) CDAD
Blinding of outcome assessment (detection bias) CDAD	Low risk	"Microbiology staff who were blind to the study grouping assessed occurrence of *C. difficile* by analysis of a stool sample from patients who had diarrhea." We

Hickson 2007 *(Continued)*

		consider the CDAD outcome to have been assessed blind in this study and the risk of 'material' detection bias to be low
Blinding of outcome assessment (detection bias) AE	Unclear risk	It appears AE were assessed by the participants and reported to study staff. It is unclear if all participants were blind. For the purposes of this review we have classified AE as a subjective outcome and therefore we assess the risk of 'material' detection bias for AE to be unclear
Blinding of outcome assessment (detection bias) AAD	Low risk	See above: Blinding of outcome assessment (detection bias) CDAD
Incomplete outcome data (attrition bias) CDAD	Low risk	"We could not complete follow-up on 16% (22/135; 12 in probiotic group, 10 in placebo group) as we were unable to contact them at home despite numerous phone calls and written communications (16) or they had withdrawn (6) from the study, thus the analysis for occurrence of antibiotic associated diarrhea included 113 patients (56 in control and 57 in probiotic group). Four patients were not tested for *C. difficile* (one in probiotic group, three in control group) and thus were not included in the analysis for occurrence of diarrhea associated with *C. difficile*" The missing data were equally distributed between the two groups and the reasons for the missing data were similar in both groups. The missing data points are less likely to affect the authors' conclusions regarding the CDAD outcome in a 'material' way considering the event rates of 0 to 9
Incomplete outcome data (attrition bias) AE	High risk	Since there were no AE reported and the actual reasons given for dropout were not known for many participants it is possible that different AE rates due to intervention might have led to some dropout and since even a few events would change the results for this outcome (the comparison was 0 to 0) we therefore consider the risk of 'material' attrition bias to be high for this outcome
Incomplete outcome data (attrition bias) AAD	Low risk	Missing data were equally distributed between the two groups and the reasons for the missing data were similar in both groups
Selective reporting (reporting bias)	High risk	The trial was registered with the National Research Register under ID N0016106821. In the register the outcomes listed were: "Proportion of patients free of diarrhea in active & placebo groups, average length of stay compared in the two groups" The outcome of length of hospital stay which was listed in the register was not reported on as an outcome in the paper. Additionally the secondary outcome of CDAD which was listed in the paper was not listed in the register. Finally, the primary outcome of rate of diarrhea was "reported using measurements, analysis methods or subsets of the data (e.g. subscales) that were not pre-specified" (Higgins 2011). This classifies as a high risk of bias according to Higgins. Considering all of these concerns we classify the risk of 'material' reporting bias to be high
Other bias	Unclear risk	"Funding: Healthcare Foundation and Hammersmith Hospital Trustees research committee and Danone Vitapole (Paris, France). The Healthcare Foundation made initial comments on the design of the study. Once funding was agreed none of the funding sources had any role in the data collection, analysis, interpretation of data, writing of the report, or the decision to submit the paper for publication. "Competing interests: CJB, MH, and ALD'S have received funding from Danone to attend Danone International Conventions on Probiotics. CJB is a member of Danone UK advisory group.

Hickson 2007 *(Continued)*

	The intervention is a product of Danone. While the study received funding from the producer of the product a clear statement was made regarding the conduct and design of the study. According to our a priori criteria for RoB assessment for funding we consider an industry/sponsor author to be a high risk of bias. In this case an author was a member of an industry/sponsor advisory group as opposed to an employee. The risk of bias in this regard is therefore unclear to us

Imase 2008

Methods	No treatment control three armed RCT (2 active arms of differing dose), follow-up: days 3 and 7 post treatment
Participants	Adult population, NS, Japan, unclear if patients with recurrent *C. difficile* were included
Interventions	Clostridium butyricum CBM588, one group 6 tablets / day x 7 days and one group 12 tablets/day x 7 days or no treatment
Outcomes	*C. difficile* detection and AAD
Notes	

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	The method of randomization was not described
Allocation concealment (selection bias)	Unclear risk	No pertinent information provided so it is unclear if allocation was successfully concealed
Blinding of participants and personnel (performance bias) C. difficile incidence	Unclear risk	No pertinent information provided
Blinding of participants and personnel (performance bias) AAD	Unclear risk	No pertinent information provided
Blinding of outcome assessment (detection bias) C. difficile incidence	Unclear risk	No pertinent information provided
Blinding of outcome assessment (detection bias) AAD	Unclear risk	No pertinent information provided
Incomplete outcome data (attrition bias) C. difficile incidence	Low risk	No missing outcome data; number randomized is clearly stated and equal to number analysed. The risk of attrition bias is considered to be low for all outcomes
Incomplete outcome data (attrition bias)	Low risk	See above: Incomplete outcome data (attrition bias) *C. difficile* incidence

Imase 2008 *(Continued)*
AAD

Selective reporting (reporting bias)	Low risk	No protocol for this study was identified. The outcomes listed and described in 'methods' were those analysed in 'results'
Other bias	High risk	No clear statement regarding financial conflict of interest or funding. "CBM588 (MIYA-BM tablets, Miyarisan Pharmaceutical, Tokyo, Japan) is a probiotic agent containing approximately 107 cfu per tablet" One of the authors is associated with the company that produces the probiotic tested. According to our a priori criteria for RoB assessment we will classify this as a high risk of bias

Klarin 2008

Methods	Placebo controlled RCT, follow-up: 2 times per week while patient was in ICU
Participants	Adult population, inpatients (ICU), Sweden, unclear if patients with recurrent *C. difficile* were included
Interventions	*Lactobacillus plantarum* 299v initially 9.6x 10^11 cfu/day and thereafter 8x10^10 cfu/day or placebo for length of ICU stay
Outcomes	*C. difficile* detection
Notes	

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	The method of randomization was not described
Allocation concealment (selection bias)	Unclear risk	"Randomisation was blinded to the investigators, the ward staff, and the sponsor (Probi AB, Lund, Sweden). Packages of the active and control study products came from an independent company." This description makes no explicit mention of allocation concealment. There is no indication that the intervention packages were sequentially numbered. Therefore, it is unclear if allocation was successfully concealed
Blinding of participants and personnel (performance bias) AE	Low risk	"Randomization was blinded to the investigators, the ward staff, and the sponsor. Packages of the active and control study products came from an independent company… The active study product consisted of a fermented oatmeal gruel containing 8 _ 108 colony-forming units (CFU)/ml of Lp299v (Probi AB). As a control, the same gruel without Lp299v bacteria but with lactic acid added to achieve the same pH was used… Enteral feeding was carried out"
Blinding of participants and personnel (performance bias) C. difficile incidence	Low risk	See above: Blinding of participants and personnel (performance bias) AE
Blinding of outcome assessment (detection bias) AE	Low risk	"Identification of C. difficile and testing for toxins were performed at the clinical microbiology departments at the hospitals. Lp299v was analysed in blinded samples… Furthermore, at the Lund University Hospital ICU, a second set of rectal swabs was collected on sampling days and sent blinded to Probi AB

Klarin 2008 *(Continued)*

		for analyses of lactobacilli, Enterobacteriaceae, sulphite-reducing clostridia, enterococci, and total viable count of anaerobes and Gram-negative bacteria"
Blinding of outcome assessment (detection bias) C. difficile incidence	Low risk	See above: Blinding of outcome assessment (detection bias) AE
Incomplete outcome data (attrition bias) AE	Low risk	"Forty-eight patients were included according to the protocol. Two patients declined participation, and two were excluded because the enteral feeding and the tested product were not given as instructed in the protocol. Thus, a total of 44 patients completed the study; 22 were given the active treatment and 22 received the control product" Only 8% missing outcome data
Incomplete outcome data (attrition bias) C. difficile incidence	Low risk	See above: Incomplete outcome data (attrition bias) AE
Selective reporting (reporting bias)	Low risk	A protocol for this study was not identified. Outcomes not explicitly stated as 'outcomes' in 'methods' although all those inferred to be outcomes were all reported in 'results'
Other bias	High risk	"Probi AB provided the study product and performed bacterial analyses as an unconditional grant. Two of the authors, B. J. and G. M., are shareholders in Probi AB." Probi AB produces the probiotic being tested. According to our a priori defined criteria for RoB assessment we assess this as a high risk of bias

Koning 2008

Methods	Placebo controlled RCT, follow-up: days 7, 14, 63
Participants	Adult population, outpatient, Netherlands, unclear if patients with recurrent *C. difficile* were included
Interventions	Multispecies (10) probiotic for total dose of 1x 10^10 cfu/day for 2 weeks or placebo
Outcomes	AAD, *C. difficile* detection and AE
Notes	

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	The method of randomization was not described
Allocation concealment (selection bias)	Unclear risk	"The treatment allocation was concealed to all investigators and volunteers, until the study had been completed and all analyses had been performed." While this trial claims that the allocation was concealed there is no description of the methods used for concealment

Koning 2008 *(Continued)*

Blinding of participants and personnel (performance bias) AE	Low risk	"The study was executed according to a parallel, randomized, placebo-controlled, double-blind design"
Blinding of participants and personnel (performance bias) C. difficile incidence	Low risk	See above: Blinding of participants and personnel (performance bias) AE
Blinding of participants and personnel (performance bias) AAD	Low risk	See above: Blinding of participants and personnel (performance bias) AE
Blinding of outcome assessment (detection bias) AE	Low risk	The outcomes of AAD and AE were assessed by the participants who were blinded
Blinding of outcome assessment (detection bias) C. difficile incidence	Low risk	There is no explicit mention of blinding of the laboratory (e.g. cytotoxin assay) personnel although this is a placebo controlled drug trial so in accordance with our a priori defined RoB criteria we will consider the risk of bias to be low here
Blinding of outcome assessment (detection bias) AAD	Low risk	See above: Blinding of outcome assessment (detection bias) AE
Incomplete outcome data (attrition bias) AE	Low risk	"One subject in the probiotic group was found to be allergic to amoxycillin and had to be excluded.. Forty healthy volunteers completed the study"
Incomplete outcome data (attrition bias) C. difficile incidence	Low risk	There is no mention of noncompliance with fecal samples and it seems all groups were over 90% compliant with the placebo, intervention and antibiotic. From data representation in the paper it seems these two participants who did not complete the questionnaire were also excluded from analysis. In regards to *C. difficile* incidence, the two missing outcome data patients were from the placebo group so any unaccounted for *C. difficile* incidence in these participants would have actually favored the intervention effect estimate
Incomplete outcome data (attrition bias) AAD	Low risk	See above: Incomplete outcome data (attrition bias) AE
Selective reporting (reporting bias)	Low risk	A protocol for this study was not identified. Outcomes were not explicitly stated as such in 'methods' although all those inferred to be outcomes were all reported in 'results'
Other bias	Unclear risk	No explicit mention of conflict of interest. However, one of the authors is associated with the company that produces the study product. Our a priori defined criteria for assessment of funding bias considers a 'sponsor' as author to be a high risk of bias. While a study author is associated with the product being evaluated the funding appears to have come from a government agency. Additionally, no information regarding the roles of each author is provided so it is impossible to assess the role of the author connected to industry in planning the study or analysing the data. So while we identified a conflict of interest not reported in the paper we are unable to assess the role of this in creating 'material' bias in the effect estimates

Kotowska 2005

Methods	Placebo controlled RCT, follow-up: 2 weeks after last study drug dose
Participants	Pediatric population, mixed inpatient and outpatient, Poland, unclear if patients with recurrent *C. difficile* were included
Interventions	*S. boulardii* 10 x 10^9 cfu/day or placebo for duration of antibiotic course
Outcomes	CDAD, AAD and AE
Notes	

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	"Investigators at the Medical University of Warsaw used computers to generate independent allocation sequences and randomization lists for each study site. To avoid a disproportionate number of patients in the experimental or placebo group, randomization at each site was performed in blocks of six (three received placebo and three, active treatment)"
Allocation concealment (selection bias)	Low risk	"To ensure allocation concealment, an independent subject prepared the randomization schedule and oversaw the packaging and labelling of trial treatments"
Blinding of participants and personnel (performance bias) CDAD	Low risk	"All investigators, participants, outcome assessors and data analysts were blinded to the assigned treatment throughout the study" "The active treatment and placebo used in this study were prepared centrally by the hospital pharmacy at the Medical University of Warsaw as identically appearing wafers"
Blinding of participants and personnel (performance bias) AE	Low risk	See above: Blinding of participants and personnel (performance bias) CDAD
Blinding of participants and personnel (performance bias) AAD	Low risk	See above: Blinding of participants and personnel (performance bias) CDAD
Blinding of outcome assessment (detection bias) CDAD	Low risk	Outcome assessors were blinded
Blinding of outcome assessment (detection bias) AE	Low risk	Blinding of outcome assessment (detection bias) CDAD
Blinding of outcome assessment (detection bias) AAD	Low risk	Blinding of outcome assessment (detection bias) CDAD
Incomplete outcome data (attrition bias)	Low risk	"Overall, 23 (8.6%) of the randomized children [13 (9.8%) in the *S. boulardii* group and 10 (7.2%) in the placebo group] withdrew before completing the

Kotowska 2005 *(Continued)*

CDAD		trial and were lost to follow-up. The reasons for not completing the trial were non-acceptance of the allocated intervention (n = 22) or damage of the study product (n = 1)" A relatively low number of participants had missing data post randomization. The missing data was balanced between groups both in number and reasons given for the missing outcome data. Additionally, an extreme case scenario regarding the missing data was calculated by the authors and shown to not influence the authors' conclusions. While it is unclear from the paper if this extreme case scenario was conducted for outcomes besides AAD (the authors' primary outcome), we consider the missing data to not realistically have a risk of 'material' bias on the authors' conclusions regarding CDAD
Incomplete outcome data (attrition bias) AE	Low risk	There were no AE reported in either group. Although an extreme disproportion in AE event rates in the missing outcome data could have affected the estimate of AE it seems highly unlikely based on the rationale given for the missing data, null event rate in both groups, as well as the overall low amount of missing data
Incomplete outcome data (attrition bias) AAD	Low risk	See above; Incomplete outcome data (attrition bias) CDAD
Selective reporting (reporting bias)	Low risk	A protocol of this trial was not located. All outcomes listed in 'methods' were analysed in 'results.' We consider the risk of reporting bias to be low
Other bias	Unclear risk	Baseline participant characteristics roughly equivalent with no significant differences noted. No financial support, funding, or conflict of interest were listed. According to our a priori criteria for risk of funding bias we consider the risk of bias here to be unclear

Lewis 1998

Methods	Placebo controlled RCT, follow-up: every four days during length of treatment
Participants	Adult (elderly) population, inpatients, Wales, unclear if patients with recurrent *C. difficile* were included
Interventions	*S. boulardii* 226 mg/day or placebo for length of antibiotic treatment
Outcomes	*C. difficile* detection and AAD
Notes	

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	The method of randomization was not described
Allocation concealment (selection bias)	Unclear risk	No pertinent information is provided so it is unclear if allocation was successfully concealed

Lewis 1998 *(Continued)*

Blinding of participants and personnel (performance bias) C. difficile incidence	Low risk	"The trial capsules were prepacked by the pharmacy such that the nursing staff dispensing them were blinded to which medication they were dispensing to the subjects. The medical management of each volunteer was by the attending physician and not influenced by the study"
Blinding of participants and personnel (performance bias) AAD	Low risk	See above: Blinding of participants and personnel (performance bias) *C. difficile* incidence
Blinding of outcome assessment (detection bias) C. difficile incidence	Low risk	Outcomes assessed by the nursing staff are assumed to be blinded as the nurses were blinded. There is no mention of blinding of the cytotoxin assay or cell culture personnel although this is a placebo controlled drug trial so in accordance with our a priori defined RoB criteria we will consider the risk of bias to be low here
Blinding of outcome assessment (detection bias) AAD	Low risk	See above: Blinding of outcome assessment (detection bias) *C. difficile* incidence
Incomplete outcome data (attrition bias) C. difficile incidence	Low risk	"Of 81 patients invited to participate in the study, 72 agreed and were randomized. Three subjects failed to complete the study because they did not wish to have stool specimens collected" From the presentation of their results it seems 69 participants were included in analysis therefore it seems the missing outcomes data are for the 3 who did not complete the study. It is not clear from which group those three belonged. However, the reason given for the missing outcome data (not wishing to collect stool specimens) is unlikely to be related to the true outcome. Additionally, even assuming high event rates for each outcome from the missing data there would be little effect on the conclusion reached by the study authors. Therefore, we will consider the risk of attrition bias here to be low for all outcomes
Incomplete outcome data (attrition bias) AAD	Low risk	See above: Incomplete outcome data (attrition bias) *C. difficile* incidence
Selective reporting (reporting bias)	Low risk	A protocol for this study was not identified. Outcomes not explicitly stated as such in 'methods' although all those inferred to be outcomes were all reported in 'results'
Other bias	Low risk	This paper appears to be free of baseline imbalances and funding conflicts. No other sources of bias identified

Lonnermark 2010

Methods	Placebo controlled RCT, follow-up: depending on outcome last day of study drug or 3 weeks post treatment
Participants	Adult population, mixed inpatient and outpatient, Sweden, unclear if patients with recurrent *C. difficile* were included
Interventions	*L. plantarum* 299v 10 x 10^9 cfu/day or placebo within 48 hours of antibiotic commencement until 7 days after discontinuation

Lonnermark 2010 *(Continued)*

Outcomes	CDAD, AAD, AE, and *C. difficile* detection
Notes	

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	"Computer-generated randomization lists were used to allocate patients to either treatment group"
Allocation concealment (selection bias)	Low risk	"Staff at Ska˚nemejerier, who at no time had direct contact with the patients or investigators, labelled the test drink packages according to the randomization schedule"
Blinding of participants and personnel (performance bias) CDAD	Low risk	"The study was double blind and placebo controlled"
Blinding of participants and personnel (performance bias) AE	Low risk	See above: Blinding of participants and personnel (performance bias) CDAD
Blinding of participants and personnel (performance bias) C. difficile incidence	Low risk	See above: Blinding of participants and personnel (performance bias) CDAD
Blinding of participants and personnel (performance bias) AAD	Low risk	See above: Blinding of participants and personnel (performance bias) CDAD
Blinding of outcome assessment (detection bias) CDAD	Low risk	The outcomes of diarrhea as well as other secondary outcomes such as A.E. were assessed by the participants who were blinded. There is no mention of blinding of the cytotoxin assay personnel although this is a placebo controlled drug trial so in accordance with our a priori defined RoB criteria we will consider the risk of bias to be low here
Blinding of outcome assessment (detection bias) AE	Low risk	See above: Blinding of outcome assessment (detection bias) CDAD
Blinding of outcome assessment (detection bias) C. difficile incidence	Low risk	See above: Blinding of outcome assessment (detection bias) CDAD
Blinding of outcome assessment (detection bias) AAD	Low risk	See above Blinding of outcome assessment (detection bias) CDAD
Incomplete outcome data (attrition bias) CDAD	Unclear risk	"Among the 76 patients who left the study, 38 were randomized to L. plantarum 299v and 38 to placebo. The reasons for not completing the study did not differ between these groups of individuals (data not shown). A comparison between the patients who remained in the study and patients who did not

Lonnermark 2010 *(Continued)*

		is presented in Table 1. The drop-outs were significantly younger than the patients completing the study (P=0.0015)"
		Missing outcome data balanced in numbers across intervention groups, with similar reasons for missing data across groups (when reasons were known). However, the number of drop outs is very large and we have no reason given for drop out for 31 participants. In addition, event rates were very low for both objective and subjective outcomes. Due to these concerns we will assess as unclear risk of attrition bias for all outcomes
Incomplete outcome data (attrition bias) AE	Unclear risk	See above: Incomplete outcome data (attrition bias) CDAD
Incomplete outcome data (attrition bias) C. difficile incidence	Unclear risk	See above: Incomplete outcome data (attrition bias) CDAD
Incomplete outcome data (attrition bias) AAD	Unclear risk	See above: Incomplete outcome data (attrition bias) CDAD
Selective reporting (reporting bias)	Low risk	Outcomes clearly stated in 'methods' all of which were analysed in 'results'
Other bias	High risk	The study product being investigated in this study is sold by Probi AB. Financial support came from Probi AB. One of the authors is associated with Probi AB. Three authors hold stock in Probi AB. According to our a priori defined RoB criteria for funding bias we assess this as a high risk of bias

McFarland 1995

Methods	Placebo controlled RCT, follwo-up: 7 weeks after last study drug dose
Participants	Adult population, inpatient, USA, unclear if patients with recurrent *C. difficile* were included
Interventions	*S. boulardii* lyophilized 30 x 10^9 cfu/day or placebo within 72 hours of antibiotic commencement until 3 days after discontinuation
Outcomes	CDAD, AAD, AE, and *C. difficile* detection
Notes	

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	The method of randomization was not described
Allocation concealment (selection bias)	Unclear risk	No pertinent information provided

McFarland 1995 *(Continued)*

Blinding of participants and personnel (performance bias) CDAD	Low risk	"A double-blinded…trial" "The appearance and odor of the capsules of the patented *S. boulardii* and placebo were identical. The 1:1 (*S. boulardii*:placebo) randomization and packaging of the blinded study kits was performed at Laboratoires Biocodex (Montrouge, France) to ensure that the study investigators did not have access to the identity of the study drug"
Blinding of participants and personnel (performance bias) AE	Low risk	See above: Blinding of participants and personnel (performance bias) CDAD
Blinding of participants and personnel (performance bias) C. difficile incidence	Low risk	See above: Blinding of participants and personnel (performance bias) CDAD
Blinding of participants and personnel (performance bias) AAD	Low risk	See above: Blinding of participants and personnel (performance bias) CDAD
Blinding of outcome assessment (detection bias) CDAD	Low risk	"The etiology of all cases of diarrhea was determined independently by three blinded investigators." The outcomes from this trial pertinent to our review include CDAD, C. diff incidence, and AE. It appears diarrhea and AE were reported by patients to study investigators, all of whom were blinded. In addition, the assessment of CDAD was explicitly described as assessed blinded. While not explicitly mentioned in the text of the paper, it would appear likely that the *C. difficile* incidence was assessed in a similar blinded manner.For these reasons we consider the risk of 'material' detection bias for the outcomes CDAD, C. diff incidence, and AE to be low
Blinding of outcome assessment (detection bias) AE	Low risk	See above: Blinding of outcome assessment (detection bias) CDAD
Blinding of outcome assessment (detection bias) C. difficile incidence	Low risk	See above: Blinding of outcome assessment (detection bias) CDAD
Blinding of outcome assessment (detection bias) AAD	Low risk	See above: Blinding of outcome assessment (detection bias) CDAD
Incomplete outcome data (attrition bias) CDAD	High risk	There are missing data from 33% of randomized participants. The authors claim that there was no significant difference in study group assignment between those censored and those remaining in the trial. In addition, while censored participants did have significantly different outcomes than the rest of randomized patients (e.g. AAD) the authors claim there was no significant difference based upon the type of study drug assigned. However, the raw numbers of missing outcome data per study group are not provided. Considering the extremely high missing outcome data rate we must consider the risk of 'material' attrition bias for the low event rate outcomes of CDAD and C. diff incidence to be high

McFarland 1995 *(Continued)*

Incomplete outcome data (attrition bias) AE	Low risk	96% of adverse event forms for all randomized patients were available for analysis. Therefore, we consider the risk of 'material' attrition bias to be low for the AE outcome
Incomplete outcome data (attrition bias) C. difficile incidence	High risk	See above: Incomplete outcome data (attrition bias) CDAD
Incomplete outcome data (attrition bias) AAD	High risk	See above: Incomplete outcome data (attrition bias) CDAD
Selective reporting (reporting bias)	Low risk	No protocol identified. 'Outcomes' were not explicitly listed although all outcomes and statistical analyses inferred from the 'methods' section were analysed in the 'results' section
Other bias	High risk	This study was free of baseline imbalances "The study was funded by grants to University of Kentucky, University of Washington, and St. Louis University Medical Center from Laboratoires Biocodex, Montrouge, France." The primary author is associated with a company that both produces S. boulardii and funded the trial. According to our a priori determined criteria for risk of funding bias we consider this to constitute a high risk of 'material' bias

Miller 2008a

Methods	Placebo controlled RCT, follow-up: not stated
Participants	Adult population, inpatient, Canada, unclear if patients with recurrent *C. difficile* were included
Interventions	*LGG* capsules (4 x 10^{10} cfu /day) or placebo for 14 days
Outcomes	CDAD and AE
Notes	unpublished

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	Follow up with authors revealed that they used computers for generation of randomization
Allocation concealment (selection bias)	Unclear risk	No pertinent information provided, therefore it is unclear if allocation was successfully concealed
Blinding of participants and personnel (performance bias) CDAD	Low risk	"We conducted two randomized, double-blind studies"

Miller 2008a *(Continued)*

Blinding of participants and personnel (performance bias) AE	Low risk	See above: Blinding of participants and personnel (performance bias) CDAD
Blinding of outcome assessment (detection bias) CDAD	Low risk	"Diarrhea stool was tested for *C. difficile* toxin" There is no mention of blinding of the cytotoxin assay personnel although this is a placebo controlled drug trial so we will consider the risk of bias to be low here
Blinding of outcome assessment (detection bias) AE	Low risk	While the only AE reported was mortality it was assessed as not related to intervention. Nevertheless mortality is an obviously objective outcome and so for both AE and CDAD we assess the risk of 'material' bias to be low
Incomplete outcome data (attrition bias) CDAD	Low risk	No loss to follow up
Incomplete outcome data (attrition bias) AE	Low risk	See above: Incomplete outcome data (attrition bias) CDAD
Selective reporting (reporting bias)	Unclear risk	No protocol identified. This was an unpublished abstract so unclear if methods section would match with results section. We consider the risk of 'material' reporting be unclear
Other bias	High risk	Conagra supported the study and produces the product. It is unclear what role or access Conagra had with design, conduct, and analysis of the studies "Dr. Miller has received research grants, acts as a consultant, or serves on an advisory board for the following: Biomerieux, ConAgra, Convatec, Genzyme, Iroko, Merck, Novartis, Optimer, Salix, Wyeth" Primary author has financial relationship with the company funding the trials and producing the trial intervention. According to our a priori defined criteria for RoB assessment we assess this as a high risk of bias

Miller 2008b

Methods	Placebo controlled RCT, follow-up: not stated
Participants	Adult population, inpatient, Canada, unclear if patients with recurrent *C. difficile* were included
Interventions	LGG 12 x 10^{10} cfu /day or placebo for 14 days
Outcomes	CDAD, AAD and AE
Notes	
Risk of bias	

Miller 2008b *(Continued)*

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Follow up with authors revealed that they used computers for generation of randomization
Allocation concealment (selection bias)	Unclear risk	No pertinent information provided, therefore it is unclear if allocation was successfully concealed
Blinding of participants and personnel (performance bias) CDAD	Low risk	"We conducted two randomized, double-blind studies"
Blinding of participants and personnel (performance bias) AE	Low risk	See above: Blinding of participants and personnel (performance bias) CDAD
Blinding of participants and personnel (performance bias) AAD	Low risk	See above: Blinding of participants and personnel (performance bias) CDAD
Blinding of outcome assessment (detection bias) CDAD	Low risk	"Diarrhea stool was tested for C. difficile toxin." There is no mention of blinding of the cytotoxin assay personnel although this is a placebo controlled drug trial so we will consider the risk of bias to be low here
Blinding of outcome assessment (detection bias) AE	Low risk	While the only AE reported was mortality it was assessed as not related to intervention. Nevertheless mortality is an obviously objective outcome and so for both AE and CDAD we assess the risk of 'material' bias to be low
Blinding of outcome assessment (detection bias) AAD	Low risk	Diarrhea assessment was from blinded personnel
Incomplete outcome data (attrition bias) CDAD	Unclear risk	There is some confusion in abstract and materials provided by authors. In Miller 2008b the abstract says there was 1 LTFU in the LGG group and 4 in placebo group. But it also says by the end there were 3 LTFU. It is unclear if these are additional LTFU and what group the 3 were in. Communication with authors could not resolve this
Incomplete outcome data (attrition bias) AE	Unclear risk	See above: Incomplete outcome data (attrition bias) CDAD
Incomplete outcome data (attrition bias) AAD	Unclear risk	See above: Incomplete outcome data (attrition bias) CDAD
Selective reporting (reporting bias)	Unclear risk	No protocol identified. This was an unpublished abstract so unclear if methods section would match with results section. We consider the risk of 'material' reporting be unclear
Other bias	High risk	Conagra supported the study and produces the product. It is unclear what role or access Conagra had with design, conduct, and analysis of the studies

Miller 2008b *(Continued)*

	"Dr. Miller has received research grants, acts as a consultant, or serves on an advisory board for the following: Biomerieux, ConAgra, Convatec, Genzyme, Iroko, Merck, Novartis, Optimer, Salix, Wyeth"
	Primary author has financial relationship with the company funding the trials and producing the trial intervention. According to our a priori defined criteria for RoB assessment we assess this as a high risk of bias

Nord 1997

Methods	Placebo controlled RCT, follow-up: 21 days post antibiotic treatment
Participants	Adult population, healthy, Sweden, unclear if patients with recurrent *C. difficile* were included
Interventions	*Bifidobacterium bifidum* and *Lactobacillus acidophilus* 2x10^10 cfu/day or placebo for 14 days
Outcomes	*C. difficile* detection and AE
Notes	

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	The method of randomization was not described
Allocation concealment (selection bias)	Unclear risk	No pertinent information provided, therefore it is unclear if allocation was successfully concealed
Blinding of participants and personnel (performance bias) AE	Low risk	"The investigation was performed as a randomized double-blind parallel group study…"
Blinding of participants and personnel (performance bias) C. difficile incidence	Low risk	See above: Blinding of participants and personnel (performance bias) AE
Blinding of outcome assessment (detection bias) AE	Low risk	The outcomes pertinent to our review from this trial include AE and *C. difficile* incidence. It appears AE were observed by study personnel and/or reported to them by the participants all of which were blinded. There is no explicit mention of blinding of laboratory personnel who would have assessed the *C. difficile* incidence outcome. However, this is a placebo controlled drug trial so in accordance with our a priori defined RoB criteria we will consider the risk of bias to be low here
Blinding of outcome assessment (detection bias) C. difficile incidence	Low risk	Blinding of outcome assessment (detection bias) AE
Incomplete outcome data (attrition bias)	Low risk	It appears all outcome data were available from all randomized participants

Nord 1997 *(Continued)*
AE

Incomplete outcome data (attrition bias) C. difficile incidence	Low risk	See above: Incomplete outcome data (attrition bias) AE
Selective reporting (reporting bias)	Low risk	No protocol identified and no explicit listing of "outcomes" in the 'methods' section. However, all assumed outcomes discussed in 'methods' were analysed in 'results'
Other bias	Unclear risk	No other source of bias identified. No mention of funding source. According to our a priori criteria for RoB assessment we will assess this as an unclear risk of bias

Ouwehand 2014

Methods	Placebo controlled RCT; Three arm randomized trial; single site; 38 to 49 days
Participants	Adult; inpatient; China
Interventions	Lactobacillus acidophilus NCFM, Lactobacillus paracasei Lpc-37, Bifidobacterium lactis Bi-07, Bifidobacterium lactis Bi-04; 4.17x10^9 CFU (low dose); 1.70x10^10 CFU (high dose); QD for length of antibiotics plus 7 days
Outcomes	CDAD; AAD; AE
Notes	

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Computer randomized
Allocation concealment (selection bias)	Low risk	Subjects were randomly allocated to one of three groups - bottles were individually labeled with unique subject ID numbers
Blinding of participants and personnel (performance bias) CDAD	Low risk	"triple blind"
Blinding of participants and personnel (performance bias) AE	Low risk	"triple blind"
Blinding of participants and personnel (performance bias) AAD	Low risk	"triple blind"
Blinding of outcome assessment (detection bias) CDAD	Low risk	Outcome assessment was by physician or pt seeing liquid stool and lab toxin result

Ouwehand 2014 *(Continued)*

Blinding of outcome assessment (detection bias) AE	Low risk	Outcome assessment was by physician or pt
Blinding of outcome assessment (detection bias) AAD	Low risk	Outcome assessment was by physician or pt
Incomplete outcome data (attrition bias) CDAD	Low risk	ITT based on number randomized. Low MOD in general
Incomplete outcome data (attrition bias) AE	Low risk	ITT based on number randomized. Low MOD in general
Incomplete outcome data (attrition bias) AAD	Low risk	ITT based on number randomized. Low MOD in general
Selective reporting (reporting bias)	Low risk	Registered with clinicaltrials.gov NCT01143623 No secondary outcomes are listed. However CDAD seems an appropriate secondary outcome
Other bias	High risk	Funded by industry making the study product. Primary author is employee of the company The design is solid but industry is very involved. We have meta-epidemiologic evidence that industry sponsored trials are associated with bias. However the mechanism is not clear how that might play out in this study. Unclear risk of bias overall

Pancheva 2009

Methods	Placebo controlled parallel group randomized trial; single site; "course of hospital stay"
Participants	Children (1-7yoa); inpatient; Bulgaria
Interventions	Lactobacillus acidophilus, Lactobacillus delbrueki subs. bulgaricus, Bifidobacterium bifidum; 3x10^10 CFU daily for "course of hospital stay"
Outcomes	CDAD; AAD; *C. difficile* detection
Notes	

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Mechanism of randomization not explained
Allocation concealment (selection bias)	Unclear risk	Not explained

Pancheva 2009 *(Continued)*

Blinding of participants and personnel (performance bias) CDAD	Low risk	"Double blind"
Blinding of participants and personnel (performance bias) C. difficile incidence	Low risk	"Double blind"
Blinding of participants and personnel (performance bias) AAD	Low risk	"Double blind"
Blinding of outcome assessment (detection bias) CDAD	Low risk	"Double blind"
Blinding of outcome assessment (detection bias) C. difficile incidence	Unclear risk	Lab personal not explicitly mentioned but assumption is that they were blinded as well
Blinding of outcome assessment (detection bias) AAD	Unclear risk	"Double blind"
Incomplete outcome data (attrition bias) CDAD	Unclear risk	not described
Incomplete outcome data (attrition bias) C. difficile incidence	Unclear risk	not described
Incomplete outcome data (attrition bias) AAD	Unclear risk	not described
Selective reporting (reporting bias)	Unclear risk	not described

Plummer 2004

Methods	Placebo controlled RCT, follow-up: last day of study drug
Participants	Adult population (elderly), inpatient, England, unclear if patients with recurrent *C. difficile* were included
Interventions	*L. acidophilus* and *B. bifidum* 20 x 10^9 cfu/day or placebo within 36 hours of antibiotic commencement then for 20 days
Outcomes	CDAD, AAD and *C. difficile* detection
Notes	

Plummer 2004 *(Continued)*

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	The method of randomization was not described
Allocation concealment (selection bias)	Unclear risk	No pertinent information provided and so it is unclear if allocation was successfully concealed
Blinding of participants and personnel (performance bias) CDAD	Low risk	"The trial was a double blind, placebo-controlled study…"
Blinding of participants and personnel (performance bias) C. difficile incidence	Low risk	See above: Blinding of participants and personnel (performance bias) CDAD
Blinding of participants and personnel (performance bias) AAD	Low risk	See above: Blinding of participants and personnel (performance bias) CDAD
Blinding of outcome assessment (detection bias) CDAD	Low risk	The outcomes of this study pertinent to our review include CDAD and *C. difficile* incidence. Both of these outcomes were assessed via culture and immunologic laboratory measures. There is no mention of blinding of the laboratory personnel although this is a placebo controlled drug trial so in accordance with our a priori defined RoB criteria we will consider the risk of bias to be low here
Blinding of outcome assessment (detection bias) C. difficile incidence	Low risk	See above: Blinding of outcome assessment (detection bias) CDAD
Blinding of outcome assessment (detection bias) AAD	Low risk	See above: Blinding of outcome assessment (detection bias) CDAD
Incomplete outcome data (attrition bias) CDAD	Low risk	"Of the randomised patients, 138 completed the study, 69 with probiotics in conjunction with antibiotics and 69 with antibiotics alone" "150 patients were recruited and 138 patients fulfilled the inclusion criteria. For these patients, bowel habit on admission and prescribed medication were recorded" It appears that for all eligible participants that were randomized all outcome data was available. We consider the risk of 'material' attrition bias to be low for all outcomes
Incomplete outcome data (attrition bias) C. difficile incidence	Low risk	See above: Incomplete outcome data (attrition bias) CDAD
Incomplete outcome data (attrition bias)	Low risk	See above: Incomplete outcome data (attrition bias) CDAD

Plummer 2004 *(Continued)*

AAD		
Selective reporting (reporting bias)	Low risk	No protocol identified. No explicit disclosure of 'outcomes' to be addressed although all inferred outcomes from 'methods' section were analyzed in 'results'
Other bias	High risk	There is no direct mention of study funding although it is disclosed that the study product was provided by Cultech. The primary author is an employee of the company (Cultech) that produces the study product. Although funding is not explicitly disclosed, we consider it likely that the trial was funded by Cultech. Due to these considerations we consider the risk of 'material' bias here to be high

Pozzoni 2012

Methods	Placebo controlled RCT, follow-up: 12 weeks after last antibiotic dose
Participants	Adult population (> 50 years of age), inpatient, Italy, unclear if patients with recurrent C. difficile were included
Interventions	*Saccharomyces Boulardii* $10x10^9$ cfu/day or placebo within 48 hours of antibiotic commencement for length of antibiotic treatment and then for 7 days afterwards
Outcomes	CDAD, AAD, and AE
Notes	

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	"...computer-generated random-number table"
Allocation concealment (selection bias)	Low risk	"Central randomisation by hospital pharmacy.."
Blinding of participants and personnel (performance bias) CDAD	Low risk	"The *S. boulardii* and placebo tablets were identical in shape, size, taste, smell, and color. The participants, researchers, and staff contributing to the study (doctors, nurses, and microbiologists) were unaware of the treatment allocations throughout the duration of the study"
Blinding of participants and personnel (performance bias) AE	Low risk	See above: Blinding of participants and personnel (performance bias) CDAD
Blinding of participants and personnel (performance bias) AAD	Low risk	See above: Blinding of participants and personnel (performance bias) CDAD
Blinding of outcome assessment (detection bias) CDAD	Low risk	"The participants, researchers, and staff contributing to the study (doctors, nurses, and microbiologists) were unaware of the treatment allocations throughout the duration of the study"

Pozzoni 2012 *(Continued)*

Blinding of outcome assessment (detection bias) AE	Low risk	See above: Blinding of outcome assessment (detection bias) CDAD
Blinding of outcome assessment (detection bias) AAD	Low risk	See above: Blinding of outcome assessment (detection bias) CDAD
Incomplete outcome data (attrition bias) CDAD	High risk	25% of patients in the treatment group were lost to follow-up and 27% of placebo patients were lost to follow-up It appears that patients were only evaluated for C. difficile if the diarrhea occurred in the hospital It seems only 29 patients developed diarrhea. Of these 22, developed diarrhea out of hospital and only 2 patients were tested for *C. difficile*. Therefore 20/29 cases of diarrhea were not tested for *C. difficile*
Incomplete outcome data (attrition bias) AE	Low risk	All patients who discontinued where investigated for rationale and none reported withdrawal due to AE
Incomplete outcome data (attrition bias) AAD	Unclear risk	High LTFU, unclear what effect this has on AAD outcome
Selective reporting (reporting bias)	Low risk	The study is registered under ISRCTN number ISRCTN86623192 (http://www.controlled-trials.com/ISRCTN86623192/). The reported outcomes are identical to those published in protocol
Other bias	Low risk	This study was supported financially by an *ad hoc* hospital fund for independent research. No funding biases noted. No significant baseline differences between groups

Psaradellis 2010

Methods	Placebo controlled RCT, follow-up: 3 weeks after last study drug dose
Participants	Adult population, mixed inpatient and outpatient, Canada, unclear if patients with recurrent *C. difficile* were included
Interventions	Placebo or*L. acidophilus* CL1285 and *L. casei* 25 x 10^9 cfu/day for 2 days then 50 x 10^9 cfu/day until 5 days after discontinuation of antibiotic
Outcomes	CDAD, AAD, and AE
Notes	

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	The method of randomization was not described

Psaradellis 2010 *(Continued)*

Allocation concealment (selection bias)	Unclear risk	Not enough information provided
Blinding of participants and personnel (performance bias) CDAD	Low risk	"This was a multicenter double-blind, randomized, placebo controlled, study…"
Blinding of participants and personnel (performance bias) AE	Low risk	See above: Blinding of participants and personnel (performance bias) CDAD
Blinding of participants and personnel (performance bias) AAD	Low risk	See above: Blinding of participants and personnel (performance bias) CDAD
Blinding of outcome assessment (detection bias) CDAD	Low risk	There is no explicit mention of outcome assessor blinding. Outcomes of interest to our review from this trial include AE and CDAD. There is no mention of blinding of the cytotoxin assay personnel although this is a placebo controlled drug trial so we will consider the risk of bias to be low here
Blinding of outcome assessment (detection bias) AE	Low risk	"Safety was assessed by the incidence of treatment emergent adverse events, which were reported according to the MedDRA (version 10.1) dictionary of terms." It appears AE were assessed by participants reporting to study personal all of whom were blinded and that an objective dictionary of terms was used for reported adverse events
Blinding of outcome assessment (detection bias) AAD	Low risk	Diarrhea assessed by blinded individuals
Incomplete outcome data (attrition bias) CDAD	High risk	"Among the 472 randomized patients, 29 patients were excluded from the ITT analysis due to antibiotic treatment duration of less than 3 days and 6 patients were excluded because diarrhea onset occurred before initiation of study treatment. Therefore a total of 437 (92.6%) were included in the ITT population…" "There were 16 patients in the BIO K+ group and 30 in the placebo group that underwent CDAD testing. Of these, 1 (6.2%) patient in the BIO K+ group and 4 (13.3%) in the placebo group were positive for the *C. difficile* toxins (odds ratio = 0.433, $p = 0.645$)." The missing data results from less than 10% of the participants and the numbers and reasons for those being excluded are balanced across groups. However, a 2:1 difference in sampling for CDAD is apparent and not representative of the difference in occurrence of AAD between groups. Therefore we must conclude a high risk of 'material' bias from incomplete and unbalanced outcome data for the CDAD outcome
Incomplete outcome data (attrition bias) AE	Low risk	The missing data results from less than 10% of the participants and the numbers and reasons for those being excluded are balanced across groups. Therefore we are not concerned about attrition bias as it relates to the AE outcomes
Incomplete outcome data (attrition bias)	Low risk	See above: Incomplete outcome data (attrition bias) AE

Psaradellis 2010 *(Continued)*

AAD

Selective reporting (reporting bias)	Low risk	While not reported in the full text article a protocol was discovered on clinicaltrials.gov. The primary outcome listed in the protocol was reported on in the paper. However a secondary outcome listed in the protocol was not mentioned in the paper: "Health outcome evaluation will look at the direct medical costs and clinical outcomes of alternative strategies in the prevention of antibiotic-associated diarrhea in hospitalized adult patients." Additionally, the primary outcome was secondarily analysed using statistical adjustments not prespecified in the protocol. However the unadjusted results are reported as well both in the body and abstract of the paper. We do not consider these concerns sufficient to consider the risk of 'material' reporting bias to be high. We therefore assess the risk of material bias here as low
Other bias	Unclear risk	"The patient demographics and baseline characteristics were similar for the BIO K+ and placebo groups" "John S. Sampalis and Eliofotisti Psaradellis are employees of JSS Medical Research Inc.; JSS Medical Research Inc. was paid by BIO K+ International Inc. to conduct and manage this study. JSS Medical Research Inc. was responsible for analyzing and interpreting the data as well as writing and reviewing the manuscript. The study was funded by a grant-in-aid of research from BIO K+ International Inc" Both study authors are employed by a CRO which was paid by the company (Bio K+) which produces the study product

Rafiq 2007

Methods	RCT (control group not stated), follow-up: not stated
Participants	NS, inpatient, USA, unclear if patients with recurrent *C. difficile* were included
Interventions	*L. acidophilus* 80%, *L. bulgaricus* 10%, *B. bifidum* 5%, *S. thermophilus* 5%. 3g/day with start of antibiotic until hospital discharge
Outcomes	CDAD
Notes	unpublished

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	The method of randomization was described
Allocation concealment (selection bias)	Unclear risk	Information not provided
Blinding of participants and personnel (performance bias)	Unclear risk	No mention of blinding in abstract and multiple contact attempts with author were unsuccessful. While AE were mentioned in abstract results regarding

Rafiq 2007 *(Continued)*

CDAD		this outcome were not reported so the only relevant outcome for our review is CDAD
Blinding of outcome assessment (detection bias) CDAD	Low risk	There is no mention of blinding of the cytotoxin assay personnel although this is a placebo controlled drug trial so we will consider the risk of bias to be low here
Incomplete outcome data (attrition bias) CDAD	Unclear risk	No mention of loss to follow-up in abstract and multiple contact attempts with author were unsuccessful. We are uncertain if there was incomplete outcome data and must assess as unclear
Selective reporting (reporting bias)	Unclear risk	No protocol identified. This is an abstract so unable to determine predefined outcomes from methods section for comparison with results
Other bias	Unclear risk	No information regarding funding is provided. According to our a priori defined criteria for RoB assessment we assess this as an unclear risk of bias

Ruszczynski 2008

Methods	Placebo controlled RCT, follow-up: 2 weeks after last study drug
Participants	Pediatric population, mixed inpatient and outpatient, Poland, unclear if patients with recurrent C. diff were included
Interventions	*L. rhamnosus GG* (2593, 2594, 2595) 2 x 10^{10} cfu/day or placebo for duration of antibiotic course
Outcomes	CDAD, AAD, and AE
Notes	

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	The study authors claim to have followed the protocol of an earlier study conducted by their research group (Kotowska 2005) "Investigators at the Medical University of Warsaw used computers to generate independent allocation sequences and randomization lists for each study site. To avoid a disproportionate number of patients in the experimental or placebo group, randomization at each site was performed in blocks of six (three received placebo and three, active treatment)." (Kotowska 2005)
Allocation concealment (selection bias)	Low risk	"To ensure allocation concealment, an independent subject prepared the randomization schedule and oversaw the packaging and labelling of trial treatments"
Blinding of participants and personnel (performance bias) CDAD	Low risk	"This was a double-blind, randomized, placebo-controlled, clinical trial…" "All investigators, participants, outcome assessors and data analysts were blinded to the assigned treatment throughout the study"
Blinding of participants and personnel (performance bias) AE	Low risk	See above: Blinding of participants and personnel (performance bias) CDAD

Ruszczynski 2008 *(Continued)*

Blinding of participants and personnel (performance bias) AAD	Low risk	See above: Blinding of participants and personnel (performance bias) CDAD
Blinding of outcome assessment (detection bias) CDAD	Low risk	"All investigators, participants, outcome assessors and data analysts were blinded to the assigned treatment throughout the study"
Blinding of outcome assessment (detection bias) AE	Low risk	See above: Blinding of outcome assessment (detection bias) CDAD
Blinding of outcome assessment (detection bias) AAD	Low risk	See above: Blinding of outcome assessment (detection bias) CDAD
Incomplete outcome data (attrition bias) CDAD	Low risk	"Of the 240 children recruited in the study, we assigned 120 children to receive L. rhamnosus and 120 to receive the placebo. Overall, three of the randomized children (one in the probiotic group and two in the placebo group) discontinued the study intervention and started to use one of the commercially available probiotic products. However, no patient was lost to follow-up. Thus, all 240 children enrolled were available for the analysis"
Incomplete outcome data (attrition bias) AE	Low risk	See above: Incomplete outcome data (attrition bias) CDAD
Incomplete outcome data (attrition bias) AAD	Low risk	See above: Incomplete outcome data (attrition bias) CDAD
Selective reporting (reporting bias)	Low risk	All primary and secondary outcomes were clearly identified in the 'methods' section and analysed in the 'results' section. In addition the study authors claim to have followed the protocol of an earlier study from their group (Kotowska 2005). The outcomes in that earlier paper were identical
Other bias	Low risk	"The study products were supplied by Biomed (Lublin, Poland), who had no role in the conception, design, or conduct of the study or in the analysis or interpretation of the data. Randomization codes were secured until all data entry was complete and data were analysed. The probiotic combination used in this study is commercially available as Lakcid Forte" "Declaration of funding interests: This study was funded in part by Biomed, Lublin, Poland, and the Medical University of Warsaw (Research Agreement UKI/224/2004)" This study appeared free of gross baseline imbalances between groups This study was partially funded by industry but there was a clear declaration of non-involvement and access to study design, conduct etc. According to our a priori defined criteria for funding bias we consider this a low risk of 'material' bias

Safdar 2008

Methods	Placebo controlled RCT, follow-up: not stated
Participants	Adult population, inpatient, USA, unclear if patients with recurrent *C. difficile* were included
Interventions	*L. acidophilus* 60 x 10^9 cfu/day or placebo during and 14 days after antibiotic course
Outcomes	CDAD, AAD, and AE
Notes	

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	The method of randomization was not described
Allocation concealment (selection bias)	Unclear risk	Not enough information provided so it is unclear if allocation was successfully concealed
Blinding of participants and personnel (performance bias) CDAD	Low risk	"This was a double-blind randomized placebo-controlled trial…" "Patients and investigators were unaware of treatment assignment"
Blinding of participants and personnel (performance bias) AE	Low risk	See above: Blinding of participants and personnel (performance bias) CDAD
Blinding of participants and personnel (performance bias) AAD	Low risk	See above: Blinding of participants and personnel (performance bias) CDAD
Blinding of outcome assessment (detection bias) CDAD	Low risk	There is no explicit mention of blinding of 'outcome assessors.' There is no mention of blinding of the cytotoxin assay personnel although this is a placebo controlled drug trial so in accordance with our a priori defined RoB criteria we will consider the risk of bias to be low here
Blinding of outcome assessment (detection bias) AE	Low risk	It appears AE was assessed by participants reporting to personnel all of whom were blinded
Blinding of outcome assessment (detection bias) AAD	Low risk	It appears diarrhea was assessed by participants reporting to personnel all of whom were blinded
Incomplete outcome data (attrition bias) CDAD	Low risk	"Analyses were intention-to-treat" "Between November 2003 and June 2005, 40 subjects were enrolled and were randomized, 23 to Florajen and 17 to placebo. One subject on placebo withdrew at his request and thus, 23 patients took Florajen and 16 took placebo"

Safdar 2008 *(Continued)*

		"*C. difficile* toxin was obtained only for seven patients with diarrhea. It was positive in one and negative in six cases. The one positive case of *C. difficile* diarrhea occurred in a patient randomized to placebo. The six negative cases were evenly distributed in the two study groups" 10 participants developed diarrhea. However only 7 of them were tested for *C. difficile*. Of the three that were not tested 2 were from the placebo group and one was from active group. It seems unlikely to us that this could have led to a 'material' bias that would have affected the authors' conclusions regarding the CDAD outcome. We consider the risk of 'material' bias for the CDAD outcome to be low
Incomplete outcome data (attrition bias) AE	Low risk	"Two subjects in the Florajen group and five in the placebo group reported adverse effects" Analysis was intention-to-treat. It appears one participant withdrew from the study. There was no loss to follow up. We consider the risk of attrition bias for the AE outcome to be low
Incomplete outcome data (attrition bias) AAD	Low risk	"Analyses were intention-to-treat" "Between November 2003 and June 2005, 40 subjects were enrolled and were randomized, 23 to Florajen and 17 to placebo. One subject on placebo withdrew at his request and thus, 23 patients took Florajen and 16 took placebo"
Selective reporting (reporting bias)	Low risk	No protocol for this study was identified. The outcomes listed and described in 'methods' were those analysed in 'results'
Other bias	Unclear risk	This study was free of baseline imbalances "We thank American Lifeline for providing study medication and placebo" No authors were associated with the company which produces the product being investigated. There is no explicit mention of study funding besides the provision of placebo and study medication. According to our a priori determined criteria for RoB we consider the lack of adequate funding disclosure to constitute an unclear risk of 'material' bias

Selinger 2013

Methods	Placebo controlled RCT, follow-up: 3 weeks after last study drug dose
Participants	Adult population, inpatient, United Kingdom, unclear if patients with recurrent C. *difficile* were included
Interventions	VSL #3 (*B. breve, B. longum, B. infantis, L. acidophilus, L. plantarum, L. paracasei, L. bulgaricus, S. thermophilus*) 900 x 10^9 cfu/day or placebo during and 7 days after antibiotic course
Outcomes	CDAD, AAD, length of hospital stay, and AE

Selinger 2013 *(Continued)*

Notes

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	"Participants were randomized 1:1 to active or placebo group using computer-generated random-permuted blocks stratified by centre, which were supplied by an independent statistician"
Allocation concealment (selection bias)	Low risk	Allocation of participants was performed by the pharmacies at each site, which remained blinded throughout the trial
Blinding of participants and personnel (performance bias) CDAD	Low risk	Double-blind; Allocation of participants was performed by the pharmacies at each site, which remained blinded throughout the trial
Blinding of participants and personnel (performance bias) AE	Low risk	Double-blind
Blinding of participants and personnel (performance bias) AAD	Low risk	“This multi-centre, randomised, double-blind, placebo-controlled trial…”
Blinding of outcome assessment (detection bias) CDAD	Low risk	“Masking: Double Blind (Subject, Caregiver, Investigator, Outcomes Assessor)” Protocol identified on clinicaltrials.gov NCT00973908. Protocol indicated outcome assessors were blinded
Blinding of outcome assessment (detection bias) AE	Low risk	See above: Blinding of outcome assessment (detection bias) CDAD
Blinding of outcome assessment (detection bias) AAD	Low risk	See above: Blinding of outcome assessment (detection bias) CDAD
Incomplete outcome data (attrition bias) CDAD	Unclear risk	Lost about 50% of patients to follow up, although they included all participants in ITT
Incomplete outcome data (attrition bias) AE	Unclear risk	Lost about 50% of patients to follow up, although they included all participants in ITT
Incomplete outcome data (attrition bias) AAD	Unclear risk	Lost about 50% of patients to follow up, although they included all participants in ITT.
Selective reporting (reporting bias)	Low risk	Protocol available: NCT00973908 Outcomes registered are the same as those reported

Selinger 2013 *(Continued)*

Other bias	Unclear risk	Conflict of interest statement C.P.S., A.B., A.C., M.L., S.S. and N.H. have support from Ferring Pharmaceuticals Ltd for the submitted work; C.P.S., A.C., M.L., S.S. and N.H. have relationships with Abbott, Dr Falk, Ferring Pharmaceuticals Ltd, Merck Smith Klyne, Procter & Gamble, Shire Warner Chillcott that might have an interest in the submitted work in the previous three years. Ferring Pharmaceuticals Ltd was not involved in study design; in the collection, analysis, and interpretation of data; in the writing of the report; and in the decision to submit the article for publication. Ferring Pharmaceuticals Ltd received the final version of the manuscript prior to submission for publication. Funding sources This investigator-led trial was funded by an unrestricted research grant from Ferring Pharmaceuticals Ltd covering the costs of providing the investigational medicinal product, a significant contribution towards the cost of the research nurses at the trial sites and the fees incurred by the MHRA. Further support was received from the National Institute for Health Research Clinical Research Network

Shan 2013

Methods	No treatment controlled RCT; single site; followed for length of Abx plus 2 weeks afterwards
Participants	Children (6 months to 14 years old); inpatient; China
Interventions	Saccharomyces boulardii; 500mg qd; taken for the length of the antibiotic prescription
Outcomes	CDAD; AAD; AE
Notes	

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	"Randomisation was done according to a computer-determined allocation to group A or B"
Allocation concealment (selection bias)	Low risk	"The sequence was concealed in an envelope, and the next neutral envelope was opened each time the next patient was included in the study"
Blinding of participants and personnel (performance bias) CDAD	High risk	Open label study
Blinding of participants and personnel (performance bias) AE	High risk	Open label study
Blinding of participants and personnel (performance bias) AAD	High risk	Open label study
Blinding of outcome assessment (detection bias) CDAD	High risk	Open label study

Shan 2013 *(Continued)*

Blinding of outcome assessment (detection bias) AE	High risk	Open label study
Blinding of outcome assessment (detection bias) AAD	High risk	Open label study
Incomplete outcome data (attrition bias) CDAD	High risk	A considerable amount of patients lost to follow up and not accounted for in the data. "In total, 50 (15%) patients were considered drop-outs (28 (16.7%) in group A and 22 (13.2%) in group B) because of withdrawal of consent (n=12), antibiotic stop within 48 h (n=8), non-compliance (n=10), and lost to follow-up (n=20)"
Incomplete outcome data (attrition bias) AE	High risk	ibid
Incomplete outcome data (attrition bias) AAD	High risk	ibid
Selective reporting (reporting bias)	Unclear risk	Outcomes in methods are reported in results, but no adverse events they are looking for are specified. No protocol available online.
Other bias	High risk	Funding bias. Senior author is consultant for the company that makes the study intervention

Shimbo 2005

Methods	No treatment control RCT, follow-up: up to 22 days after starting study drug
Participants	Adult population, outpatients, China, unclear if patients with recurrent *C. difficile* were included
Interventions	Clostridium butyricum MIYAIRI 588, 360 mg/day for 1 week prior to eradication therapy for 14 days
Outcomes	*C. difficile* detection, AAD and AE
Notes	

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Unclear risk	The method of randomization was not described
Allocation concealment (selection bias)	Unclear risk	No pertinent information provided
Blinding of participants and personnel (performance bias) AE	Unclear risk	"The patients were blindly and randomly allocated to two groups" The study claims the participants were blindly allocated. It is unclear but assumed this refers to blinding of patients to what group they were in. Howev-

Shimbo 2005 *(Continued)*

		er there was no placebo. The arms were standard therapy versus standard therapy plus probiotics. There is no further explanation as to how the medications were dispensed so it is unclear if blinding could have been assured. Also there is no statement on blinding of study personnel and no mention of double blinding or double dummy. We must assess the risk of 'material' performance bias to be unclear for both *C. difficile* incidence and AE
Blinding of participants and personnel (performance bias) C. difficile incidence	Unclear risk	See above: Blinding of participants and personnel (performance bias) AE
Blinding of participants and personnel (performance bias) AAD	Unclear risk	See above: Blinding of participants and personnel (performance bias) AE
Blinding of outcome assessment (detection bias) AE	Unclear risk	No pertinent information provided
Blinding of outcome assessment (detection bias) C. difficile incidence	Unclear risk	See above: Blinding of outcome assessment (detection bias) AE
Blinding of outcome assessment (detection bias) AAD	Unclear risk	See above: Blinding of outcome assessment (detection bias) AE
Incomplete outcome data (attrition bias) AE	Unclear risk	No pertinent information provided
Incomplete outcome data (attrition bias) C. difficile incidence	Unclear risk	See above:Incomplete outcome data (attrition bias) AE
Incomplete outcome data (attrition bias) AAD	Unclear risk	See above:Incomplete outcome data (attrition bias) AE
Selective reporting (reporting bias)	Low risk	No protocol identified. All outcomes discussed in the 'methods' section were analysed in the 'results' section
Other bias	Unclear risk	There is no mention of a funding source. According to our a priori determined RoB criteria for funding bias we consider this an unclear risk of 'material' bias

Siitonen 1990

Methods	Placebo controlled RCT, follow-up: last day of treatment
Participants	Adult population, age not stated, Finland, unclear if patients with recurrent *C. difficile* were included
Interventions	LGG yogurt 250 ml/day or placebo for 7 days
Outcomes	AE and *C. difficile* detection

Siitonen 1990 *(Continued)*

Notes

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	The method of randomization was not described
Allocation concealment (selection bias)	Unclear risk	No pertinent information provided and so it is unclear if allocation was successfully concealed
Blinding of participants and personnel (performance bias) AE	Low risk	There is no mention of blinding in this study. However this is a placebo controlled drug trial and so in accordance with our a priori determined RoB criteria for performance bias we will consider this as constituting a low risk of 'material' performance bias
Blinding of participants and personnel (performance bias) C. difficile incidence	Low risk	See above: Blinding of participants and personnel (performance bias) AE
Blinding of outcome assessment (detection bias) AE	Low risk	There is no mention of blinding in this study. However this is a placebo controlled drug trial and so in accordance with our a priori determined RoB criteria for detection bias we will consider this as constituting a low risk of 'material' detection bias
Blinding of outcome assessment (detection bias) C. difficile incidence	Low risk	See above: Blinding of outcome assessment (detection bias) AE
Incomplete outcome data (attrition bias) AE	Low risk	It appears there are no missing outcome data
Incomplete outcome data (attrition bias) C. difficile incidence	Low risk	See above: Incomplete outcome data (attrition bias) AE
Selective reporting (reporting bias)	Low risk	A protocol for this study was not identified. Outcomes were not explicitly mentioned as such but all inferred outcomes discussed in 'methods' were reported in 'results'
Other bias	High risk	Four authors are associated with either Valio Finnish Co-operative Dairies' Association or Orion Pharmaceutica. Lactobacillus is an organism found in many fermented dairy products. Orion is an industry that promotes and sells products with Lactobacillus GG which was the study intervention. There is no explicit mention of funding in this trial. However we believe it is likely this study was sponsored by either of the two aforementioned companies. We believe the conflict of interest and likely funding bias makes the risk of 'material' bias high

Sullivan 2004

Methods	Placebo controlled RCT, follow-up: up to 1 month after start of treatment

Sullivan 2004 *(Continued)*

Participants	Adult population, inpatients, Sweden, unclear if patients with recurrent *C. difficile* were included
Interventions	20 x 10^9 cfu/day *Lactobacillus paracasei spp. paracasei* F19 or placebo for 14 days
Outcomes	*C. difficile* detection and AE
Notes	

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	The method of randomization was not described
Allocation concealment (selection bias)	Unclear risk	No pertinent information provided so it is unclear if allocation was successfully concealed
Blinding of participants and personnel (performance bias) AE	Low risk	"The two treatment groups were randomized into one placebo and one active group regarding the probiotic supplement in a double-blind fashion" "A similar product was given to patients in the placebo groups but with no added microorganisms"
Blinding of participants and personnel (performance bias) C. difficile incidence	Low risk	See above: Blinding of participants and personnel (performance bias) AE
Blinding of outcome assessment (detection bias) AE	Low risk	It is assumed that AE were reported either by participants to personnel or observed by personnel all of whom where blinded. Therefore, we consider the AE outcome to have been assessed blind
Blinding of outcome assessment (detection bias) C. difficile incidence	Low risk	assay personnel although this is a placebo controlled drug trial so in accordance with our a priori determined RoB criteria we will consider the risk of bias to be low here
Incomplete outcome data (attrition bias) AE	High risk	44% of randomized participants did not complete the study and therefore had missing outcome data. This high missing outcome percentage leads us to consider the risk of 'material' attrition bias to be high for all outcomes
Incomplete outcome data (attrition bias) C. difficile incidence	High risk	See above: Incomplete outcome data (attrition bias) AE
Selective reporting (reporting bias)	Low risk	A protocol of this trial was not located. All outcomes listed in 'methods' were analysed in 'results.' We consider the risk of reporting bias to be low
Other bias	Unclear risk	No financial support, funding, or conflict of interest were listed. According to our a priori criteria for risk of funding bias we consider the risk of bias here to be unclear

Surawicz 1989

Methods	Placebo controlled RCT, follow-up: Mean 17.3 days (SD 8.6)
Participants	Adult population, inpatients, USA, unclear if patients with recurrent *C. difficile* were included
Interventions	*S. boulardii* lyophilized 20 x 10^9cfu/day or placebo within 48 hours of antibiotic commencement until 2 weeks after discontinuation
Outcomes	CDAD, AAD, AE, and *C. difficile* detection
Notes	

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	The method of randomization was not described
Allocation concealment (selection bias)	Unclear risk	No pertinent information provided and so it is unclear if allocation was successfully concealed
Blinding of participants and personnel (performance bias) CDAD	Low risk	Quotes: "The study was performed double-blindly" "The placebo was an inert composition formulated to be indistinguishable from the capsules of yeast"
Blinding of participants and personnel (performance bias) AE	Low risk	See above: Blinding of participants and personnel (performance bias) CDAD
Blinding of participants and personnel (performance bias) C. difficile incidence	Low risk	See above: Blinding of participants and personnel (performance bias) CDAD
Blinding of participants and personnel (performance bias) AAD	Low risk	See above: Blinding of participants and personnel (performance bias) CDAD
Blinding of outcome assessment (detection bias) CDAD	Low risk	*C. difficile* and the presence of *C. difficile* in those patients with diarrhea (CDAD) were determined via culture and toxin assay laboratory methods. There is no mention of blinding of the laboratory personnel although this is a placebo controlled drug trial so in accordance with our a priori defined RoB criteria we will consider the risk of bias to be low here
Blinding of outcome assessment (detection bias) AE	Low risk	While the diarrhea outcome was observed by study personnel as well as reported by participants to study personnel it is unclear how AE were assessed. While we consider this outcome to be a 'subjective' outcome which may be more susceptible to inadequate blinding we assume AE were reported by participants to trial personnel all of whom were blinded. So despite lack of clarity in the reporting of AE outcome assessment and its subjective nature we consider the overall risk of 'material' detection bias for AE to be low
Blinding of outcome assessment (detection bias)	Low risk	See above: Blinding of outcome assessment (detection bias) CDAD

Surawicz 1989 *(Continued)*
C. difficile incidence

Blinding of outcome assessment (detection bias) AAD	Low risk	See above: Blinding of outcome assessment (detection bias) CDAD
Incomplete outcome data (attrition bias) CDAD	High risk	"Of the 318 patients enrolled, 138 could not be evaluated for the following reasons: never received study drug or missed >3 doses (26 patients), developed diarrhea within 24 h of starting study (15 patients) or ˜72 h of antibiotic therapy (12 patients), exclusion drug started (9 patients], radiation therapy started (2 patients), or were monitored for <8 days (74 patients)" There is missing outcome data on 43% of randomized participants. This represents a potential for bias especially with the low reported event rate outcomes of CDAD and AE. While some of the missing data appears to have been due to randomized participants not being eligible for the trial due to predefined eligibility criteria there is still a large number of participants with missing outcomes data not due to exclusion criteria. The breakdown of how many missing outcome participants randomized into each group is unclear. Additionally, not all of the 180 evaluated participants were evaluated for C. diff (138 of 180 were). For all of these reasons we consider the risk of 'material' attrition bias to be high in all outcomes
Incomplete outcome data (attrition bias) AE	High risk	See above: Incomplete outcome data (attrition bias) CDAD
Incomplete outcome data (attrition bias) C. difficile incidence	High risk	See above: Incomplete outcome data (attrition bias) CDAD
Incomplete outcome data (attrition bias) AAD	High risk	See above: Incomplete outcome data (attrition bias) CDAD
Selective reporting (reporting bias)	Unclear risk	No protocol identified "The effectiveness of diarrhea prevention by the yeast was also evaluated in two subgroups of the study population: patients not receiving nasogastric tube feeding and patients infected with *C. difficile*. Patients on nasogastric tube feeding constituted a population with an increased risk of diarrhea (discussed later), and we wanted to evaluate patients in the absence of this risk factor for diarrhea. When patients who received tube feedings were eliminated from the calculations, the rate of diarrhea in the *S. boulardii* group was 5 of 109 (4.6%) compared with 13 of 59 (22%) for placebo (Figure 1); x2 = 10.42, P < 0.001" 'Outcomes' were not explicitly listed as such in the methods section. Therefore it is difficult to assess whether apparent subgroup analyses such as that evaluating participants with naso-gastric tubes separately constitute "one or more primary outcomes [being] reported using measurements, analysis methods or subsets of the data (e.g. subscales) that were not pre-specified." (Higgins 2011). We therefore consider the risk of 'material' reporting bias to be unclear
Other bias	Low risk	Baseline differences appeared roughly equivalent for the variables analysed

Surawicz 1989 *(Continued)*

	"This work was supported by a grant from Laboratoire Biocodex. Montrouge, France" Sponsor acknowledged but no author is associated with sponsor. According to our a priori determined criteria for RoB assessment we consider the risk of 'material' bias to be low here

Thomas 2001

Methods	Placebo controlled RCT, follow-up: 7 days after last study drug dose
Participants	Adult population, inpatient, USA, 2 patients in group 1 and 3 in the control group had a history of *C. difficile* infection
Interventions	*L. rhamnosus GG* 20 x 10^9 cfu/day or placebo within 24 hours of antibiotic commencement then for 14 days
Outcomes	CDAD, AAD and AE
Notes	

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	"A randomization schedule was generated by the Section of Biostatistics and stratified on 3 parameters, including baseline daily bowel movement frequency (<1 vs >1), use of beta-lactams as initial antibiotic therapy, and age at entry (<65 vs >65 years)" While the exact mechanism of randomization is not described we consider the involvement of the biostatistics department to be sufficient to assume a low risk of 'material' selection bias due to inadequate sequence generation
Allocation concealment (selection bias)	Low risk	"A pharmacist who at no time had direct contact with the patients or investigators dispensed active and placebo capsules according to the randomization schedule"
Blinding of participants and personnel (performance bias) CDAD	Low risk	Quotes: "Patients and investigators were blinded to the treatment" "Placebo capsules appeared identical to the active capsules..."
Blinding of participants and personnel (performance bias) AE	Low risk	See above: Blinding of participants and personnel (performance bias) CDAD
Blinding of participants and personnel (performance bias) AAD	Low risk	See above: Blinding of participants and personnel (performance bias) CDAD

Thomas 2001 *(Continued)*

Blinding of outcome assessment (detection bias) CDAD	Low risk	It appears that CDAD was determined by following up with the participants' primary care physicians and comparing hospital records of *C. difficile* positive patients which those enrolled in the trial. While it is not completely clear it seems as though the trial personnel were not those involved in assessing *C. difficile* but rather that those managing the patients ordered the tests themselves. Although this is unclear, this is a placebo controlled drug trial which is described as double blind. Based on our a priori determined criteria for the risk of bias for outcome assessor blinding this is sufficient to assess the risk of 'material' bias to be low here
Blinding of outcome assessment (detection bias) AE	Low risk	It appears AE was reported by participants to study personnel all of whom were blinded
Blinding of outcome assessment (detection bias) AAD	Low risk	It appears AAD was reported by blinded individuals
Incomplete outcome data (attrition bias) CDAD	Low risk	"Of the 302 patients who consented to participate, 34 failed to complete the study, and 1 patient enrolled but discontinued antibiotics after 1 dose, so was therefore determined to be ineligible. Thus, 267 patients completed the study" 12% missing data. The placebo group had 16 participants withdrawn (Dropped out (n=9), Insufficient follow-up (n=7)) and the treatment group had 19 participants withdrawn (Dropped out (n=14), Insufficient follow-up (n=4), Discontinued antibiotic after 1 dose (n=1) The numbers of missing data are grossly even between groups and not extreme. The reasons for withdrawal and dropout are not described "A chart review and a list of all patients with a positive *C. difficile* toxin assay since July 1998 obtained from the Mayo Clinic microbiology laboratory revealed 5 study patients diagnosed as having and treated for *C. difficile* colitis at our institution. Two of these patients were randomized to *Lactobacillus* GG, and 3 were randomized to placebo" The chart review displayed infrequent CDAD and seemingly same frequency in both groups. While the reasons for withdrawal and drop out were not clear, in light of the authors' negative findings we elected not to rate down here
Incomplete outcome data (attrition bias) AE	Unclear risk	"There was no difference in the proportion of patients experiencing nausea or abdominal cramping between the groups (*P*=.40 and *P*=.74, respectively). The patients receiving placebo tended to report gas or bloating more often than those receiving *Lactobacillus* GG (38.8% vs 28.0%), but this difference was not statistically significant (P=.06)" Numbers of patients from each group experiencing the AE of nausea and abdominal cramping cannot be calculated from the presented data. The event rates for gas and bloating can be calculated and the event rates are frequent enough to most likely not be significantly influenced by the relatively low amount of missing data. However, not all event rates are clear and so it is difficult to assess the risk of attrition bias in this instance

Thomas 2001 *(Continued)*

Incomplete outcome data (attrition bias) AAD	Unclear risk	The numbers of missing data are grossly even between groups and not extreme. The reasons for withdrawal and dropout are not described
Selective reporting (reporting bias)	Low risk	"The primary outcome was the proportion of patients experiencing diarrhea in the first 21 days after enrolment" "Two secondary outcomes were also assessed. The first was the proportion of patients who had either stool cultures or additional testing to determine the cause of diarrhea in the first 21 days after enrolment. These tests included fecal leukocyte counts, stool osmolality, and stool electrolytes. The second assessment was to determine the number of patients who were diagnosed as having AAD due to *C. difficile* in the first 21 days or at any time after enrolment" Despite the clear declaration of outcomes in the 'methods' section the primary outcome was assessed with multiple subgroup analyses not discussed in 'methods.' Examples include subgroups based on type of antibiotic, differing definitions of diarrhea, duration of antibiotic treatment, severe diarrhea and length of hospitalization. Therefore the primary outcome of rate of diarrhea was "reported using measurements, analysis methods or subsets of the data (e.g. subscales) that were not pre-specified". This classifies as a high risk of bias (Higgins 2011). This being said it is important to note that none of the subgroups resulted in significant findings either so this concern would be unlikely to bias the authors' conclusion. Considering the authors' conclusions, the direction of expected bias, and that these subgroups were not pertinent to our review, we consider the risk of a 'material' reporting bias that could influence our cumulative effect estimate in meta-analysis to be low
Other bias	Low risk	"The treatment (n=133) and placebo (n=134) groups were similar in terms of their demographics and medical profiles at enrolment." The study appears free of baseline imbalances "This study was supported in part by a grant from ConAgra Foods, Inc, Omaha, Neb" "Active capsules (CAG Functional Foods, Omaha, Neb)…" The study product is produced by a division of the sponsoring company (ConAgra). The sponsor is acknowledged and no one from the sponsoring agency was an author so based on our a priori defined criteria for funding bias we consider the risk of 'material' bias to be low

Wenus 2008

Methods	Placebo controlled RCT
Participants	Adult population, NS, Norway, unclear if patients with recurrent *C. difficile* were included
Interventions	Mixture of LGG, Lactobacillus acidophilus, and bifidobacterium 52.5 x 10^9 cfu/day or placebo for 14 days

Wenus 2008 *(Continued)*

Outcomes	CDAD, AAD and *C. difficile* detection
Notes	

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	The method of randomization was not described
Allocation concealment (selection bias)	Unclear risk	No pertinent information provided and so it is unclear if allocation was successfully concealed
Blinding of participants and personnel (performance bias) CDAD	Low risk	"In this double-blind placebo controlled study…" "Both products had a neutral taste…"
Blinding of participants and personnel (performance bias) C. difficile incidence	Low risk	See above: Blinding of participants and personnel (performance bias) CDAD
Blinding of participants and personnel (performance bias) AAD	Low risk	See above: Blinding of participants and personnel (performance bias) CDAD
Blinding of outcome assessment (detection bias) CDAD	Low risk	There is no mention of blinding of the cytotoxin assay personnel although this is a placebo controlled drug trial so in accordance with our a priori defined RoB criteria we will consider the risk of bias to be low here
Blinding of outcome assessment (detection bias) C. difficile incidence	Low risk	See above: Blinding of outcome assessment (detection bias) CDAD
Blinding of outcome assessment (detection bias) AAD	Low risk	Diarrhea assessed by participants who were blinded
Incomplete outcome data (attrition bias) CDAD	High risk	"The remaining 87 intention-to-treat patients were randomized to probiotic (n = 46) or placebo (n = 41) treatment. Groups were well balanced at study entry (Table 1). During the study there were 12 withdrawals/drop-outs in each treatment group (Figure 1). The remaining 34 and 29 patients in the active and placebo group, respectively, completed the study according to the protocol. The withdrawal/drop-out group did not differ from the per-protocol group with respect to age, sex, usual number of stools/day and previous experiences of AAD (data not shown) "Stool samples were collected twice during the study period" "Owing to low patient compliance, only 55 stool samples were examined. In the non-AAD/probiotic group one sample was positive for *C. difficile* culture,

Wenus 2008 *(Continued)*

		and another sample was positive for *C. difficile* toxin A. In the AAD/placebo group one sample was positive for *C. difficile* culture" "Our study was based on per-protocol analysis. Intention-to-treat analysis could not be obtained as end point data was lacking for several patients owing to withdrawal or drop-out" Comment: There are missing outcome data on 28% of randomized participants. Additionally it appears that of the remaining 63 participants there should have been 126 stool samples for *C. difficile* analysis. Only 55 were analysed. These concerns coupled with an extremely low *C. difficile* incidence event rate lead us to consider the risk of 'material' attrition bias to be high
Incomplete outcome data (attrition bias) C. difficile incidence	High risk	See above: Incomplete outcome data (attrition bias) CDAD
Incomplete outcome data (attrition bias) AAD	High risk	See above: Incomplete outcome data (attrition bias) CDAD
Selective reporting (reporting bias)	Low risk	No protocol for this study was identified. All outcomes listed in the 'methods' section were analysed in the 'results' section
Other bias	Low risk	Groups apparently free of baseline imbalances. The investigated product is produced by the sponsor of this trial (Biola: TINE BA, Oslo, Norway). No author was associated with the sponsoring company

Wong 2014

Methods	No treatment controlled trial; follow-up to 30 days from the end of antibiotic prescription
Participants	Adults with spinal cord injuries; inpatient; United Kingdom
Interventions	*Lactobacillus casei shirota* (Probiotic drink - Yakult Light™: 65 ml); 6.5 x 10^9 CFU qd; for length of antibiotic and 7 days afterwards
Outcomes	CDAD; AAD; AE
Notes	

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Unclear risk	Mechanism of randomization is not mentioned
Allocation concealment (selection bias)	Unclear risk	Not specified

Wong 2014 *(Continued)*

Blinding of participants and personnel (performance bias) CDAD	High risk	Standard care (no placebo)
Blinding of participants and personnel (performance bias) AE	High risk	Standard care (no placebo)
Blinding of participants and personnel (performance bias) AAD	High risk	Standard care (no placebo)
Blinding of outcome assessment (detection bias) CDAD	High risk	Standard care (no placebo)
Blinding of outcome assessment (detection bias) AE	High risk	Standard care (no placebo)
Blinding of outcome assessment (detection bias) AAD	High risk	Standard care (no placebo)
Incomplete outcome data (attrition bias) CDAD	Low risk	Little missing outcome data
Incomplete outcome data (attrition bias) AE	Low risk	Little missing outcome data
Incomplete outcome data (attrition bias) AAD	Low risk	Little missing outcome data
Selective reporting (reporting bias)	Low risk	Methods match results. No pre-published protocol mentioned
Other bias	Unclear risk	Sponsor of study is the company that makes the product being evaluated. Explicit statement that not involved in design and analysis

Methods: Randomized controlled trial (RCT)
Interventions: Colony-forming units (cfu)
Outcomes: Clostridium difficile-associated diarrhea (CDAD), antibiotic-associated diarrhea (AAD), adverse events (AE)

Characteristics of excluded studies *[ordered by study ID]*

Study	Reason for exclusion
Agarwal 2003	*C. difficile* or CDAD not measured
Allen 2012	Not an RCT

Study	Reason for exclusion
Anukam 2006	*C. difficile* or CDAD not measured
Armuzzi 2001	*C. difficile* or CDAD not measured
Avadhani 2011	Not an RCT
Basu 2007	*C. difficile* or CDAD not measured
Bekar 2011	*C. difficile* or CDAD not measured
Bellomo 1980	*C. difficile* or CDAD not measured
Benhamou 1999	*C. difficile* or CDAD not measured
Beniwal 2003	*C. difficile* or CDAD not measured
Berni 2011	Not an RCT
Black 1991	*C. difficile* or CDAD not measured
Bleichner 1997	*C. difficile* or CDAD not measured
Brunser 2006	*C. difficile* or CDAD not measured
Butler 2012	Not an RCT
Chapman 2011	Not an RCT
Chen 2011	Not an RCT
Cimperman 2011	*C. difficile* or CDAD not measured
Clements 1981	*C. difficile* or CDAD not measured
Contardi 1991	*C. difficile* or CDAD not measured
Conway 2007	*C. difficile* or CDAD not measured
Correa 2005	*C. difficile* or CDAD not measured
Cremonini 2002	*C. difficile* or CDAD not measured
de Bortoli 2007	*C. difficile* or CDAD not measured
de Vrese 2011	*C. difficile* or CDAD not measured
Elmer 1999	Patients had active diarrhea or CDAD
Erdeve 2004	*C. difficile* or CDAD not measured
Felley 2001	*C. difficile* or CDAD not measured
Forestier 2008	*C. difficile* or CDAD not measured
Francavilla 2008	*C. difficile* or CDAD not measured

Study	Reason for exclusion
Friedman 2010	Describing a previously included trial
Goldman 2006	*C. difficile* or CDAD not measured
Gotteland 2005	*C. difficile* or CDAD not measured
Gotz 1979	*C. difficile* or CDAD not measured
Guandalini 2000	*C. difficile* or CDAD not measured
Hafeez 2002	*C. difficile* or CDAD not measured
Hatakka 2001	*C. difficile* or CDAD not measured
Heimburger 1994	*C. difficile* or CDAD not measured
Hotz 1990	*C. difficile* or CDAD not measured
Hurduc 2009	*C. difficile* or CDAD not measured
Jacobi 2011	Not an RCT
Jirapinyo 2002	*C. difficile* or CDAD not measured
Kato 2004	*C. difficile* or CDAD not measured
Kollaritsch 1993	*C. difficile* or CDAD not measured
Kruis 2012	*C. difficile* or CDAD not measured
La Rosa 2003	*C. difficile* or CDAD not measured
Lawrence 2005	Patients had active diarrhea or CDAD
Lei 2006	*C. difficile* or CDAD not measured
Lionetti 2006	*C. difficile* or CDAD not measured
Madden 2005	*C. difficile* or CDAD not measured
Madeo 1999	*C. difficile* or CDAD not measured
Marcone 2008	*C. difficile* or CDAD not measured
Marshall 2008	Not an RCT
Martinez 2009	*C. difficile* or CDAD not measured
McFarland 1994a	Patients had active diarrhea or CDAD
Merenstein 2009	*C. difficile* or CDAD not measured
Mihatsch 2010	*C. difficile* or CDAD not measured
Mohan 2008	*C. difficile* or CDAD not measured

Study	Reason for exclusion
Myllyluoma 2005	*C. difficile* or CDAD not measured
Myllyluoma 2007	*C. difficile* or CDAD not measured
Nista 2004	*C. difficile* or CDAD not measured
Oleinichenko 1999	*C. difficile* or CDAD not measured
Ozdil 2011	*C. difficile* or CDAD not measured
Park 2007	*C. difficile* or CDAD not measured
Pereg 2005	*C. difficile* or CDAD not measured
Pirotta 2004	*C. difficile* or CDAD not measured
Plewinska 2006	*C. difficile* or CDAD not measured
Pochapin 2000	Patients had active diarrhea or CDAD
Potts 1996	*C. difficile* or CDAD not measured
Pushkarev 2005	*C. difficile* or CDAD not measured
Ranasinghe 2007	*C. difficile* or CDAD not measured
Rayes 2002a	*C. difficile* or CDAD not measured
Rayes 2002b	*C. difficile* or CDAD not measured
Rayes 2002c	*C. difficile* or CDAD not measured
Reddy 2007	*C. difficile* or CDAD not measured
Robertson 2000	Not an RCT
Sahagún-Flores 2007	*C. difficile* or CDAD not measured
Schrezenmeir 2002	*C. difficile* or CDAD not measured
Schrezenmeir 2004	*C. difficile* or CDAD not measured
Sepp 2011	Not an RCT
Souza 2012	*C. difficile* or CDAD not measured
Stein 2007	*C. difficile* or CDAD not measured
Stockenhuber 2008	Not an RCT
Tankanow 1990	*C. difficile* or CDAD not measured
Tursi 2004	*C. difficile* or CDAD not measured
Vandenplas 2011	*C. difficile* or CDAD not measured

Study	Reason for exclusion
Wilhelm 2011	Not an RCT
Witsell 1995	*C. difficile* or CDAD not measured
Woo 2008	Not an RCT
Wullt 2007	Patients had active diarrhea or CDAD
Yoon 2011	*C. difficile* or CDAD not measured
Yost 1985	*C. difficile* or CDAD not measured
Ziemniak 2006	*C. difficile* or CDAD not measured

DATA AND ANALYSES

Comparison 1. Probiotics versus control

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 Incidence CDAD: complete case	31	8672	Risk Ratio (M-H, Random, 95% CI)	0.40 [0.30, 0.52]
2 Incidence CDAD: complete case - fixed effects	31	8672	Risk Ratio (M-H, Fixed, 95% CI)	0.38 [0.30, 0.50]
3 Incidence CDAD: Sensitivity (1.5:1)	31	9637	Risk Ratio (M-H, Random, 95% CI)	0.41 [0.32, 0.54]
4 Incidence CDAD: Sensitivity (2:1)	31	9637	Risk Ratio (M-H, Random, 95% CI)	0.44 [0.34, 0.58]
5 Incidence CDAD: Sensitivity (3:1)	31	9637	Risk Ratio (M-H, Random, 95% CI)	0.48 [0.36, 0.63]
6 Incidence CDAD: Sensitivity (5:1)	31	9637	Risk Ratio (M-H, Random, 95% CI)	0.54 [0.39, 0.74]
7 Incidence CDAD: Subgroup: Inpatient versus outpatient populations	28	8691	Risk Ratio (M-H, Random, 95% CI)	0.40 [0.30, 0.53]
7.1 Inpatient	21	7024	Risk Ratio (M-H, Random, 95% CI)	0.39 [0.29, 0.53]
7.2 Outpatient	2	462	Risk Ratio (M-H, Random, 95% CI)	0.31 [0.01, 7.47]
7.3 Mixed	5	1205	Risk Ratio (M-H, Random, 95% CI)	0.43 [0.20, 0.94]

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
8 Incidence CDAD: Subgroup: Species: all	31	8672	Risk Ratio (M-H, Random, 95% CI)	0.40 [0.30, 0.52]
8.1 S. boulardii	9	1755	Risk Ratio (M-H, Random, 95% CI)	0.41 [0.22, 0.79]
8.2 S. cerevisiae	1	477	Risk Ratio (M-H, Random, 95% CI)	0.94 [0.13, 6.61]
8.3 Lactobacillus GG	5	1126	Risk Ratio (M-H, Random, 95% CI)	0.63 [0.30, 1.32]
8.4 L. acidophilus + L. casei	3	707	Risk Ratio (M-H, Random, 95% CI)	0.22 [0.11, 0.42]
8.5 L. acidophilus + B. bifidum	2	2948	Risk Ratio (M-H, Random, 95% CI)	0.64 [0.33, 1.25]
8.6 L. acidophilus	1	36	Risk Ratio (M-H, Random, 95% CI)	0.22 [0.01, 4.99]
8.7 L. acidophilus + L. delbrueki subs. bulgaricus + B. bifidum	1	156	Risk Ratio (M-H, Random, 95% CI)	0.35 [0.15, 0.85]
8.8 L. acidophilus + L. bulgaricus + B. bifidum + S. thermophilus	1	100	Risk Ratio (M-H, Random, 95% CI)	0.28 [0.11, 0.67]
8.9 L. acidophilus + L. paracasei + B. lactis	1	447	Risk Ratio (M-H, Random, 95% CI)	0.35 [0.12, 1.00]
8.10 B. breve + B. Longum + B. infantis + L. acidophilus + L. plantarum + L. paracasei + L. bulgaricus + S. thermophilus	1	217	Risk Ratio (M-H, Random, 95% CI)	0.0 [0.0, 0.0]
8.11 L. casei + L. bulgaris + S. thermophilus	1	109	Risk Ratio (M-H, Random, 95% CI)	0.05 [0.00, 0.84]
8.12 L. plantarum	1	156	Risk Ratio (M-H, Random, 95% CI)	3.16 [0.13, 76.30]
8.13 L. reuteri	1	97	Risk Ratio (M-H, Random, 95% CI)	0.0 [0.0, 0.0]
8.14 Lactobacillus GG + L. acidophilus + B. animalis	1	63	Risk Ratio (M-H, Random, 95% CI)	0.29 [0.01, 6.76]
8.15 L. casei shirota	1	158	Risk Ratio (M-H, Random, 95% CI)	0.36 [0.01, 8.69]
8.16 Lactococcus lactis, Lactobacillus, Bifidobacterium and Streptococcus thermophilus	1	120	Risk Ratio (M-H, Random, 95% CI)	0.0 [0.0, 0.0]

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
9 Incidence CDAD: Subgroup: Species: LGG versus SB	13	2921	Risk Ratio (M-H, Random, 95% CI)	0.50 [0.31, 0.82]
9.1 Lactobacillus GG	5	1131	Risk Ratio (M-H, Random, 95% CI)	0.63 [0.30, 1.33]
9.2 S. boulardii	8	1790	Risk Ratio (M-H, Random, 95% CI)	0.42 [0.22, 0.80]
10 Incidence CDAD: Subgroup: Species: LGG versus LA + LC	8	1912	Risk Ratio (M-H, Random, 95% CI)	0.35 [0.21, 0.57]
10.1 Lactobacillus GG	5	1131	Risk Ratio (M-H, Random, 95% CI)	0.63 [0.30, 1.33]
10.2 L. acidophilus + L. casei	3	781	Risk Ratio (M-H, Random, 95% CI)	0.21 [0.11, 0.42]
11 Incidence CDAD: Subgroup: Risk of Bias	31	8672	Risk Ratio (M-H, Random, 95% CI)	0.40 [0.30, 0.52]
11.1 Low risk of bias	10	4688	Risk Ratio (M-H, Random, 95% CI)	0.39 [0.25, 0.61]
11.2 High or unclear risk of bias	21	3984	Risk Ratio (M-H, Random, 95% CI)	0.40 [0.28, 0.58]
12 Incidence CDAD: Subgroup: Adult versus child	30	8941	Risk Ratio (M-H, Random, 95% CI)	0.40 [0.30, 0.52]
12.1 Adult studies	24	7800	Risk Ratio (M-H, Random, 95% CI)	0.41 [0.30, 0.57]
12.2 Pediatric studies	6	1141	Risk Ratio (M-H, Random, 95% CI)	0.35 [0.19, 0.63]
13 Incidence CDAD: Baseline Risk	31	8672	Risk Ratio (M-H, Random, 95% CI)	0.40 [0.30, 0.52]
13.1 0-2% Baseline Risk	15	5845	Risk Ratio (M-H, Random, 95% CI)	0.77 [0.45, 1.32]
13.2 3-5% Baseline risk	3	373	Risk Ratio (M-H, Random, 95% CI)	0.53 [0.16, 1.77]
13.3 >5% Baseline Risk	13	2454	Risk Ratio (M-H, Random, 95% CI)	0.30 [0.21, 0.42]
14 Incidence CDAD: worst case	20	4317	Risk Ratio (M-H, Random, 95% CI)	1.90 [0.90, 4.01]
15 Incidence CDAD: 0-2% baseline risk RoB	15	5845	Risk Ratio (M-H, Random, 95% CI)	0.77 [0.45, 1.32]

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
15.1 Low Risk	4	3651	Risk Ratio (M-H, Random, 95% CI)	0.72 [0.38, 1.38]
15.2 High/Unclear	11	2194	Risk Ratio (M-H, Random, 95% CI)	0.89 [0.34, 2.34]
16 Incidence CDAD: 3-5% baseline risk RoB	3	373	Risk Ratio (M-H, Random, 95% CI)	0.53 [0.16, 1.77]
16.1 Low Risk	1	151	Risk Ratio (M-H, Random, 95% CI)	0.21 [0.01, 4.37]
16.2 High/Unclear	2	222	Risk Ratio (M-H, Random, 95% CI)	0.63 [0.17, 2.36]
17 Incidence CDAD: >5% baseline risk RoB	13	2454	Risk Ratio (M-H, Random, 95% CI)	0.30 [0.21, 0.42]
17.1 Low Risk	5	886	Risk Ratio (M-H, Random, 95% CI)	0.25 [0.14, 0.44]
17.2 Unclear/High	8	1568	Risk Ratio (M-H, Random, 95% CI)	0.33 [0.21, 0.50]
18 Incidence of infection: complete case	15	1214	Risk Ratio (M-H, Random, 95% CI)	0.86 [0.67, 1.10]
19 Incidence of infection: Subgroup: Risk of Bias	15	1214	Risk Ratio (M-H, Random, 95% CI)	0.86 [0.67, 1.10]
19.1 Low Risk of Bias	4	227	Risk Ratio (M-H, Random, 95% CI)	0.89 [0.54, 1.48]
19.2 High or Unclear Risk of Bias	11	987	Risk Ratio (M-H, Random, 95% CI)	0.85 [0.64, 1.13]
20 Incidence of infection: Inpatient versus outpatient	13	1132	Risk Ratio (M-H, Random, 95% CI)	0.85 [0.66, 1.09]
20.1 Inpatient	8	870	Risk Ratio (M-H, Random, 95% CI)	0.87 [0.67, 1.13]
20.2 Outpatient	4	112	Risk Ratio (M-H, Random, 95% CI)	0.45 [0.13, 1.52]
20.3 Mixed	1	150	Risk Ratio (M-H, Random, 95% CI)	1.03 [0.21, 4.93]
21 Incidence of infection: Species: all	15	1214	Risk Ratio (M-H, Random, 95% CI)	0.86 [0.67, 1.10]
21.1 Lactobacillus reuteri DSM 17938	1	97	Risk Ratio (M-H, Random, 95% CI)	0.91 [0.50, 1.68]

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
21.2 Clostridium butyricum	2	54	Risk Ratio (M-H, Random, 95% CI)	1.17 [0.13, 10.66]
21.3 B. bifidum, B. lactis, B. longum, E. faecium, L. acidophilus, L. paracasei, L. plantarum, L. rhamnosus, and L. salivarius	1	38	Risk Ratio (M-H, Random, 95% CI)	0.5 [0.05, 5.06]
21.4 S. boulardii	3	400	Risk Ratio (M-H, Random, 95% CI)	0.98 [0.66, 1.45]
21.5 Bifidobacterium bifidum and Lactobacillus acidophilus	1	23	Risk Ratio (M-H, Random, 95% CI)	0.44 [0.11, 1.81]
21.6 Lactobacillus acidophilus, Lactobacillus delbrueki subs. bulgaricus, Bifidobacterium bifidum	1	156	Risk Ratio (M-H, Random, 95% CI)	0.77 [0.47, 1.26]
21.7 Lactobacillus paracasei spp. paracasei F19	1	36	Risk Ratio (M-H, Random, 95% CI)	1.0 [0.16, 6.35]
21.8 LGG	1	16	Risk Ratio (M-H, Random, 95% CI)	0.0 [0.0, 0.0]
21.9 L. acidophilus and B. bifidum	1	138	Risk Ratio (M-H, Random, 95% CI)	0.71 [0.24, 2.14]
21.10 LGG, Lactobacillus acidophilus, and bifidobacterium	1	63	Risk Ratio (M-H, Random, 95% CI)	1.71 [0.16, 17.87]
21.11 L. plantarum 299v	2	193	Risk Ratio (M-H, Random, 95% CI)	0.45 [0.05, 4.14]
22 Incidence of infection: Subgroup: Species: LGG versus SB	4	416	Risk Ratio (M-H, Random, 95% CI)	0.98 [0.66, 1.45]
22.1 LGG	1	16	Risk Ratio (M-H, Random, 95% CI)	0.0 [0.0, 0.0]
22.2 SB	3	400	Risk Ratio (M-H, Random, 95% CI)	0.98 [0.66, 1.45]
23 Incidence of infection: Adult versus child	15	1214	Risk Ratio (M-H, Random, 95% CI)	0.86 [0.67, 1.10]
23.1 Children	2	253	Risk Ratio (M-H, Random, 95% CI)	0.82 [0.56, 1.21]
23.2 Adult	13	961	Risk Ratio (M-H, Random, 95% CI)	0.89 [0.64, 1.24]
24 Adverse Events: complete case	32	8305	Risk Ratio (M-H, Random, 95% CI)	0.83 [0.71, 0.97]

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
25 Adverse Events: Subgroup: Risk of Bias	32	8305	Risk Ratio (M-H, Random, 95% CI)	0.83 [0.71, 0.97]
25.1 Low Risk of Bias	14	5281	Risk Ratio (M-H, Random, 95% CI)	0.89 [0.76, 1.04]
25.2 High/Unclear risk of bias	18	3024	Risk Ratio (M-H, Random, 95% CI)	0.56 [0.36, 0.88]
26 Adverse Events: Sensitivity 1.5:1	32	9151	Risk Ratio (M-H, Random, 95% CI)	0.86 [0.74, 1.01]
27 Adverse Events: Sensitivity 2:1	32	9151	Risk Ratio (M-H, Random, 95% CI)	0.87 [0.74, 1.01]
28 Adverse Events: Sensitivity 3:1	32	9151	Risk Ratio (M-H, Random, 95% CI)	0.89 [0.75, 1.06]
29 Adverse Events: Sensitivity 5:1	32	9151	Risk Ratio (M-H, Random, 95% CI)	0.96 [0.78, 1.17]
30 Adverse Events: Species: all	32	8305	Risk Ratio (M-H, Random, 95% CI)	0.83 [0.71, 0.97]
30.1 L. plantarum	2	207	Risk Ratio (M-H, Random, 95% CI)	1.04 [0.22, 4.99]
30.2 LGG	6	1142	Risk Ratio (M-H, Random, 95% CI)	0.74 [0.48, 1.14]
30.3 SB	8	1684	Risk Ratio (M-H, Random, 95% CI)	0.76 [0.48, 1.19]
30.4 LA	1	39	Risk Ratio (M-H, Random, 95% CI)	0.28 [0.06, 1.26]
30.5 S. cerevisiae	1	292	Risk Ratio (M-H, Random, 95% CI)	1.5 [0.75, 3.00]
30.6 Clostridium butyricum	2	54	Risk Ratio (M-H, Random, 95% CI)	0.31 [0.15, 0.65]
30.7 L. casei shirota	1	158	Risk Ratio (M-H, Random, 95% CI)	0.0 [0.0, 0.0]
30.8 Lactobacillus paracasei spp. paracasei F19	1	36	Risk Ratio (M-H, Random, 95% CI)	0.0 [0.0, 0.0]
30.9 LA + BB	2	2964	Risk Ratio (M-H, Random, 95% CI)	1.03 [0.90, 1.18]
30.10 LA + LC	3	781	Risk Ratio (M-H, Random, 95% CI)	0.92 [0.76, 1.11]

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
30.11 L. acidophilus + L. paracasei + B. lactis	1	448	Risk Ratio (M-H, Random, 95% CI)	0.55 [0.26, 1.16]
30.12 L. casei + L. bulgaris + S. thermophilus	1	113	Risk Ratio (M-H, Random, 95% CI)	0.0 [0.0, 0.0]
30.13 Lactococcus lactis, Lactobacillus, Bifidobacterium and Streptococcus thermophilus	1	120	Risk Ratio (M-H, Random, 95% CI)	0.0 [0.0, 0.0]
30.14 B. bifidum, B. lactis, B. longum, E. faecium, L. acidophilus, L. paracasei, L. plantarum, L. rhamnosus, and L. salivarius	1	38	Risk Ratio (M-H, Random, 95% CI)	0.88 [0.67, 1.17]
30.15 B. breve + B. Longum + B. infantis + L. acidophilus + L. plantarum + L. paracasei + L. bulgaricus + S. thermophilus	1	229	Risk Ratio (M-H, Random, 95% CI)	0.84 [0.43, 1.63]
31 Adverse Events: Species: LGG versus SB	14	2826	Risk Ratio (M-H, Random, 95% CI)	0.76 [0.58, 1.01]
31.1 LGG	6	1142	Risk Ratio (M-H, Random, 95% CI)	0.74 [0.48, 1.14]
31.2 SB	8	1684	Risk Ratio (M-H, Random, 95% CI)	0.76 [0.48, 1.19]
32 Adverse Events: Species: LGG versus LA + LC	9	1923	Risk Ratio (M-H, Random, 95% CI)	0.86 [0.72, 1.03]
32.1 LGG	6	1142	Risk Ratio (M-H, Random, 95% CI)	0.74 [0.48, 1.14]
32.2 LA + LC	3	781	Risk Ratio (M-H, Random, 95% CI)	0.92 [0.76, 1.11]
33 Adverse Events: Inpatient versus outpatient	29	8042	Risk Ratio (M-H, Random, 95% CI)	0.88 [0.77, 1.02]
33.1 Inpatient	18	6263	Risk Ratio (M-H, Random, 95% CI)	0.89 [0.71, 1.13]
33.2 Outpatient	6	574	Risk Ratio (M-H, Random, 95% CI)	0.77 [0.54, 1.10]
33.3 Mixed	5	1205	Risk Ratio (M-H, Random, 95% CI)	0.90 [0.73, 1.11]
34 Adverse Events: Adult versus child	32	8305	Risk Ratio (M-H, Random, 95% CI)	0.83 [0.71, 0.97]

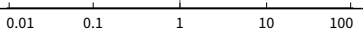
Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
34.1 Adult	28	7417	Risk Ratio (M-H, Random, 95% CI)	0.83 [0.71, 0.97]
34.2 Children	4	888	Risk Ratio (M-H, Random, 95% CI)	0.0 [0.0, 0.0]
35 Incidence AAD: complete case	33	8870	Risk Ratio (M-H, Random, 95% CI)	0.58 [0.48, 0.70]
36 Incidence AAD: Subgroup: Risk of Bias	33	8870	Risk Ratio (M-H, Random, 95% CI)	0.58 [0.48, 0.70]
36.1 Low risk of bias	16	5669	Risk Ratio (M-H, Random, 95% CI)	0.65 [0.51, 0.82]
36.2 High or Unclear risk of bias	17	3201	Risk Ratio (M-H, Random, 95% CI)	0.52 [0.40, 0.67]
37 Incidence AAD: sensitivity (1.5:1)	33	9516	Risk Ratio (M-H, Random, 95% CI)	0.60 [0.50, 0.73]
38 Incidence AAD: sensitivity (2:1)	33	9516	Risk Ratio (M-H, Random, 95% CI)	0.63 [0.52, 0.75]
39 Incidence AAD: sensitivity (3:1)	33	9516	Risk Ratio (M-H, Random, 95% CI)	0.66 [0.55, 0.80]
40 Incidence AAD: sensitivity (5:1)	33	9516	Risk Ratio (M-H, Random, 95% CI)	0.74 [0.60, 0.90]
41 Incidence AAD: Patient population	26	4995	Risk Ratio (M-H, Random, 95% CI)	0.56 [0.46, 0.68]
41.1 Inpatient	18	3280	Risk Ratio (M-H, Random, 95% CI)	0.56 [0.44, 0.72]
41.2 Outpatient	3	510	Risk Ratio (M-H, Random, 95% CI)	0.51 [0.30, 0.87]
41.3 Mixed	5	1205	Risk Ratio (M-H, Random, 95% CI)	0.55 [0.35, 0.86]
42 Incidence AAD: Species: all	31	5359	Risk Ratio (M-H, Random, 95% CI)	0.55 [0.46, 0.65]
42.1 Lactobacillus GG	4	942	Risk Ratio (M-H, Random, 95% CI)	0.75 [0.39, 1.43]
42.2 S. boulardi	10	1925	Risk Ratio (M-H, Random, 95% CI)	0.51 [0.36, 0.73]
42.3 Clostridium butyricum	2	54	Risk Ratio (M-H, Random, 95% CI)	0.29 [0.06, 1.34]

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
42.4 L. acidophilus	1	39	Risk Ratio (M-H, Random, 95% CI)	0.46 [0.16, 1.38]
42.5 L. acidophilus + L. casei	3	781	Risk Ratio (M-H, Random, 95% CI)	0.59 [0.42, 0.81]
42.6 L. acidophilus + B. bifidum	1	138	Risk Ratio (M-H, Random, 95% CI)	0.83 [0.51, 1.36]
42.7 B. breve + B. longum + B. infantis + L. acidophilus + L. plantarum	1	124	Risk Ratio (M-H, Random, 95% CI)	0.4 [0.08, 1.98]
42.8 L. casei + L. bulgaris + S. thermophilus	1	113	Risk Ratio (M-H, Random, 95% CI)	0.36 [0.17, 0.79]
42.9 L. plantarum	1	163	Risk Ratio (M-H, Random, 95% CI)	1.25 [0.40, 3.92]
42.10 Lactobacillus GG + L. acidophilus + B. animalis	1	63	Risk Ratio (M-H, Random, 95% CI)	0.21 [0.05, 0.93]
42.11 B. bifidum + B. lactis + B. longum + E. faecium + L. acidophilus + L. paracasei + L. plantarum + L. rhamnosus + L. sativarius	1	38	Risk Ratio (M-H, Random, 95% CI)	0.6 [0.35, 1.02]
42.12 L. reuteri	1	97	Risk Ratio (M-H, Random, 95% CI)	0.98 [0.06, 15.22]
42.13 L. acidophilus + L. paracasei + B. lactis	1	448	Risk Ratio (M-H, Random, 95% CI)	0.62 [0.44, 0.89]
42.14 L. acidophilus + L. delbrueki subs. bulgaricus + B. bifidum	1	156	Risk Ratio (M-H, Random, 95% CI)	0.42 [0.29, 0.61]
42.15 L. casei shirota	1	158	Risk Ratio (M-H, Random, 95% CI)	0.31 [0.18, 0.53]
42.16 Lactococcus lactis, Lactobacillus, Bifidobacterium and Streptococcus thermophilus	1	120	Risk Ratio (M-H, Random, 95% CI)	0.0 [0.0, 0.0]
43 Incidence AAD: Species: LGG versus SB	14	2867	Risk Ratio (M-H, Random, 95% CI)	0.57 [0.41, 0.80]
43.1 LGG	4	942	Risk Ratio (M-H, Random, 95% CI)	0.75 [0.39, 1.43]
43.2 SB	10	1925	Risk Ratio (M-H, Random, 95% CI)	0.51 [0.36, 0.73]
44 Incidence AAD: Species: LGG versus LA + LC	7	1723	Risk Ratio (M-H, Random, 95% CI)	0.65 [0.47, 0.91]

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
44.1 LGG	4	942	Risk Ratio (M-H, Random, 95% CI)	0.75 [0.39, 1.43]
44.2 LA + LC	3	781	Risk Ratio (M-H, Random, 95% CI)	0.59 [0.42, 0.81]
45 Incidence AAD: Adult versus child	29	8177	Risk Ratio (M-H, Random, 95% CI)	0.56 [0.46, 0.68]
45.1 Adult	23	7036	Risk Ratio (M-H, Random, 95% CI)	0.62 [0.51, 0.76]
45.2 Child	6	1141	Risk Ratio (M-H, Random, 95% CI)	0.38 [0.29, 0.49]
46 Incidence AAD: Adult (RoB)	23	7036	Risk Ratio (M-H, Random, 95% CI)	0.62 [0.51, 0.76]
46.1 Low Risk of Bias	11	4540	Risk Ratio (M-H, Random, 95% CI)	0.64 [0.49, 0.84]
46.2 Unclear/High Risk Of Bias	12	2496	Risk Ratio (M-H, Random, 95% CI)	0.60 [0.44, 0.83]
47 Incidence AAD: Child (RoB)	6	1141	Risk Ratio (M-H, Random, 95% CI)	0.38 [0.29, 0.49]
47.1 Low Risk of Bias	4	739	Risk Ratio (M-H, Random, 95% CI)	0.41 [0.30, 0.55]
47.2 Unclear/High Risk of Bias	2	402	Risk Ratio (M-H, Random, 95% CI)	0.28 [0.16, 0.49]
48 Length of Hospital Stay: complete case	4	3484	Mean Difference (IV, Random, 95% CI)	-0.17 [-1.03, 0.68]

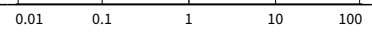
Analysis 1.1. Comparison 1 Probiotics versus control, Outcome 1 Incidence CDAD: complete case.

Study or subgroup	Experimental n/N	Control n/N	Risk Ratio M-H, Random, 95% CI	Weight	Risk Ratio M-H, Random, 95% CI
Allen 2013	12/1404	17/1406		14.25%	0.71[0.34,1.47]
Arvola 1999	1/61	1/58		1.02%	0.95[0.06,14.85]
Beausoleil 2007	1/39	7/42		1.83%	0.15[0.02,1.19]
Bravo 2008	0/41	0/41			Not estimable
Can 2006	0/73	2/78		0.85%	0.21[0.01,4.37]
Cindoruk 2007	0/59	0/51			Not estimable
Duman 2005	0/185	1/161		0.76%	0.29[0.01,7.08]
Ehrhardt 2016	2/246	2/231		2.02%	0.94[0.13,6.61]
Fominykh 2013	0/80	0/40			Not estimable
Gao 2010	9/171	20/84		13.99%	0.22[0.11,0.46]



Favours experimental — Favours control

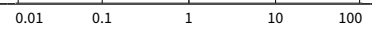
Study or subgroup	Experimental n/N	Control n/N	Risk Ratio M-H, Random, 95% CI	Weight	Risk Ratio M-H, Random, 95% CI
Georgieva 2015	0/49	0/48			Not estimable
Hickson 2007	0/56	9/53		0.97%	0.05[0,0.84]
Kotowska 2005	3/119	10/127		4.81%	0.32[0.09,1.14]
Lonnermark 2010	1/76	0/80		0.76%	3.16[0.13,76.3]
McFarland 1995	3/80	4/79		3.59%	0.74[0.17,3.2]
Miller 2008a	4/95	7/94		5.4%	0.57[0.17,1.87]
Miller 2008b	2/156	0/155		0.84%	4.97[0.24,102.65]
Ouwehand 2014	6/304	8/143		7.13%	0.35[0.12,1]
Pancheva 2009	6/78	17/78		10.04%	0.35[0.15,0.85]
Plummer 2004	2/69	5/69		2.99%	0.4[0.08,1.99]
Pozzoni 2012	3/106	2/98		2.47%	1.39[0.24,8.13]
Psaradellis 2010	1/185	4/186		1.62%	0.25[0.03,2.23]
Rafiq 2007	5/45	22/55		9.78%	0.28[0.11,0.67]
Ruszczynski 2008	3/120	7/120		4.37%	0.43[0.11,1.62]
Safdar 2008	0/22	1/14		0.78%	0.22[0.01,4.99]
Selinger 2013	0/111	0/106			Not estimable
Shan 2013	1/139	8/144		1.81%	0.13[0.02,1.02]
Surawicz 1989	3/113	5/61		3.95%	0.32[0.08,1.31]
Thomas 2001	2/133	3/134		2.45%	0.67[0.11,3.96]
Wenus 2008	0/34	1/29		0.77%	0.29[0.01,6.76]
Wong 2014	0/76	1/82		0.76%	0.36[0.01,8.69]
Total (95% CI)	**4525**	**4147**		**100%**	**0.4[0.3,0.52]**
Total events: 70 (Experimental), 164 (Control)					
Heterogeneity: Tau^2=0; Chi^2=19.06, df=25(P=0.79); I^2=0%					
Test for overall effect: Z=6.54(P<0.0001)					



Favours experimental — Favours control

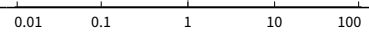
Analysis 1.2. Comparison 1 Probiotics versus control, Outcome 2 Incidence CDAD: complete case - fixed effects.

Study or subgroup	Experimental n/N	Control n/N	Risk Ratio M-H, Fixed, 95% CI	Weight	Risk Ratio M-H, Fixed, 95% CI
Allen 2013	12/1404	17/1406		9.59%	0.71[0.34,1.47]
Arvola 1999	1/61	1/58		0.58%	0.95[0.06,14.85]
Beausoleil 2007	1/39	7/42		3.81%	0.15[0.02,1.19]
Bravo 2008	0/41	0/41			Not estimable
Can 2006	0/73	2/78		1.37%	0.21[0.01,4.37]
Cindoruk 2007	0/59	0/51			Not estimable
Duman 2005	0/185	1/161		0.91%	0.29[0.01,7.08]
Ehrhardt 2016	2/246	2/231		1.16%	0.94[0.13,6.61]
Fominykh 2013	0/80	0/40			Not estimable
Gao 2010	9/171	20/84		15.15%	0.22[0.11,0.46]
Georgieva 2015	0/49	0/48			Not estimable
Hickson 2007	0/56	9/53		5.51%	0.05[0,0.84]
Kotowska 2005	3/119	10/127		5.46%	0.32[0.09,1.14]
Lonnermark 2010	1/76	0/80		0.28%	3.16[0.13,76.3]
McFarland 1995	3/80	4/79		2.27%	0.74[0.17,3.2]
Miller 2008a	4/95	7/94		3.97%	0.57[0.17,1.87]
Miller 2008b	2/156	0/155		0.28%	4.97[0.24,102.65]



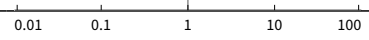
Favours experimental — Favours control

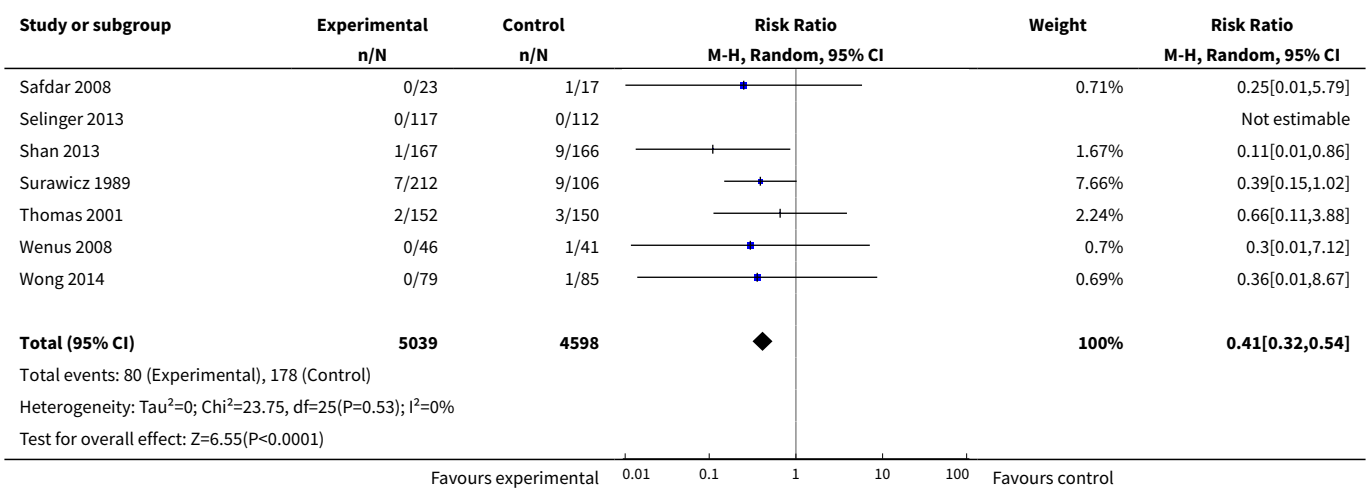
Study or subgroup	Experimental n/N	Control n/N	Risk Ratio M-H, Fixed, 95% CI	Weight	Risk Ratio M-H, Fixed, 95% CI
Ouwehand 2014	6/304	8/143		6.14%	0.35[0.12,1]
Pancheva 2009	6/78	17/78		9.6%	0.35[0.15,0.85]
Plummer 2004	2/69	5/69		2.82%	0.4[0.08,1.99]
Pozzoni 2012	3/106	2/98		1.17%	1.39[0.24,8.13]
Psaradellis 2010	1/185	4/186		2.25%	0.25[0.03,2.23]
Rafiq 2007	5/45	22/55		11.18%	0.28[0.11,0.67]
Ruszczynski 2008	3/120	7/120		3.95%	0.43[0.11,1.62]
Safdar 2008	0/22	1/14		1.03%	0.22[0.01,4.99]
Selinger 2013	0/111	0/106			Not estimable
Shan 2013	1/139	8/144		4.44%	0.13[0.02,1.02]
Surawicz 1989	3/113	5/61		3.67%	0.32[0.08,1.31]
Thomas 2001	2/133	3/134		1.69%	0.67[0.11,3.96]
Wenus 2008	0/34	1/29		0.91%	0.29[0.01,6.76]
Wong 2014	0/76	1/82		0.82%	0.36[0.01,8.69]
Total (95% CI)	**4525**	**4147**		**100%**	**0.38[0.3,0.5]**
Total events: 70 (Experimental), 164 (Control)					
Heterogeneity: Tau^2=0; Chi^2=19.06, df=25(P=0.79); I^2=0%					
Test for overall effect: Z=7.05(P<0.0001)					



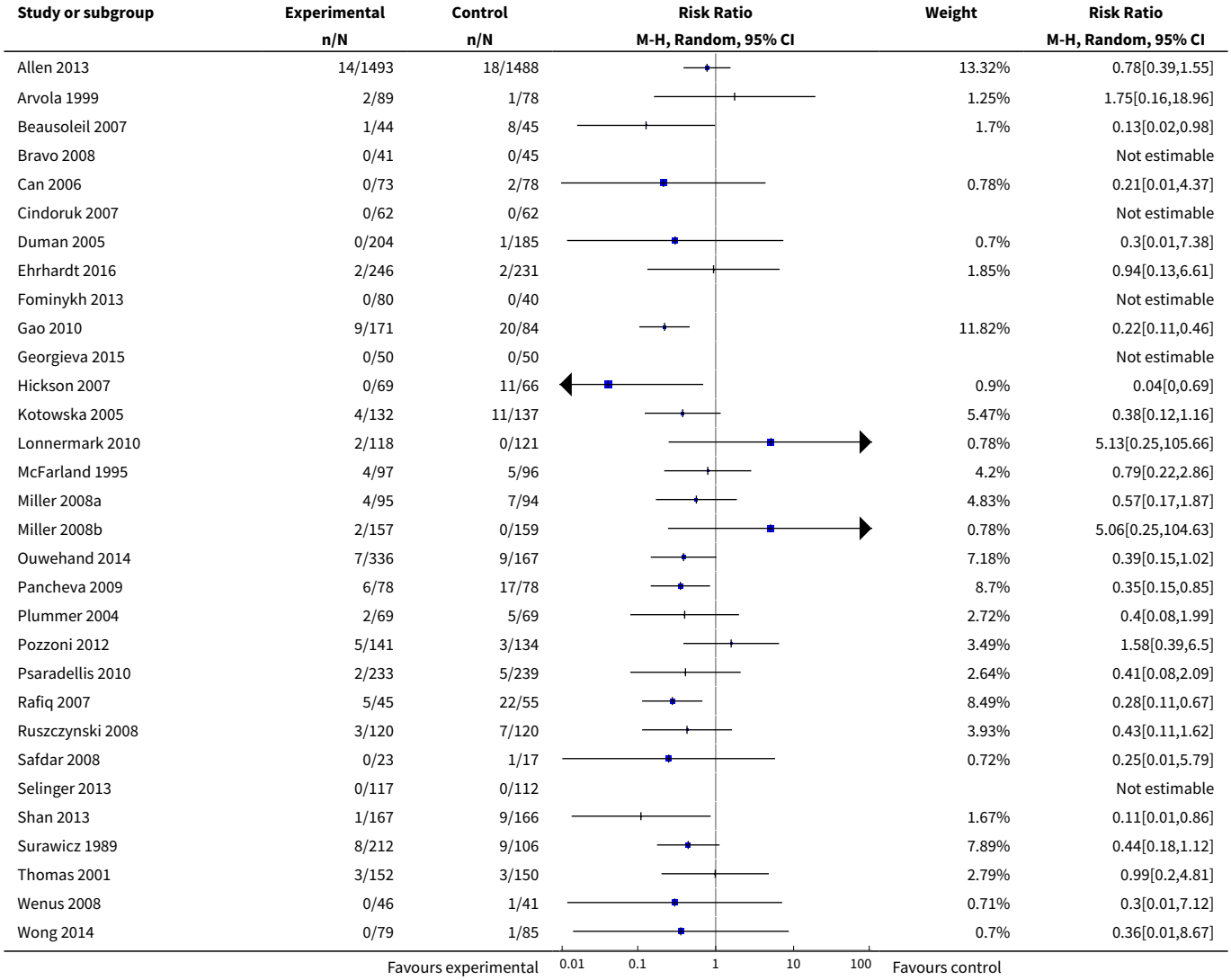
Analysis 1.3. Comparison 1 Probiotics versus control, Outcome 3 Incidence CDAD: Sensitivity (1.5:1).

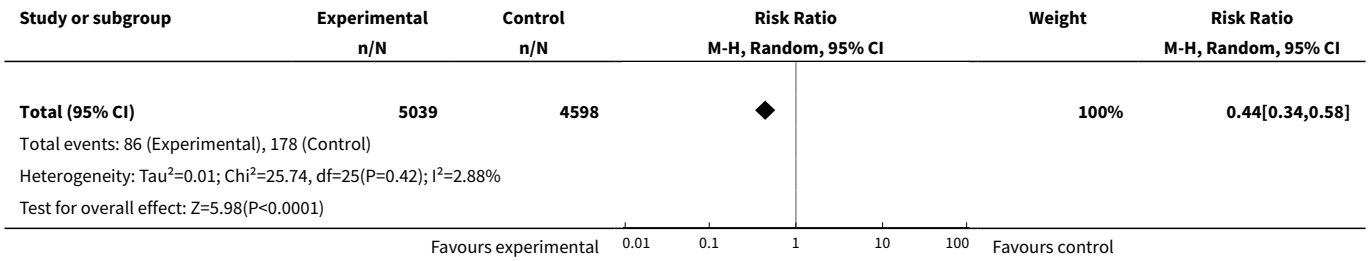
Study or subgroup	Experimental n/N	Control n/N	Risk Ratio M-H, Random, 95% CI	Weight	Risk Ratio M-H, Random, 95% CI
Allen 2013	13/1493	18/1488		14%	0.72[0.35,1.46]
Arvola 1999	2/89	1/78		1.24%	1.75[0.16,18.96]
Beausoleil 2007	1/44	8/45		1.7%	0.13[0.02,0.98]
Bravo 2008	0/41	0/45			Not estimable
Can 2006	0/73	2/78		0.77%	0.21[0.01,4.37]
Cindoruk 2007	0/62	0/62			Not estimable
Duman 2005	0/204	1/185		0.69%	0.3[0.01,7.38]
Ehrhardt 2016	2/246	2/231		1.85%	0.94[0.13,6.61]
Fominykh 2013	0/80	0/40			Not estimable
Gao 2010	9/171	20/84		12.81%	0.22[0.11,0.46]
Georgieva 2015	0/50	0/50			Not estimable
Hickson 2007	0/69	11/66		0.89%	0.04[0,0.69]
Kotowska 2005	3/132	11/137		4.48%	0.28[0.08,0.99]
Lonnermark 2010	2/118	0/121		0.77%	5.13[0.25,105.66]
McFarland 1995	4/97	5/96		4.28%	0.79[0.22,2.86]
Miller 2008a	4/95	7/94		4.94%	0.57[0.17,1.87]
Miller 2008b	2/157	0/159		0.77%	5.06[0.25,104.63]
Ouwehand 2014	7/336	9/167		7.49%	0.39[0.15,1.02]
Pancheva 2009	6/78	17/78		9.19%	0.35[0.15,0.85]
Plummer 2004	2/69	5/69		2.74%	0.4[0.08,1.99]
Pozzoni 2012	4/141	3/134		3.23%	1.27[0.29,5.56]
Psaradellis 2010	1/233	5/239		1.54%	0.21[0.02,1.74]
Rafiq 2007	5/45	22/55		8.95%	0.28[0.11,0.67]
Ruszczynski 2008	3/120	7/120		4%	0.43[0.11,1.62]



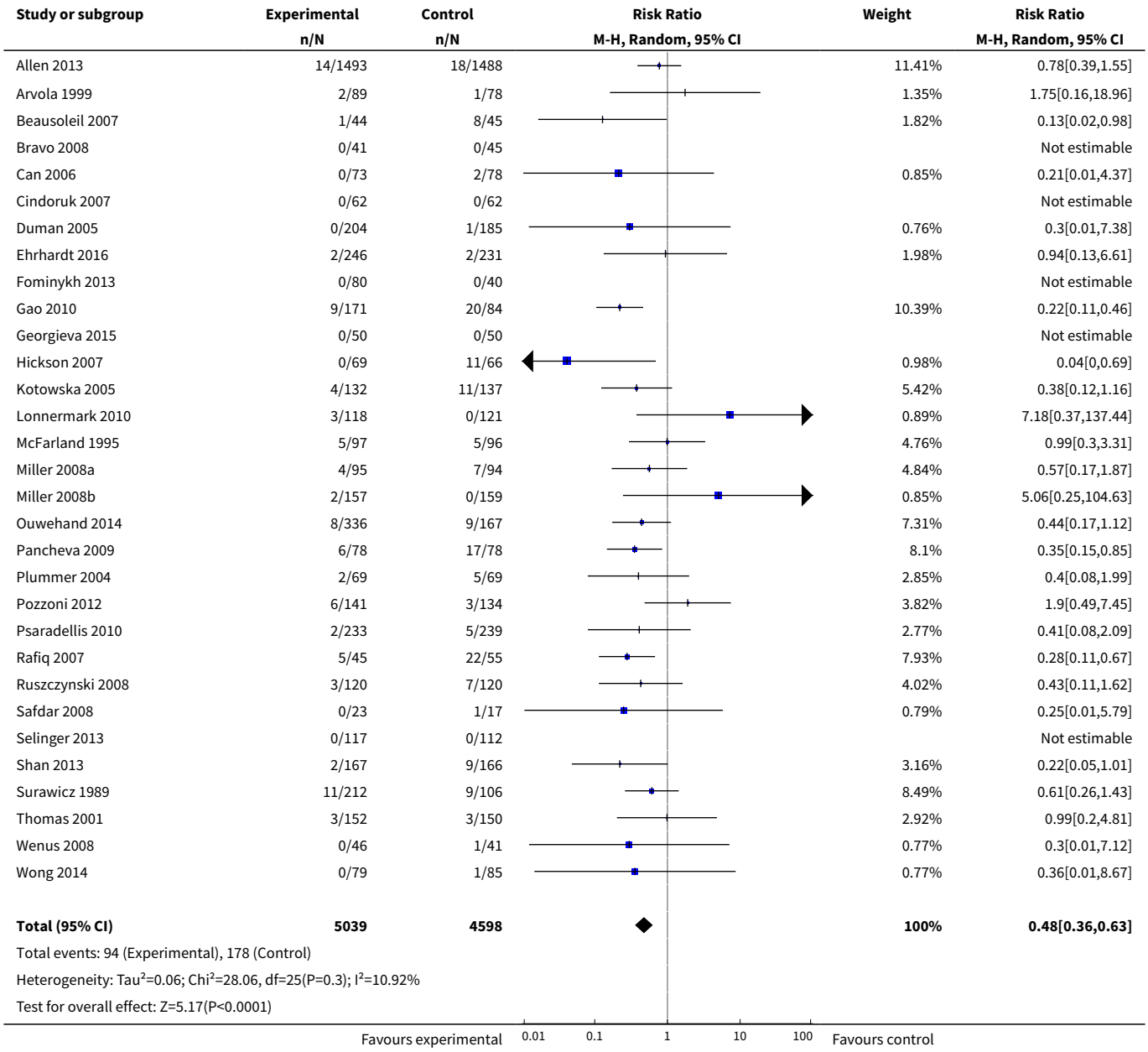


Analysis 1.4. Comparison 1 Probiotics versus control, Outcome 4 Incidence CDAD: Sensitivity (2:1).



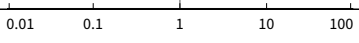


Analysis 1.5. Comparison 1 Probiotics versus control, Outcome 5 Incidence CDAD: Sensitivity (3:1).



Analysis 1.6. Comparison 1 Probiotics versus control, Outcome 6 Incidence CDAD: Sensitivity (5:1).

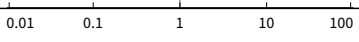
Study or subgroup	Experimental n/N	Control n/N	Risk Ratio M-H, Random, 95% CI	Weight	Risk Ratio M-H, Random, 95% CI
Allen 2013	16/1493	18/1488		9.25%	0.89[0.45,1.73]
Arvola 1999	3/89	1/78		1.79%	2.63[0.28,24.76]
Beausoleil 2007	2/44	8/45		3.53%	0.26[0.06,1.14]
Bravo 2008	0/41	0/45			Not estimable
Can 2006	0/73	2/78		1.04%	0.21[0.01,4.37]
Cindoruk 2007	0/62	0/62			Not estimable
Duman 2005	0/204	1/185		0.93%	0.3[0.01,7.38]
Ehrhardt 2016	2/246	2/231		2.28%	0.94[0.13,6.61]
Fominykh 2013	0/80	0/40			Not estimable
Gao 2010	9/171	20/84		8.46%	0.22[0.11,0.46]
Georgieva 2015	0/50	0/50			Not estimable
Hickson 2007	0/69	11/66		1.19%	0.04[0,0.69]
Kotowska 2005	5/132	11/137		5.94%	0.47[0.17,1.32]
Lonnermark 2010	4/118	0/121		1.11%	9.23[0.5,169.51]
McFarland 1995	6/97	5/96		5.13%	1.19[0.38,3.76]
Miller 2008a	4/95	7/94		4.89%	0.57[0.17,1.87]
Miller 2008b	2/157	0/159		1.03%	5.06[0.25,104.63]
Ouwehand 2014	9/336	9/167		6.91%	0.5[0.2,1.23]
Pancheva 2009	6/78	17/78		7.17%	0.35[0.15,0.85]
Plummer 2004	2/69	5/69		3.15%	0.4[0.08,1.99]
Pozzoni 2012	8/141	3/134		4.31%	2.53[0.69,9.35]
Psaradellis 2010	2/233	5/239		3.07%	0.41[0.08,2.09]
Rafiq 2007	5/45	22/55		7.07%	0.28[0.11,0.67]
Ruszczynski 2008	3/120	7/120		4.2%	0.43[0.11,1.62]
Safdar 2008	0/23	1/17		0.96%	0.25[0.01,5.79]
Selinger 2013	0/117	0/112			Not estimable
Shan 2013	2/167	9/166		3.44%	0.22[0.05,1.01]
Surawicz 1989	16/212	9/106		8.05%	0.89[0.41,1.94]
Thomas 2001	3/152	3/150		3.21%	0.99[0.2,4.81]
Wenus 2008	0/46	1/41		0.95%	0.3[0.01,7.12]
Wong 2014	0/79	1/85		0.94%	0.36[0.01,8.67]
Total (95% CI)	**5039**	**4598**		**100%**	**0.54[0.39,0.74]**
Total events: 109 (Experimental), 178 (Control)					
Heterogeneity: Tau^2=0.17; Chi^2=34.86, df=25(P=0.09); I^2=28.28%					
Test for overall effect: Z=3.81(P=0)					



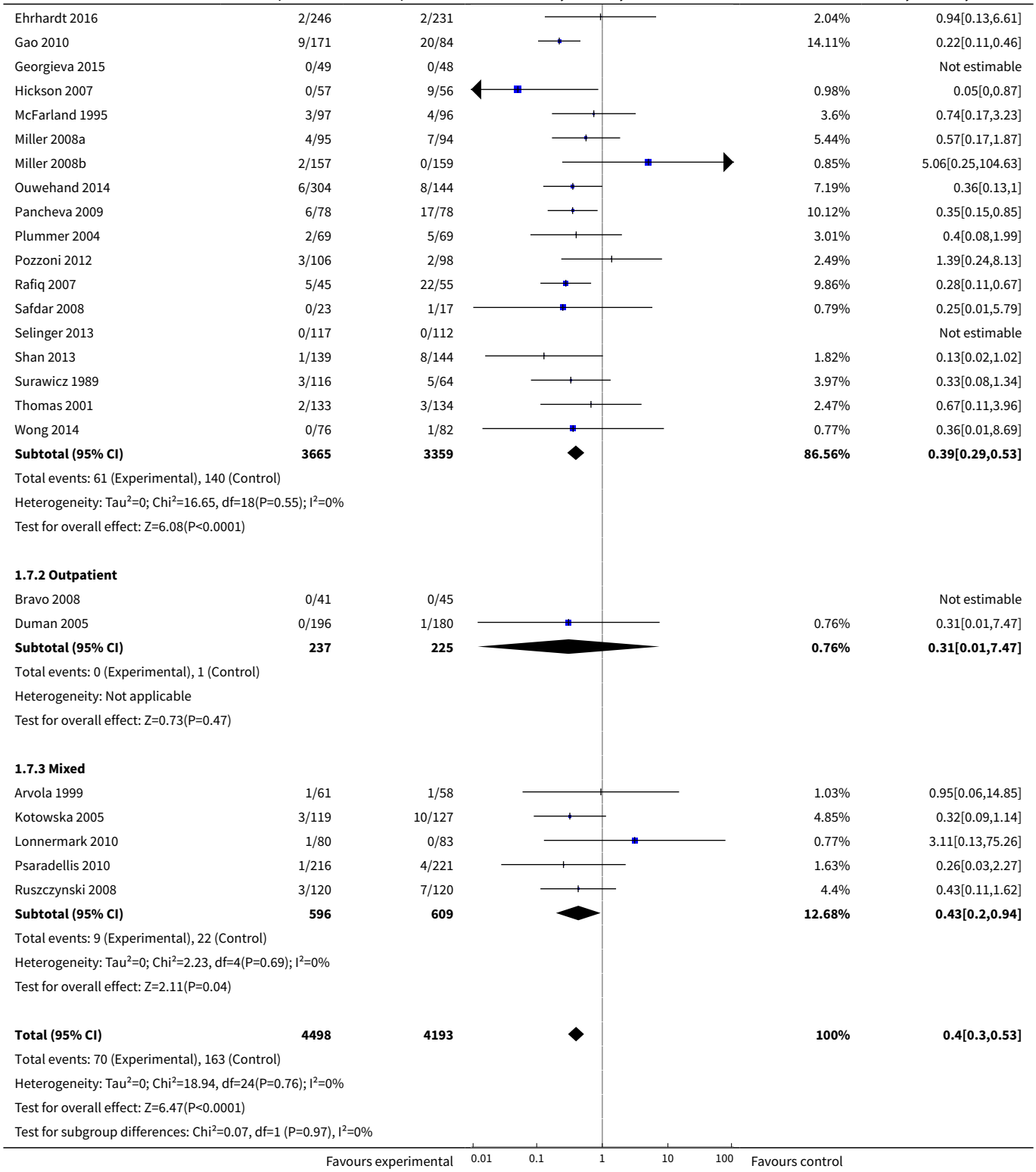
Favours experimental — Favours control

Analysis 1.7. Comparison 1 Probiotics versus control, Outcome 7 Incidence CDAD: Subgroup: Inpatient versus outpatient populations.

Study or subgroup	Experimental n/N	Control n/N	Risk Ratio M-H, Random, 95% CI	Weight	Risk Ratio M-H, Random, 95% CI
1.7.1 Inpatient					
Allen 2013	12/1470	17/1471		14.37%	0.71[0.34,1.47]
Beausoleil 2007	1/44	7/45		1.84%	0.15[0.02,1.14]
Can 2006	0/73	2/78		0.85%	0.21[0.01,4.37]

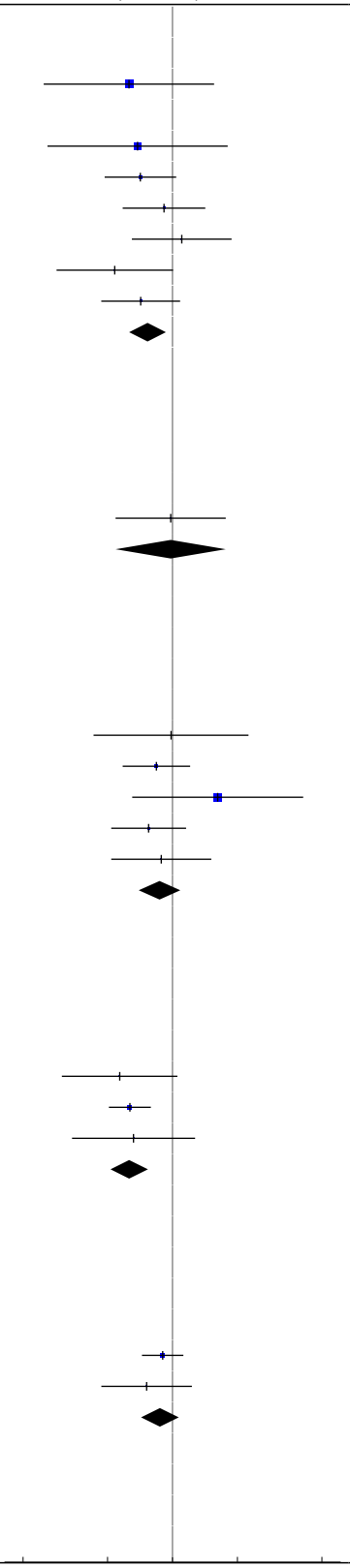


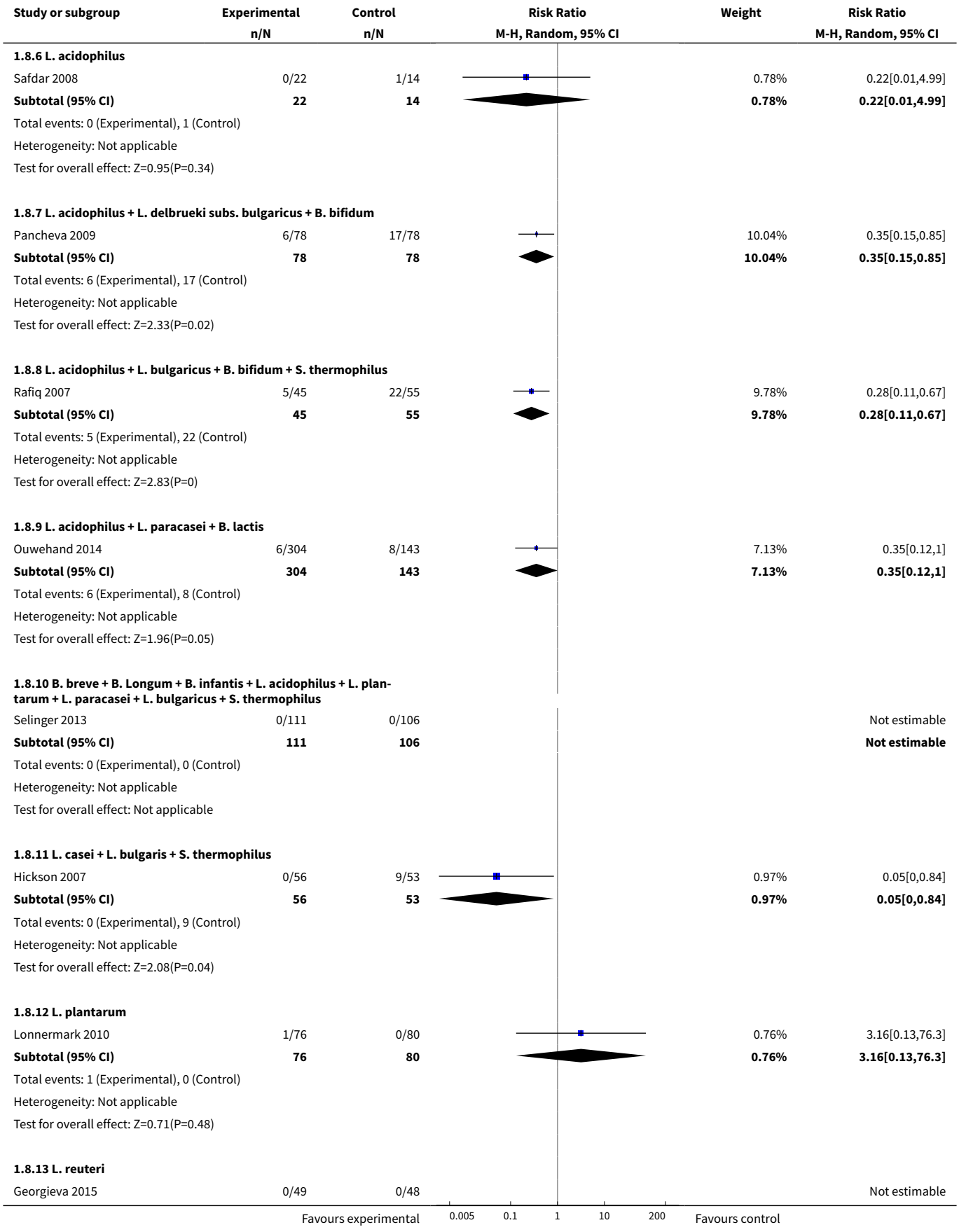
Favours experimental — Favours control

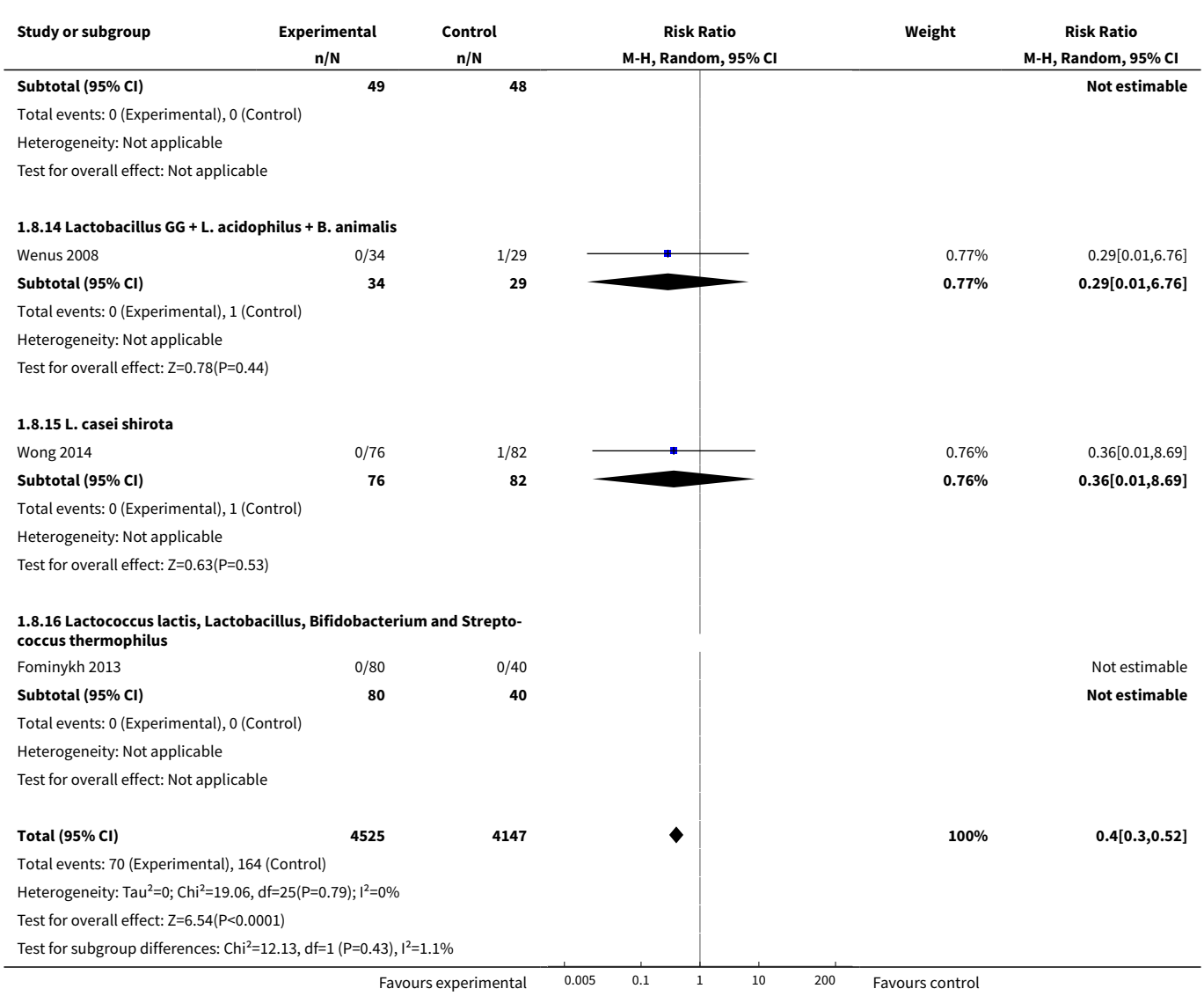


Analysis 1.8. Comparison 1 Probiotics versus control, Outcome 8 Incidence CDAD: Subgroup: Species: all.

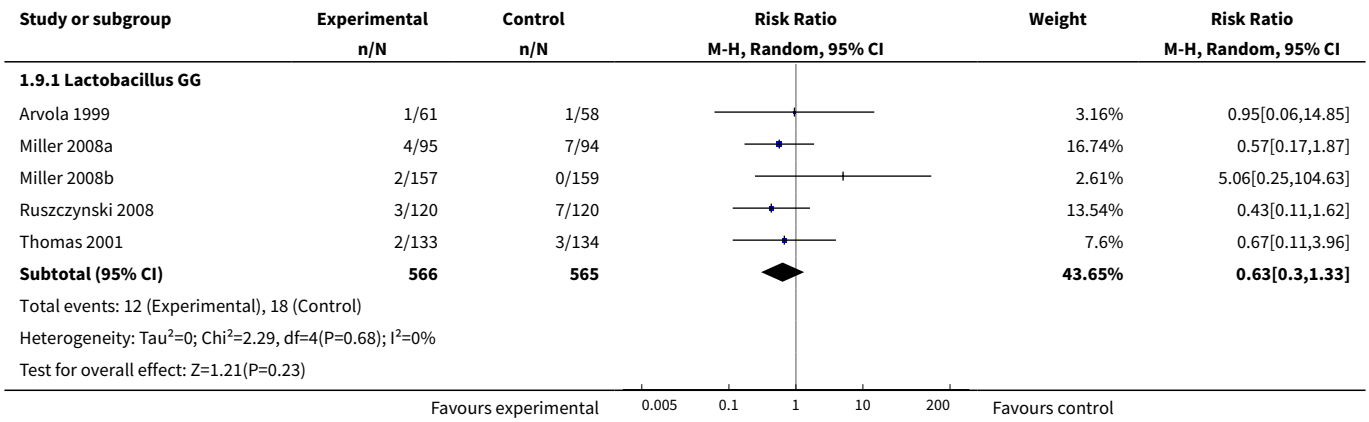
Study or subgroup	Experimental n/N	Control n/N	Risk Ratio M-H, Random, 95% CI	Weight	Risk Ratio M-H, Random, 95% CI
1.8.1 S. boulardii					
Bravo 2008	0/41	0/41			Not estimable
Can 2006	0/73	2/78		0.85%	0.21[0.01,4.37]
Cindoruk 2007	0/59	0/51			Not estimable
Duman 2005	0/185	1/161		0.76%	0.29[0.01,7.08]
Kotowska 2005	3/119	10/127		4.81%	0.32[0.09,1.14]
McFarland 1995	3/80	4/79		3.59%	0.74[0.17,3.2]
Pozzoni 2012	3/106	2/98		2.47%	1.39[0.24,8.13]
Shan 2013	1/139	8/144		1.81%	0.13[0.02,1.02]
Surawicz 1989	3/113	5/61		3.95%	0.32[0.08,1.31]
Subtotal (95% CI)	**915**	**840**		**18.22%**	**0.41[0.22,0.79]**
Total events: 13 (Experimental), 32 (Control)					
Heterogeneity: Tau^2=0; Chi^2=4.18, df=6(P=0.65); I^2=0%					
Test for overall effect: Z=2.67(P=0.01)					
1.8.2 S. cerevisiae					
Ehrhardt 2016	2/246	2/231		2.02%	0.94[0.13,6.61]
Subtotal (95% CI)	**246**	**231**		**2.02%**	**0.94[0.13,6.61]**
Total events: 2 (Experimental), 2 (Control)					
Heterogeneity: Not applicable					
Test for overall effect: Z=0.06(P=0.95)					
1.8.3 Lactobacillus GG					
Arvola 1999	1/61	1/58		1.02%	0.95[0.06,14.85]
Miller 2008a	4/95	7/94		5.4%	0.57[0.17,1.87]
Miller 2008b	2/156	0/155		0.84%	4.97[0.24,102.65]
Ruszczynski 2008	3/120	7/120		4.37%	0.43[0.11,1.62]
Thomas 2001	2/133	3/134		2.45%	0.67[0.11,3.96]
Subtotal (95% CI)	**565**	**561**		**14.07%**	**0.63[0.3,1.32]**
Total events: 12 (Experimental), 18 (Control)					
Heterogeneity: Tau^2=0; Chi^2=2.26, df=4(P=0.69); I^2=0%					
Test for overall effect: Z=1.22(P=0.22)					
1.8.4 L. acidophilus + L. casei					
Beausoleil 2007	1/39	7/42		1.83%	0.15[0.02,1.19]
Gao 2010	9/171	20/84		13.99%	0.22[0.11,0.46]
Psaradellis 2010	1/185	4/186		1.62%	0.25[0.03,2.23]
Subtotal (95% CI)	**395**	**312**		**17.45%**	**0.22[0.11,0.42]**
Total events: 11 (Experimental), 31 (Control)					
Heterogeneity: Tau^2=0; Chi^2=0.13, df=2(P=0.94); I^2=0%					
Test for overall effect: Z=4.53(P<0.0001)					
1.8.5 L. acidophilus + B. bifidum					
Allen 2013	12/1404	17/1406		14.25%	0.71[0.34,1.47]
Plummer 2004	2/69	5/69		2.99%	0.4[0.08,1.99]
Subtotal (95% CI)	**1473**	**1475**		**17.24%**	**0.64[0.33,1.25]**
Total events: 14 (Experimental), 22 (Control)					
Heterogeneity: Tau^2=0; Chi^2=0.4, df=1(P=0.53); I^2=0%					
Test for overall effect: Z=1.31(P=0.19)					

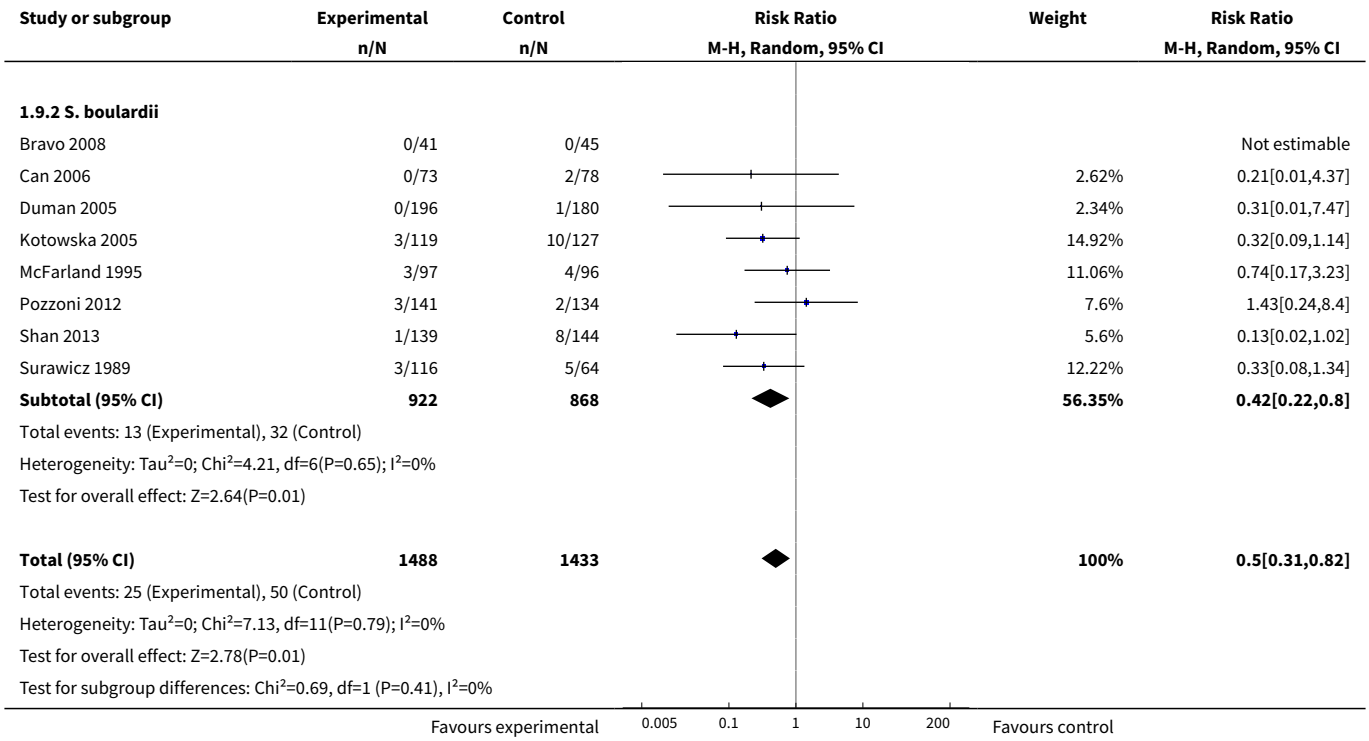




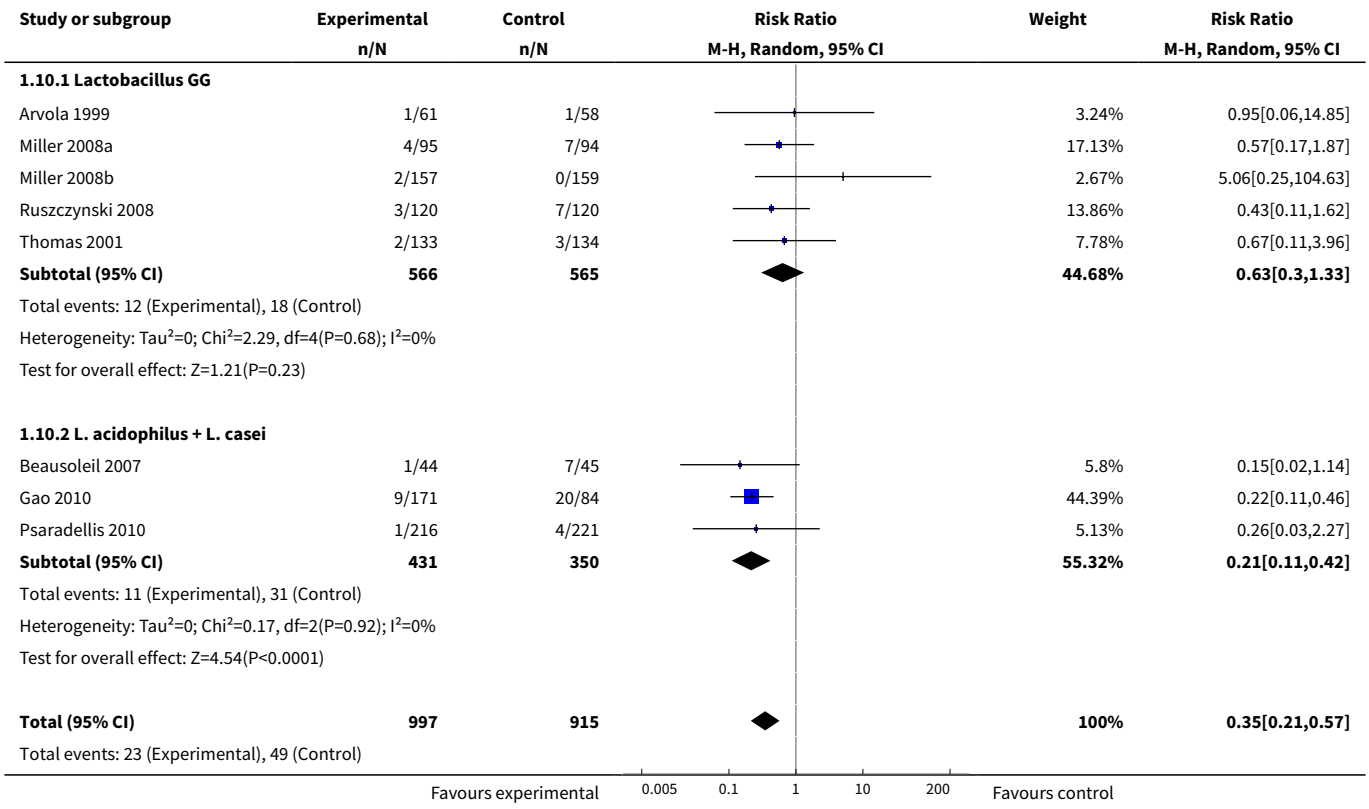


Analysis 1.9. Comparison 1 Probiotics versus control, Outcome 9 Incidence CDAD: Subgroup: Species: LGG versus SB.

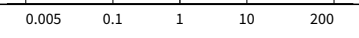




Analysis 1.10. Comparison 1 Probiotics versus control, Outcome 10 Incidence CDAD: Subgroup: Species: LGG versus LA + LC.



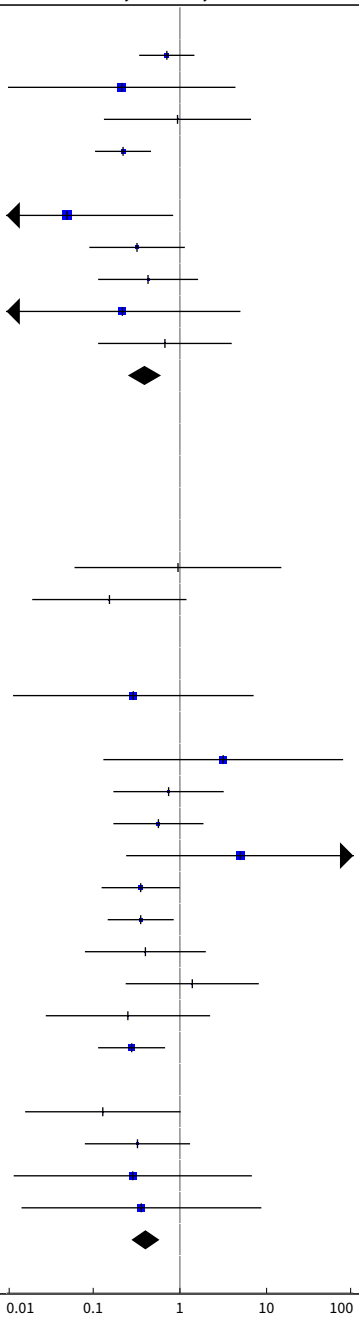
Study or subgroup	Experimental n/N	Control n/N	Risk Ratio M-H, Random, 95% CI	Weight	Risk Ratio M-H, Random, 95% CI
Heterogeneity: Tau²=0; Chi²=7, df=7(P=0.43); I²=0.02%					
Test for overall effect: Z=4.18(P<0.0001)					
Test for subgroup differences: Chi²=4.54, df=1 (P=0.03), I²=77.98%					



Favours experimental — Favours control

Analysis 1.11. Comparison 1 Probiotics versus control, Outcome 11 Incidence CDAD: Subgroup: Risk of Bias.

Study or subgroup	Experimental n/N	Control n/N	Risk Ratio M-H, Random, 95% CI	Weight	Risk Ratio M-H, Random, 95% CI
1.11.1 Low risk of bias					
Allen 2013	12/1404	17/1406		14.25%	0.71[0.34,1.47]
Can 2006	0/73	2/78		0.85%	0.21[0.01,4.37]
Ehrhardt 2016	2/246	2/231		2.02%	0.94[0.13,6.61]
Gao 2010	9/171	20/84		13.99%	0.22[0.11,0.46]
Georgieva 2015	0/49	0/48			Not estimable
Hickson 2007	0/56	9/53		0.97%	0.05[0,0.84]
Kotowska 2005	3/119	10/127		4.81%	0.32[0.09,1.14]
Ruszczynski 2008	3/120	7/120		4.37%	0.43[0.11,1.62]
Safdar 2008	0/22	1/14		0.78%	0.22[0.01,4.99]
Thomas 2001	2/133	3/134		2.45%	0.67[0.11,3.96]
Subtotal (95% CI)	**2393**	**2295**		**44.49%**	**0.39[0.25,0.61]**
Total events: 31 (Experimental), 71 (Control)					
Heterogeneity: Tau²=0.02; Chi²=8.42, df=8(P=0.39); I²=4.97%					
Test for overall effect: Z=4.19(P<0.0001)					
1.11.2 High or unclear risk of bias					
Arvola 1999	1/61	1/58		1.02%	0.95[0.06,14.85]
Beausoleil 2007	1/39	7/42		1.83%	0.15[0.02,1.19]
Bravo 2008	0/41	0/41			Not estimable
Cindoruk 2007	0/59	0/51			Not estimable
Duman 2005	0/185	1/161		0.76%	0.29[0.01,7.08]
Fominykh 2013	0/80	0/40			Not estimable
Lonnermark 2010	1/76	0/80		0.76%	3.16[0.13,76.3]
McFarland 1995	3/80	4/79		3.59%	0.74[0.17,3.2]
Miller 2008a	4/95	7/94		5.4%	0.57[0.17,1.87]
Miller 2008b	2/156	0/155		0.84%	4.97[0.24,102.65]
Ouwehand 2014	6/304	8/143		7.13%	0.35[0.12,1]
Pancheva 2009	6/78	17/78		10.04%	0.35[0.15,0.85]
Plummer 2004	2/69	5/69		2.99%	0.4[0.08,1.99]
Pozzoni 2012	3/106	2/98		2.47%	1.39[0.24,8.13]
Psaradellis 2010	1/185	4/186		1.62%	0.25[0.03,2.23]
Rafiq 2007	5/45	22/55		9.78%	0.28[0.11,0.67]
Selinger 2013	0/111	0/106			Not estimable
Shan 2013	1/139	8/144		1.81%	0.13[0.02,1.02]
Surawicz 1989	3/113	5/61		3.95%	0.32[0.08,1.31]
Wenus 2008	0/34	1/29		0.77%	0.29[0.01,6.76]
Wong 2014	0/76	1/82		0.76%	0.36[0.01,8.69]
Subtotal (95% CI)	**2132**	**1852**		**55.51%**	**0.4[0.28,0.58]**
Total events: 39 (Experimental), 93 (Control)					



Favours experimental — Favours control

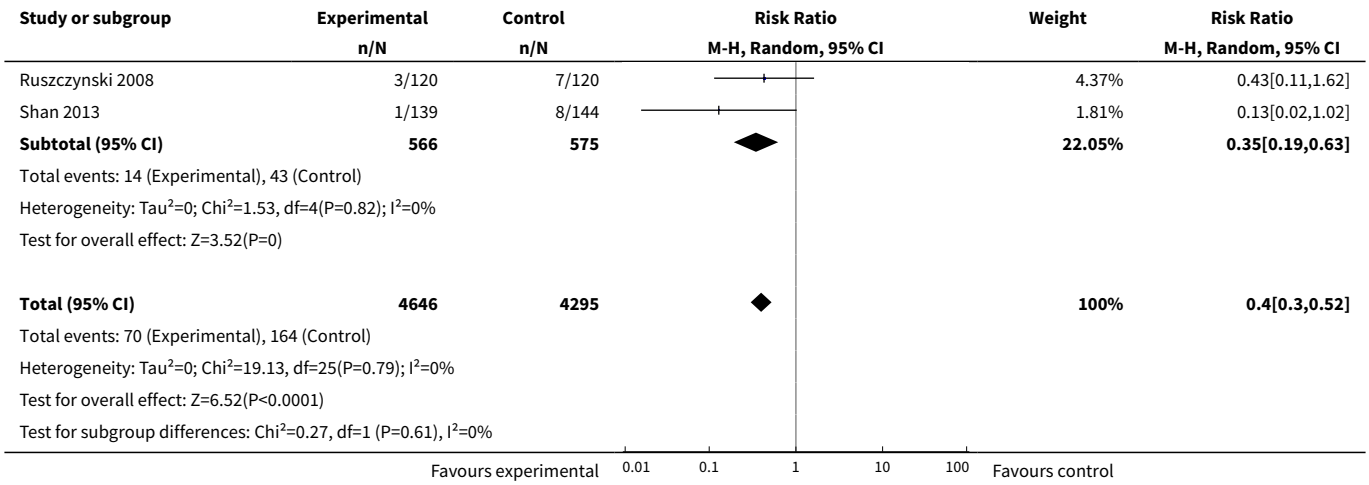
Study or subgroup	Experimental n/N	Control n/N	Risk Ratio M-H, Random, 95% CI	Weight	Risk Ratio M-H, Random, 95% CI
Heterogeneity: Tau²=0; Chi²=10.67, df=16(P=0.83); I²=0%					
Test for overall effect: Z=4.82(P<0.0001)					
Total (95% CI)	**4525**	**4147**	◆	**100%**	**0.4[0.3,0.52]**
Total events: 70 (Experimental), 164 (Control)					
Heterogeneity: Tau²=0; Chi²=19.06, df=25(P=0.79); I²=0%					
Test for overall effect: Z=6.54(P<0.0001)					
Test for subgroup differences: Chi²=0.01, df=1 (P=0.93), I²=0%					

Favours experimental 0.01 0.1 1 10 100 Favours control

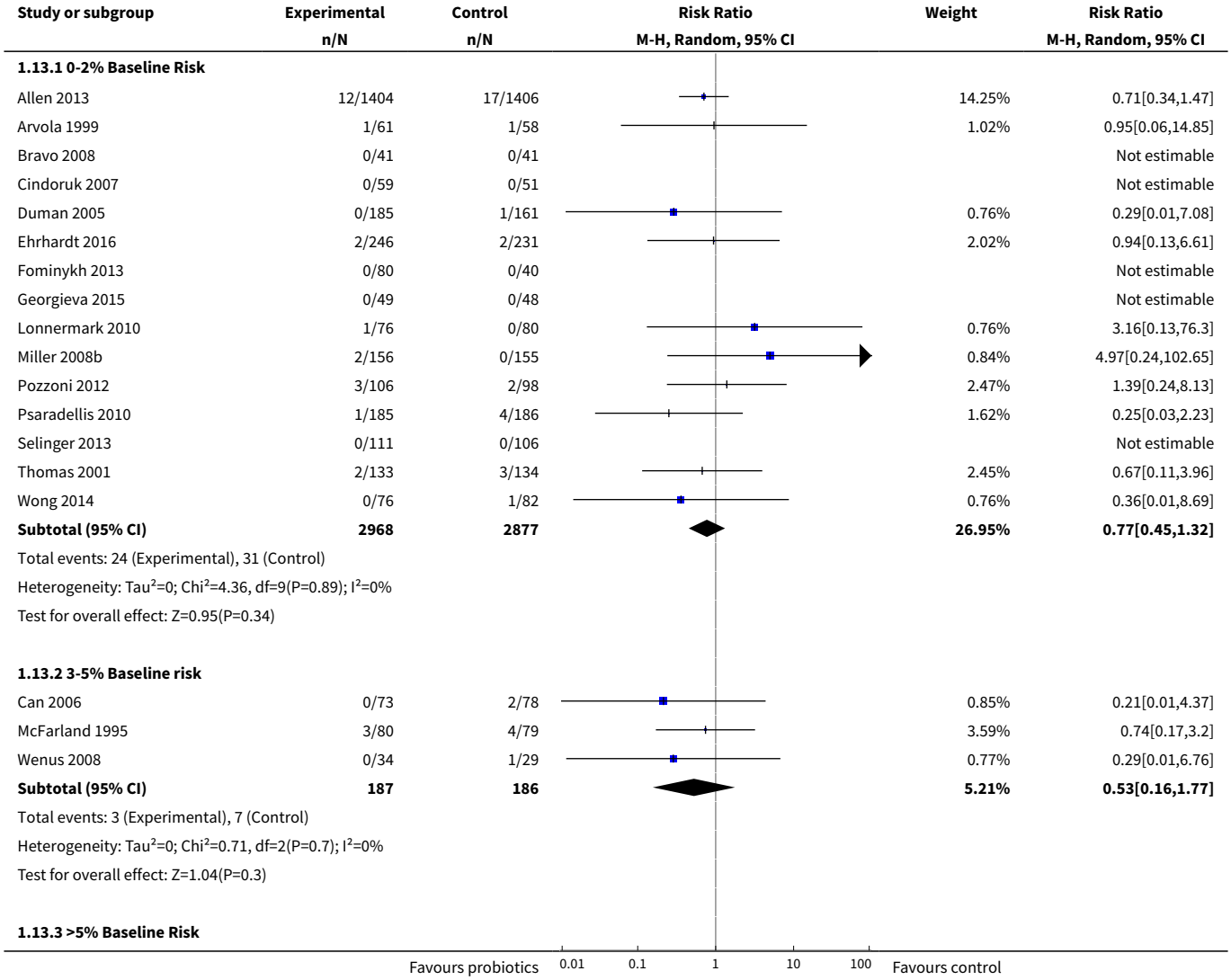
Analysis 1.12. Comparison 1 Probiotics versus control, Outcome 12 Incidence CDAD: Subgroup: Adult versus child.

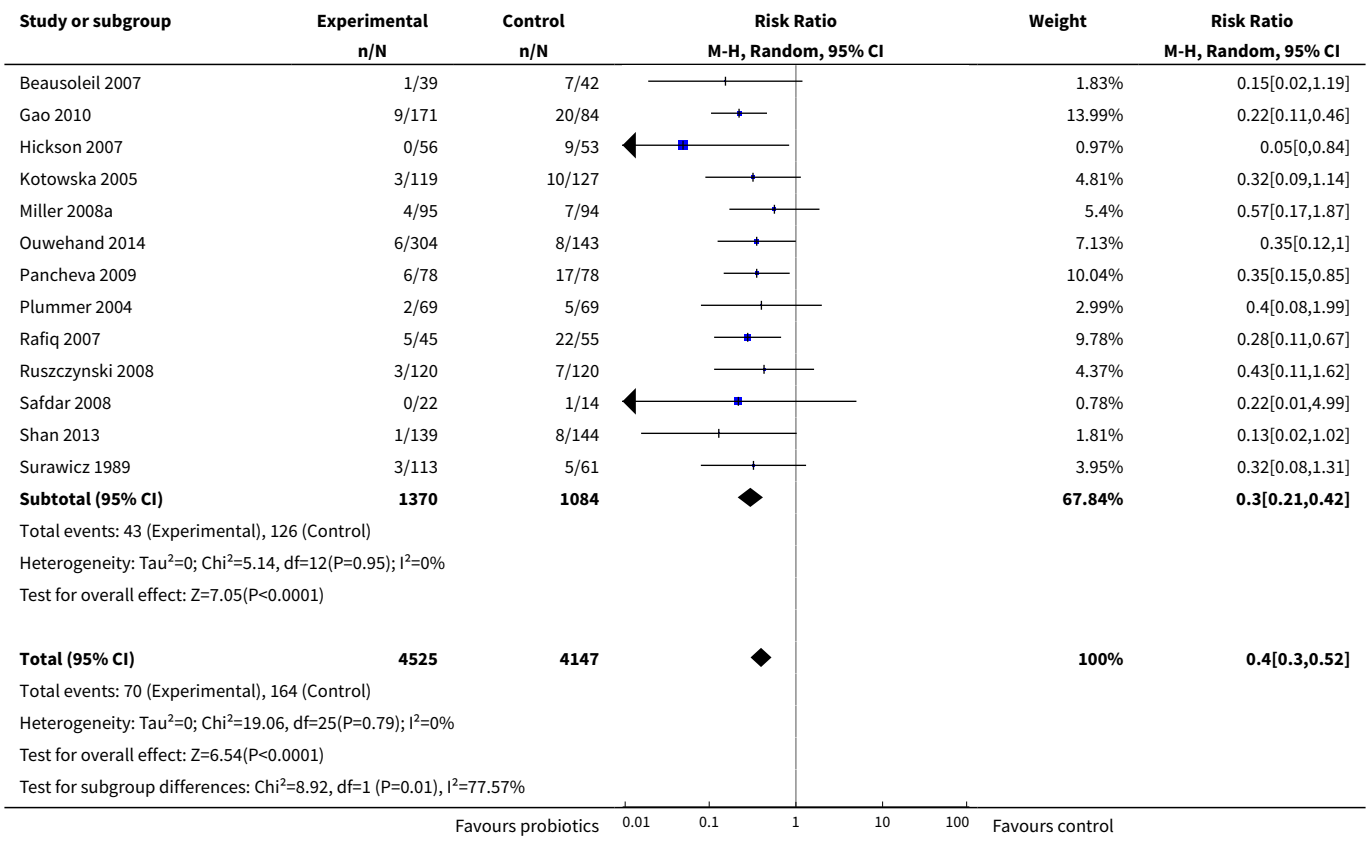
Study or subgroup	Experimental n/N	Control n/N	Risk Ratio M-H, Random, 95% CI	Weight	Risk Ratio M-H, Random, 95% CI
1.12.1 Adult studies					
Allen 2013	12/1470	17/1471		14.26%	0.71[0.34,1.47]
Beausoleil 2007	1/44	7/45		1.83%	0.15[0.02,1.14]
Bravo 2008	0/41	0/45			Not estimable
Can 2006	0/73	2/78		0.85%	0.21[0.01,4.37]
Duman 2005	0/196	1/180		0.76%	0.31[0.01,7.47]
Ehrhardt 2016	2/246	2/231		2.02%	0.94[0.13,6.61]
Fominykh 2013	0/80	0/40			Not estimable
Gao 2010	9/171	20/84		14%	0.22[0.11,0.46]
Hickson 2007	0/56	9/53		0.97%	0.05[0,0.84]
Lonnermark 2010	1/80	0/83		0.76%	3.11[0.13,75.26]
McFarland 1995	3/97	4/96		3.57%	0.74[0.17,3.23]
Miller 2008a	4/95	7/94		5.4%	0.57[0.17,1.87]
Miller 2008b	2/157	0/159		0.84%	5.06[0.25,104.63]
Ouwehand 2014	6/304	8/144		7.13%	0.36[0.13,1]
Plummer 2004	2/69	5/69		2.99%	0.4[0.08,1.99]
Pozzoni 2012	3/141	2/134		2.45%	1.43[0.24,8.4]
Psaradellis 2010	1/216	4/221		1.62%	0.26[0.03,2.27]
Rafiq 2007	5/45	22/55		9.79%	0.28[0.11,0.67]
Safdar 2008	0/23	1/17		0.78%	0.25[0.01,5.79]
Selinger 2013	0/117	0/112			Not estimable
Surawicz 1989	3/116	5/64		3.94%	0.33[0.08,1.34]
Thomas 2001	2/133	3/134		2.45%	0.67[0.11,3.96]
Wenus 2008	0/34	1/29		0.77%	0.29[0.01,6.76]
Wong 2014	0/76	1/82		0.76%	0.36[0.01,8.69]
Subtotal (95% CI)	**4080**	**3720**	◆	**77.95%**	**0.41[0.3,0.57]**
Total events: 56 (Experimental), 121 (Control)					
Heterogeneity: Tau²=0; Chi²=17.34, df=20(P=0.63); I²=0%					
Test for overall effect: Z=5.51(P<0.0001)					
1.12.2 Pediatric studies					
Arvola 1999	1/61	1/58		1.02%	0.95[0.06,14.85]
Georgieva 2015	0/49	0/48			Not estimable
Kotowska 2005	3/119	10/127		4.81%	0.32[0.09,1.14]
Pancheva 2009	6/78	17/78		10.04%	0.35[0.15,0.85]

Favours experimental 0.01 0.1 1 10 100 Favours control

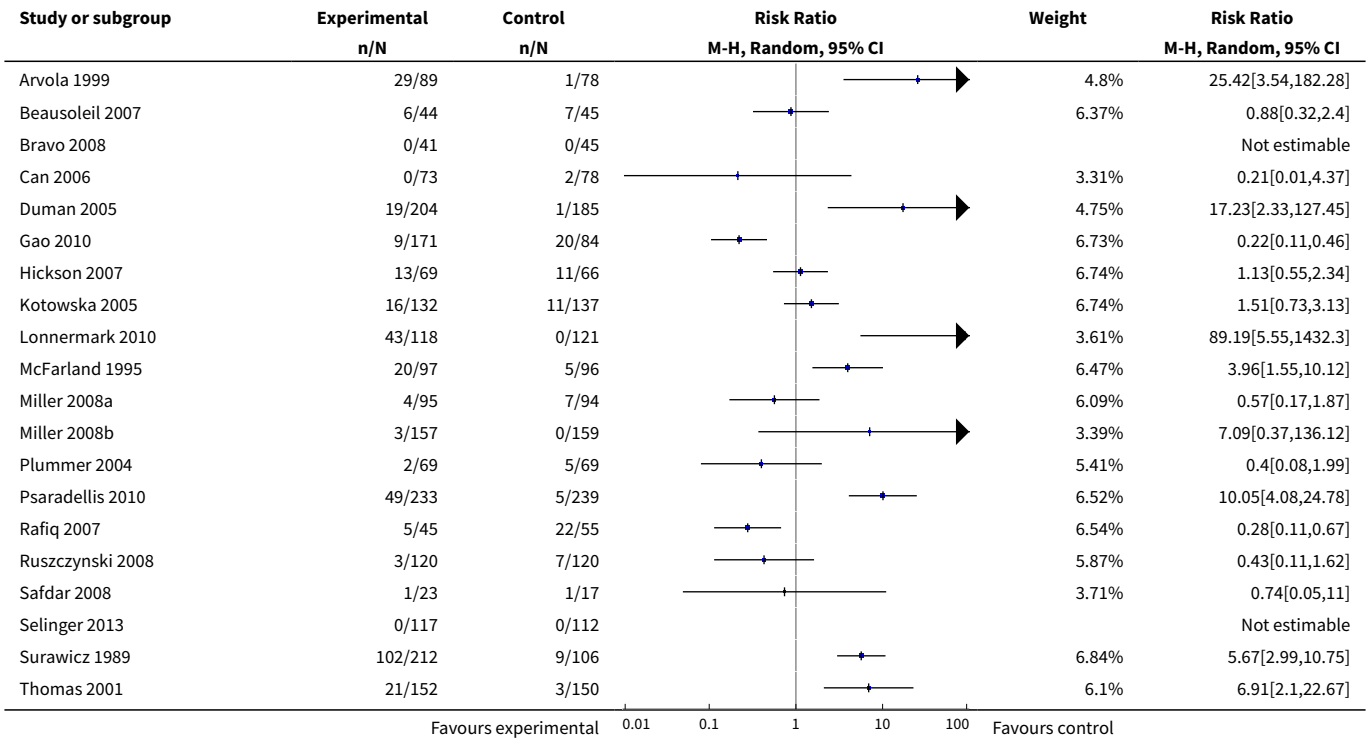


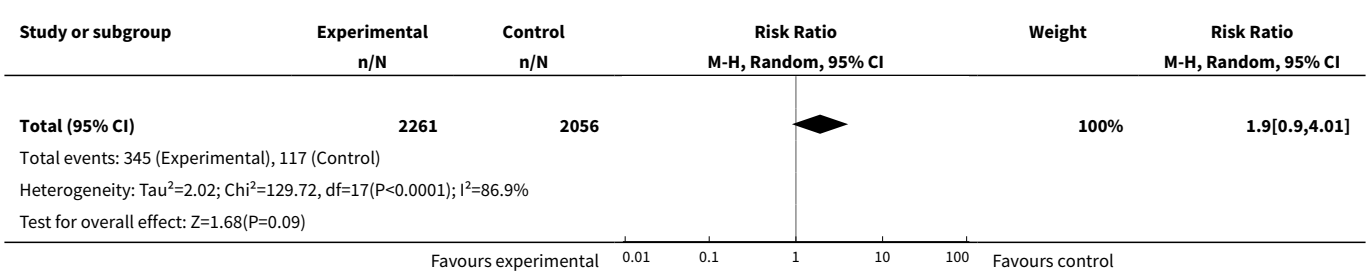
Analysis 1.13. Comparison 1 Probiotics versus control, Outcome 13 Incidence CDAD: Baseline Risk.



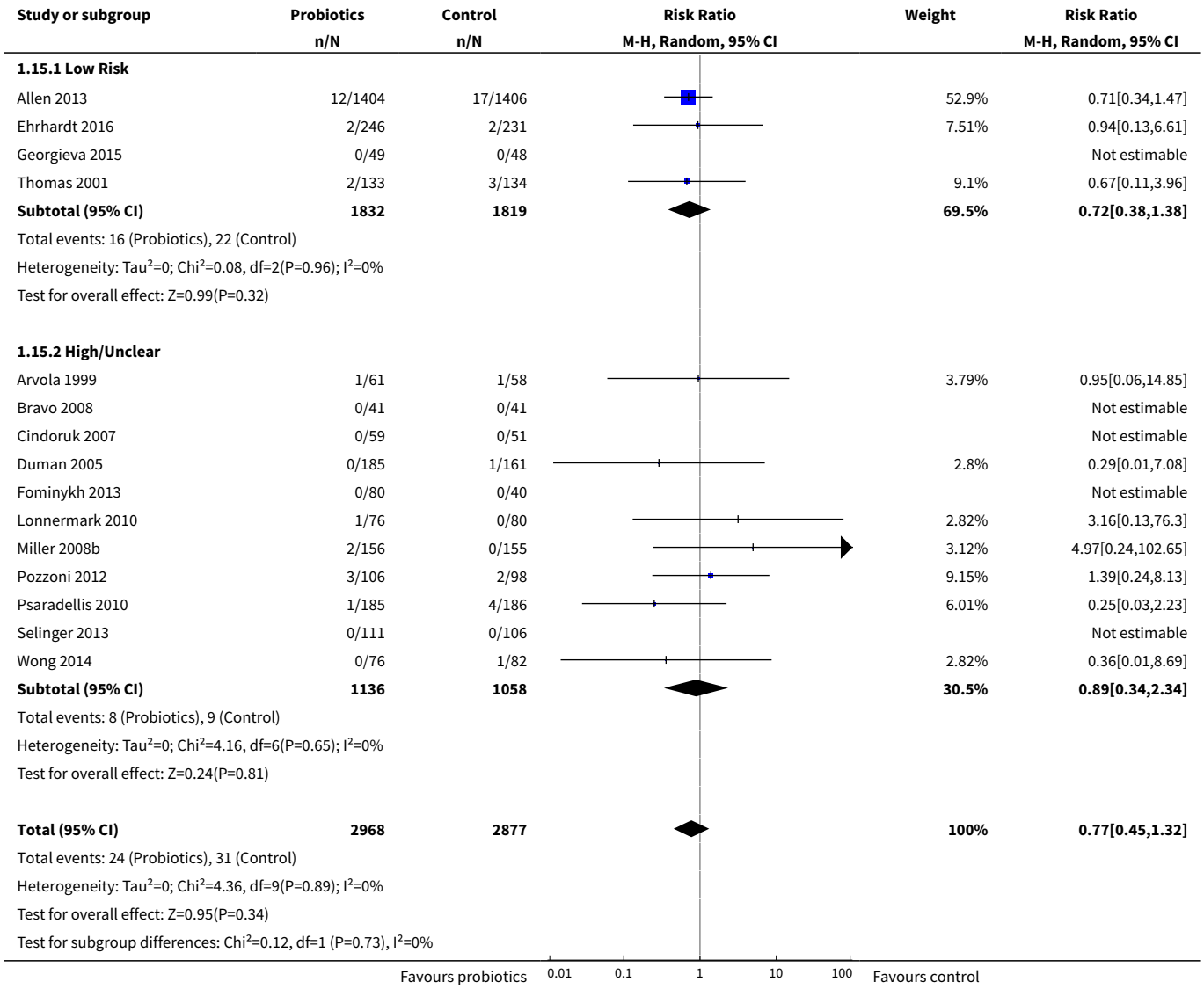


Analysis 1.14. Comparison 1 Probiotics versus control, Outcome 14 Incidence CDAD: worst case.



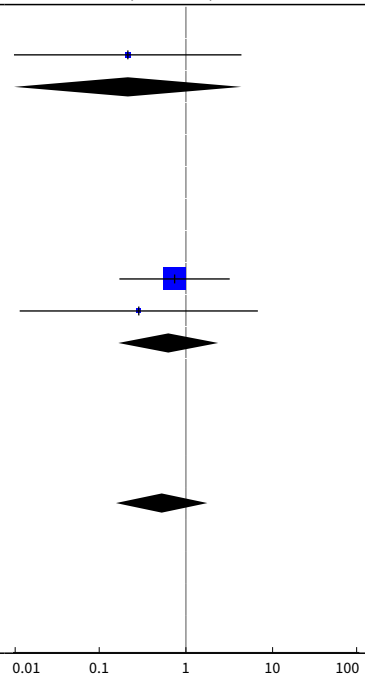


Analysis 1.15. Comparison 1 Probiotics versus control, Outcome 15 Incidence CDAD: 0-2% baseline risk RoB.



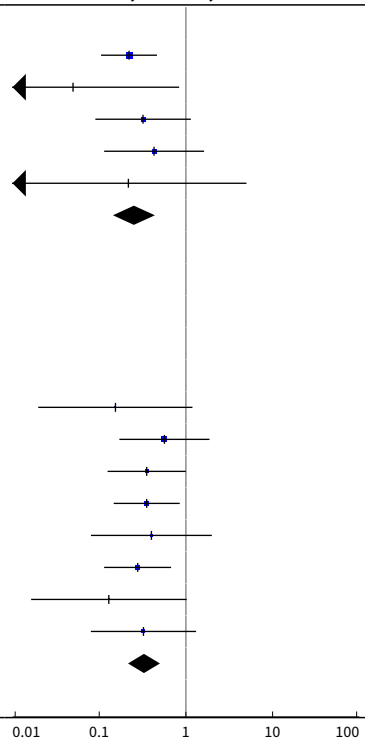
Analysis 1.16. Comparison 1 Probiotics versus control, Outcome 16 Incidence CDAD: 3-5% baseline risk RoB.

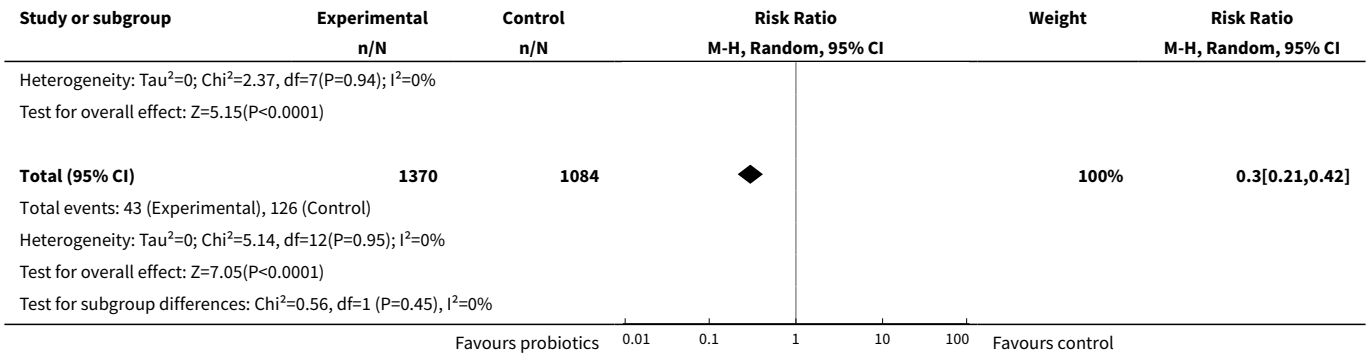
Study or subgroup	Experimental n/N	Control n/N	Risk Ratio M-H, Random, 95% CI	Weight	Risk Ratio M-H, Random, 95% CI
1.16.1 Low Risk					
Can 2006	0/73	2/78		16.22%	0.21[0.01,4.37]
Subtotal (95% CI)	**73**	**78**		**16.22%**	**0.21[0.01,4.37]**
Total events: 0 (Experimental), 2 (Control)					
Heterogeneity: Tau^2=0; Chi^2=0, df=0(P<0.0001); I^2=100%					
Test for overall effect: Z=1(P=0.32)					
1.16.2 High/Unclear					
McFarland 1995	3/80	4/79		68.99%	0.74[0.17,3.2]
Wenus 2008	0/34	1/29		14.78%	0.29[0.01,6.76]
Subtotal (95% CI)	**114**	**108**		**83.78%**	**0.63[0.17,2.36]**
Total events: 3 (Experimental), 5 (Control)					
Heterogeneity: Tau^2=0; Chi^2=0.29, df=1(P=0.59); I^2=0%					
Test for overall effect: Z=0.69(P=0.49)					
Total (95% CI)	**187**	**186**		**100%**	**0.53[0.16,1.77]**
Total events: 3 (Experimental), 7 (Control)					
Heterogeneity: Tau^2=0; Chi^2=0.71, df=2(P=0.7); I^2=0%					
Test for overall effect: Z=1.04(P=0.3)					
Test for subgroup differences: Chi^2=0.41, df=1 (P=0.52), I^2=0%					



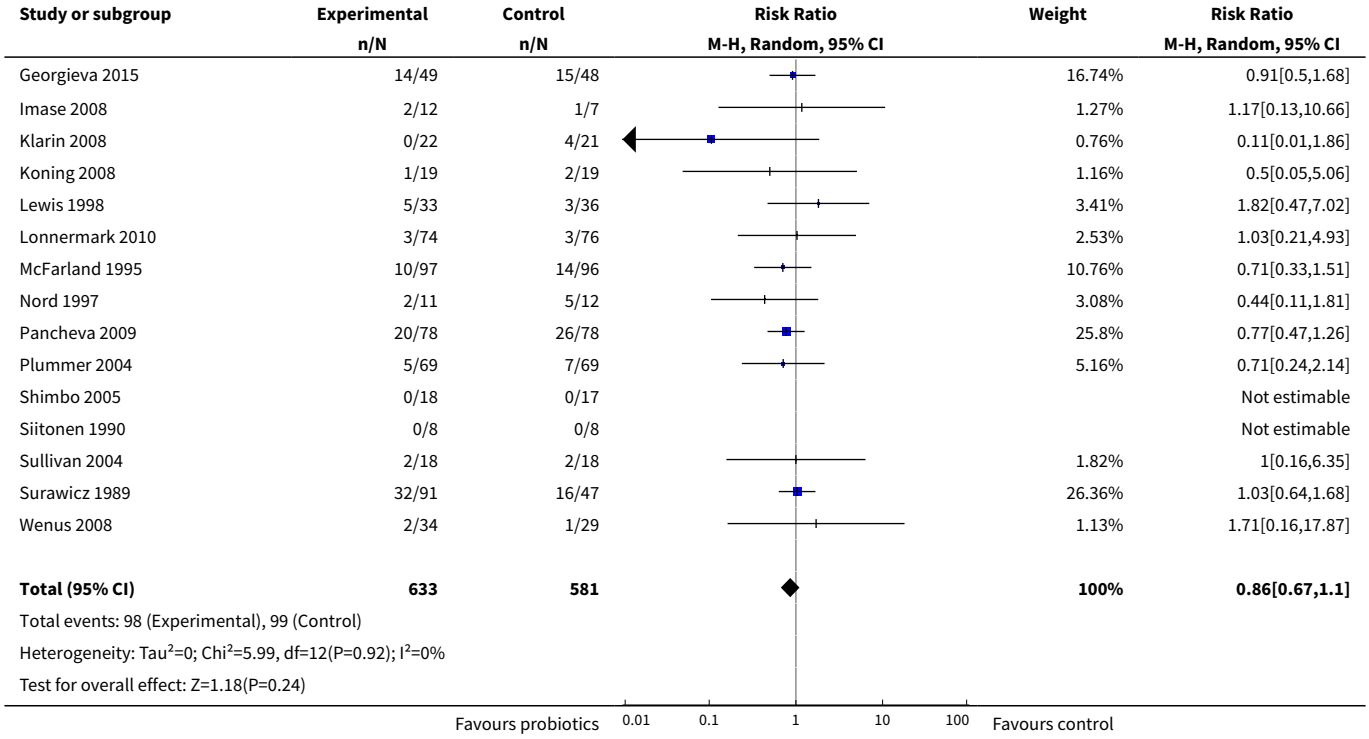
Analysis 1.17. Comparison 1 Probiotics versus control, Outcome 17 Incidence CDAD: >5% baseline risk RoB.

Study or subgroup	Experimental n/N	Control n/N	Risk Ratio M-H, Random, 95% CI	Weight	Risk Ratio M-H, Random, 95% CI
1.17.1 Low Risk					
Gao 2010	9/171	20/84		20.62%	0.22[0.11,0.46]
Hickson 2007	0/56	9/53		1.43%	0.05[0,0.84]
Kotowska 2005	3/119	10/127		7.09%	0.32[0.09,1.14]
Ruszczynski 2008	3/120	7/120		6.43%	0.43[0.11,1.62]
Safdar 2008	0/22	1/14		1.16%	0.22[0.01,4.99]
Subtotal (95% CI)	**488**	**398**		**36.73%**	**0.25[0.14,0.44]**
Total events: 15 (Experimental), 47 (Control)					
Heterogeneity: Tau^2=0; Chi^2=2.21, df=4(P=0.7); I^2=0%					
Test for overall effect: Z=4.87(P<0.0001)					
1.17.2 Unclear/High					
Beausoleil 2007	1/39	7/42		2.7%	0.15[0.02,1.19]
Miller 2008a	4/95	7/94		7.95%	0.57[0.17,1.87]
Ouwehand 2014	6/304	8/143		10.51%	0.35[0.12,1]
Pancheva 2009	6/78	17/78		14.79%	0.35[0.15,0.85]
Plummer 2004	2/69	5/69		4.41%	0.4[0.08,1.99]
Rafiq 2007	5/45	22/55		14.42%	0.28[0.11,0.67]
Shan 2013	1/139	8/144		2.66%	0.13[0.02,1.02]
Surawicz 1989	3/113	5/61		5.82%	0.32[0.08,1.31]
Subtotal (95% CI)	**882**	**686**		**63.27%**	**0.33[0.21,0.5]**
Total events: 28 (Experimental), 79 (Control)					

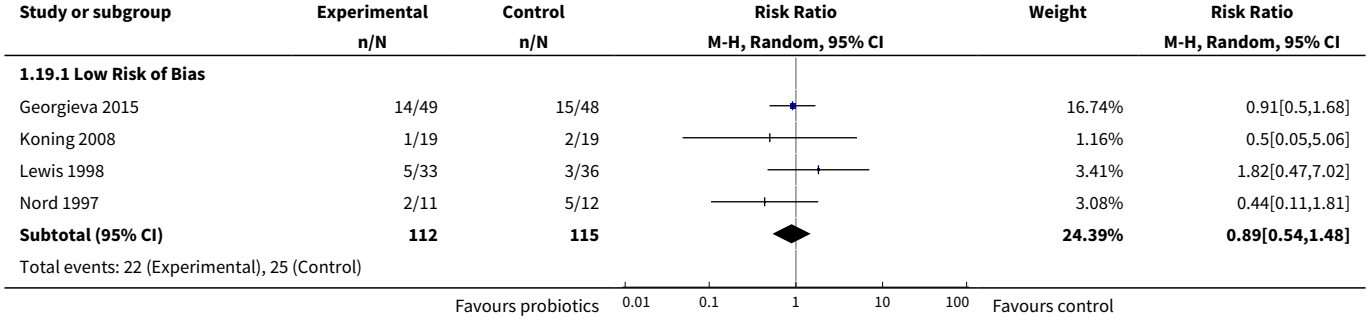


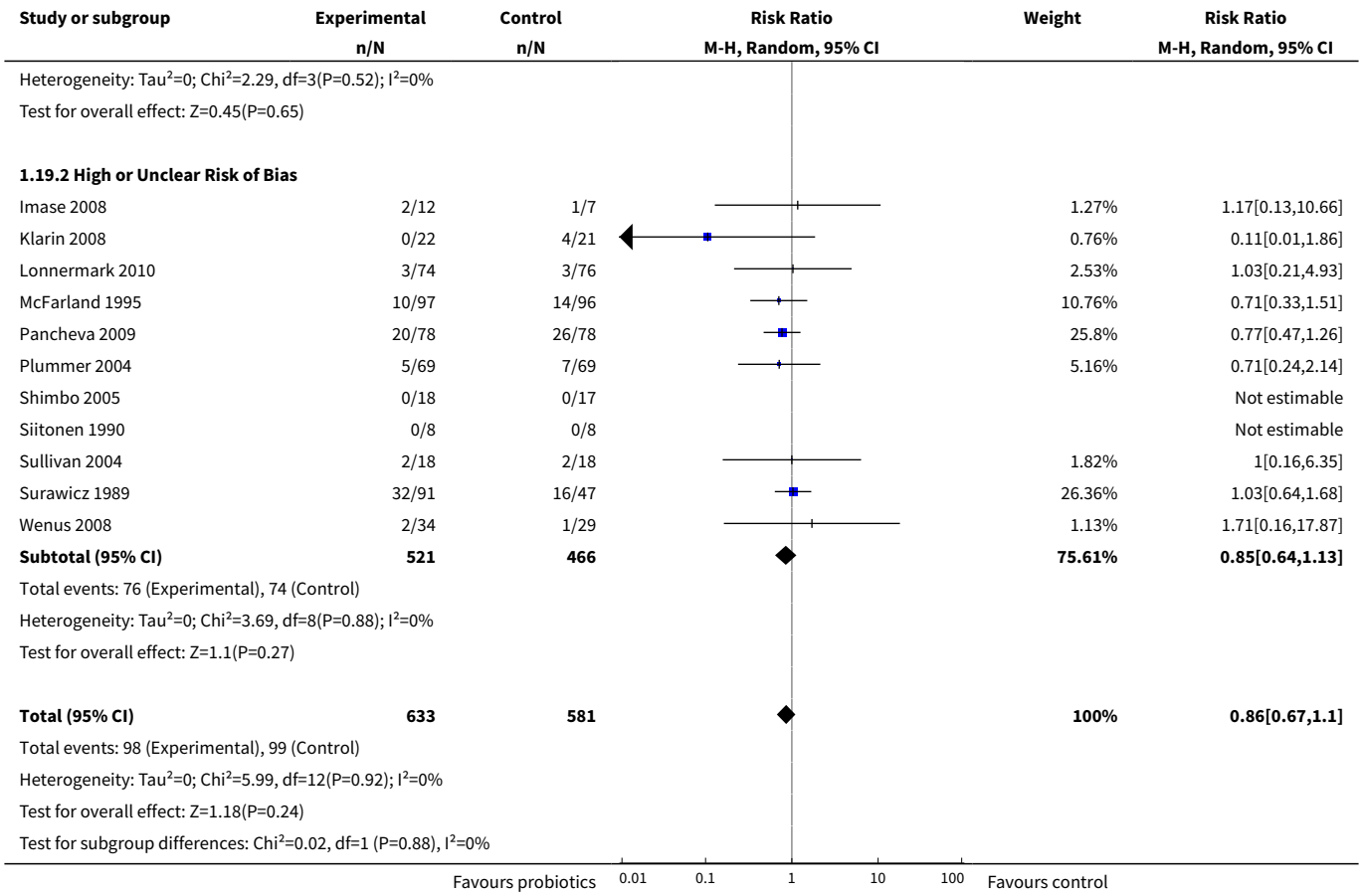


Analysis 1.18. Comparison 1 Probiotics versus control, Outcome 18 Incidence of infection: complete case.

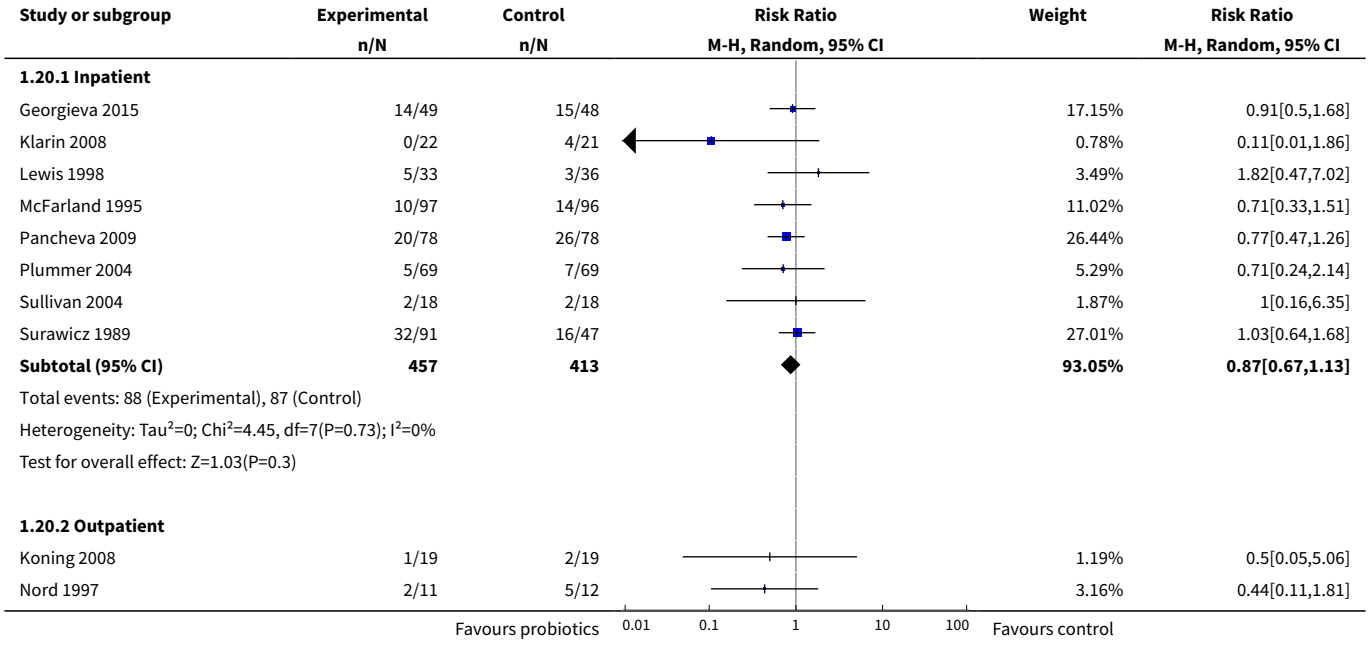


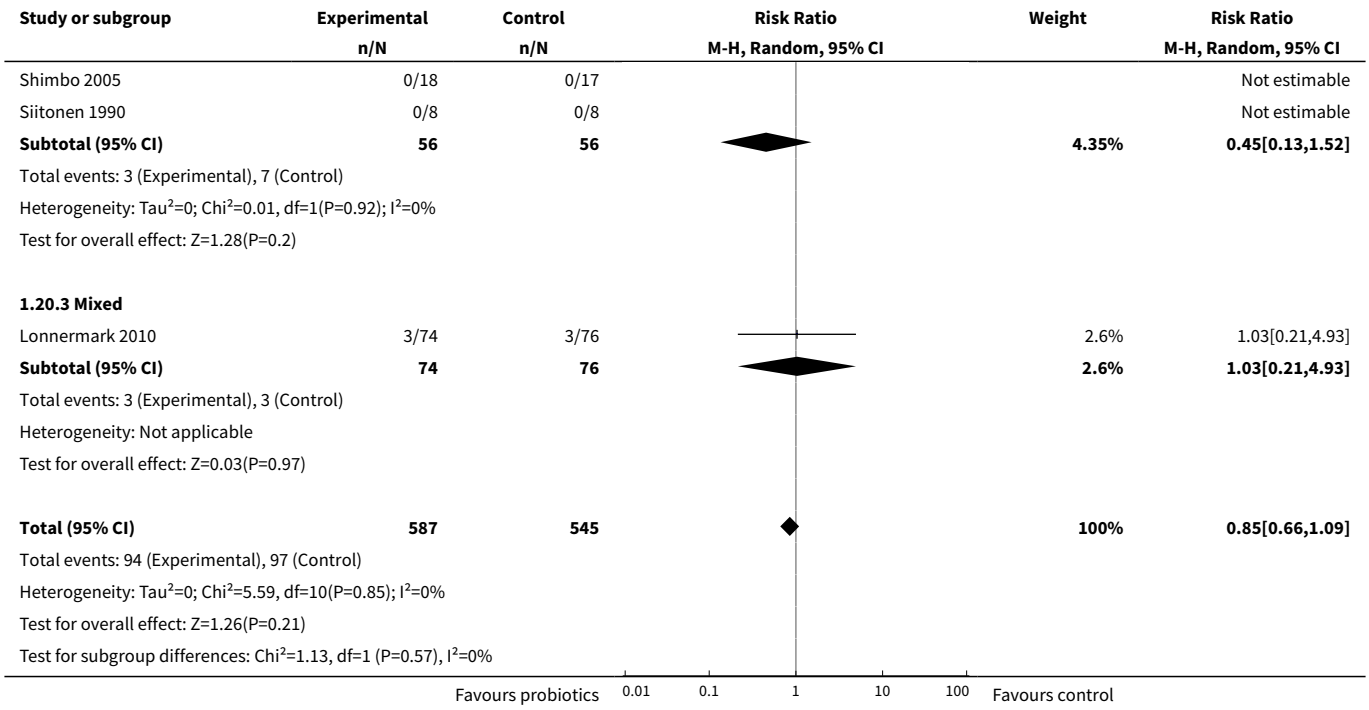
Analysis 1.19. Comparison 1 Probiotics versus control, Outcome 19 Incidence of infection: Subgroup: Risk of Bias.



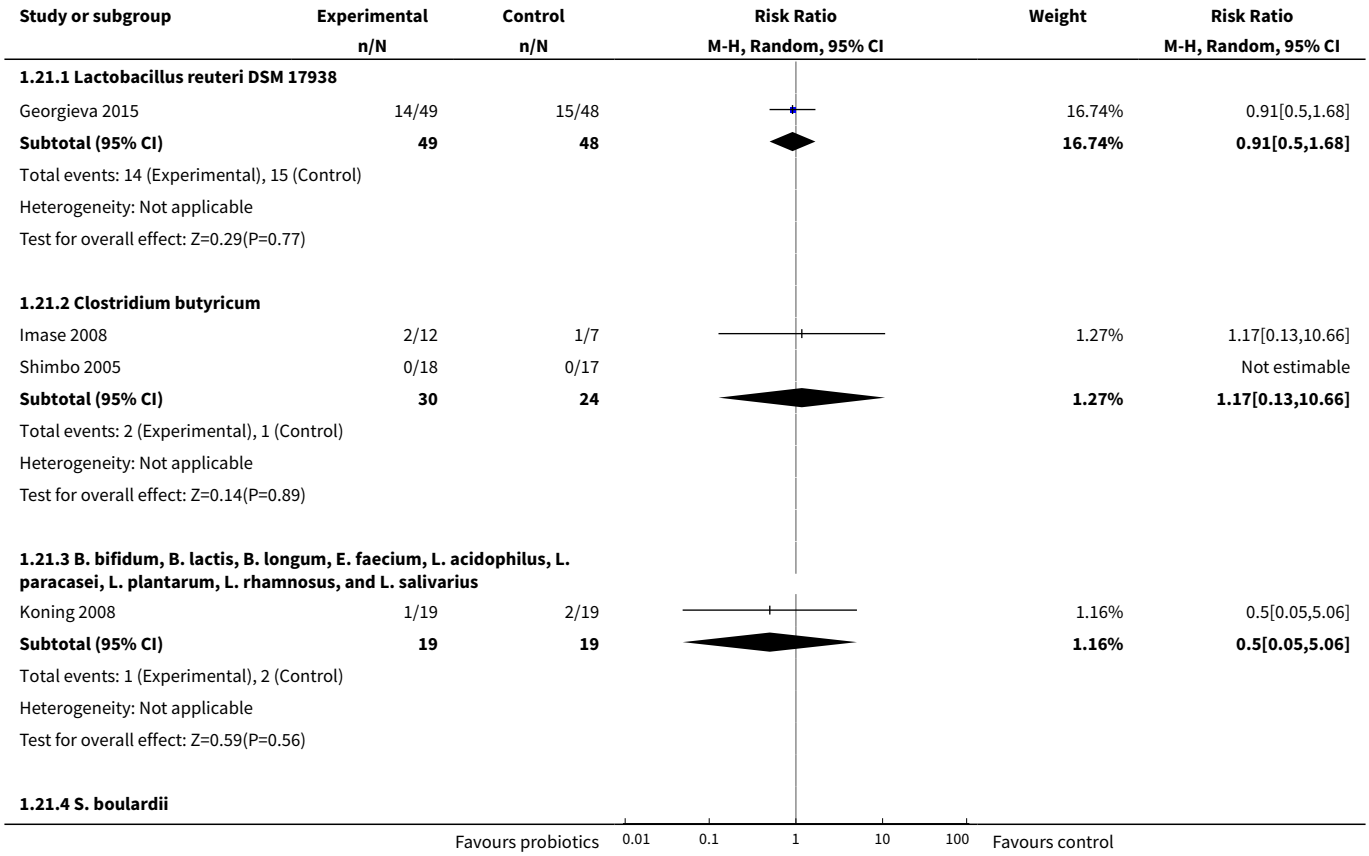


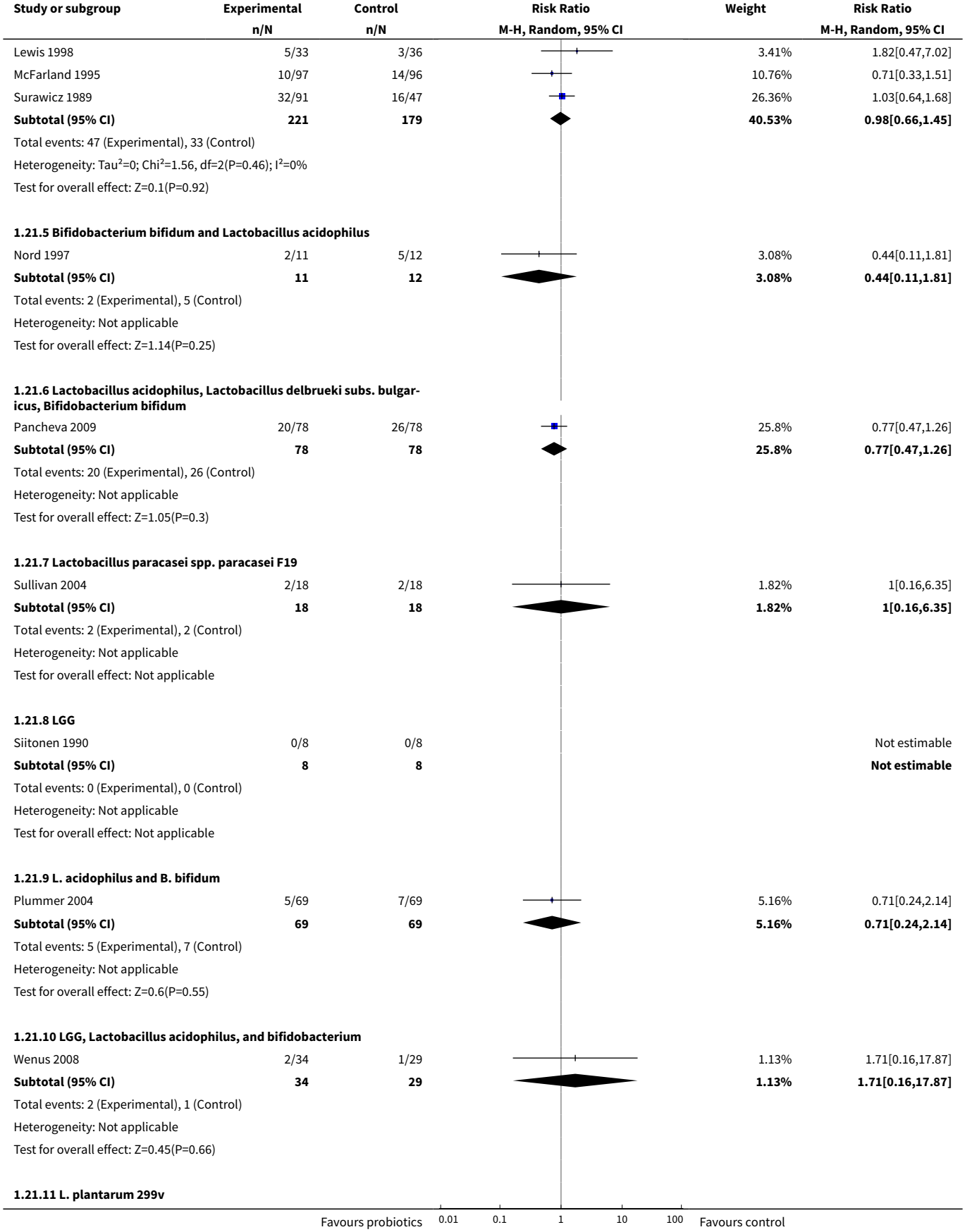
Analysis 1.20. Comparison 1 Probiotics versus control, Outcome 20 Incidence of infection: Inpatient versus outpatient.

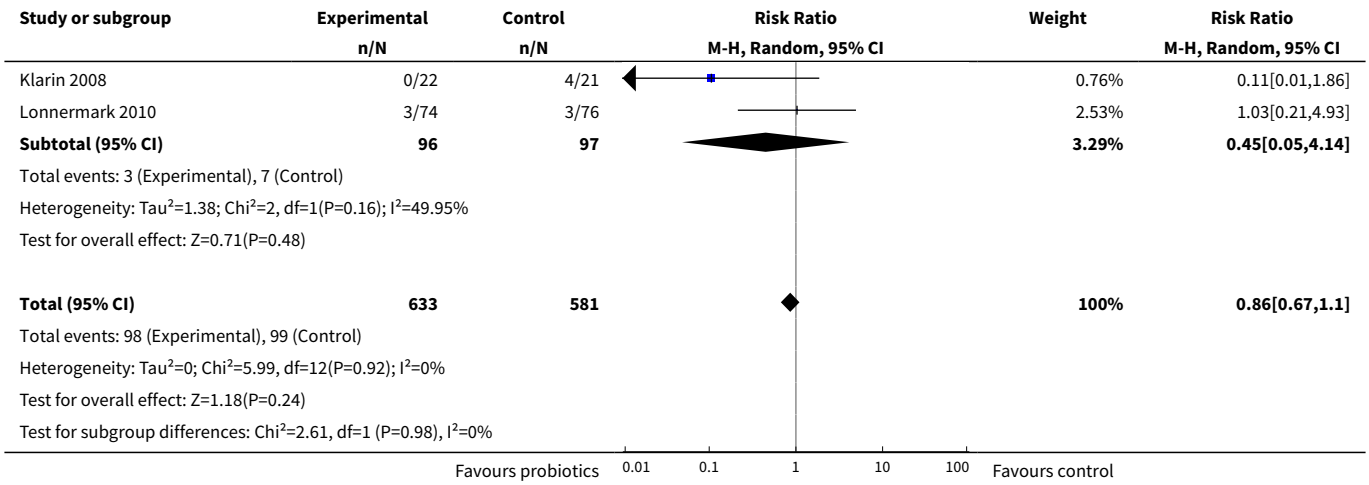




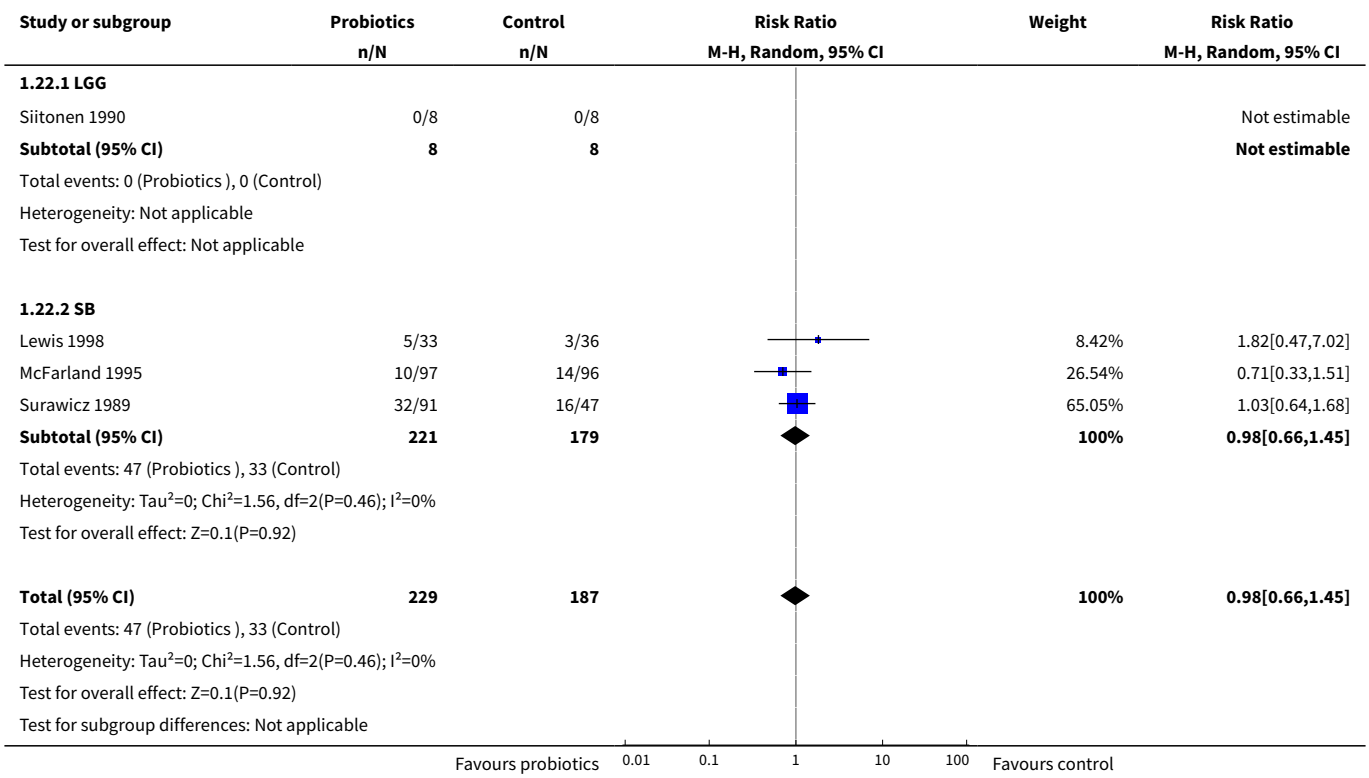
Analysis 1.21. Comparison 1 Probiotics versus control, Outcome 21 Incidence of infection: Species: all.



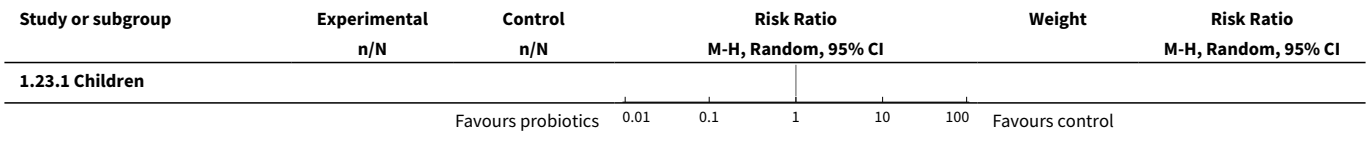


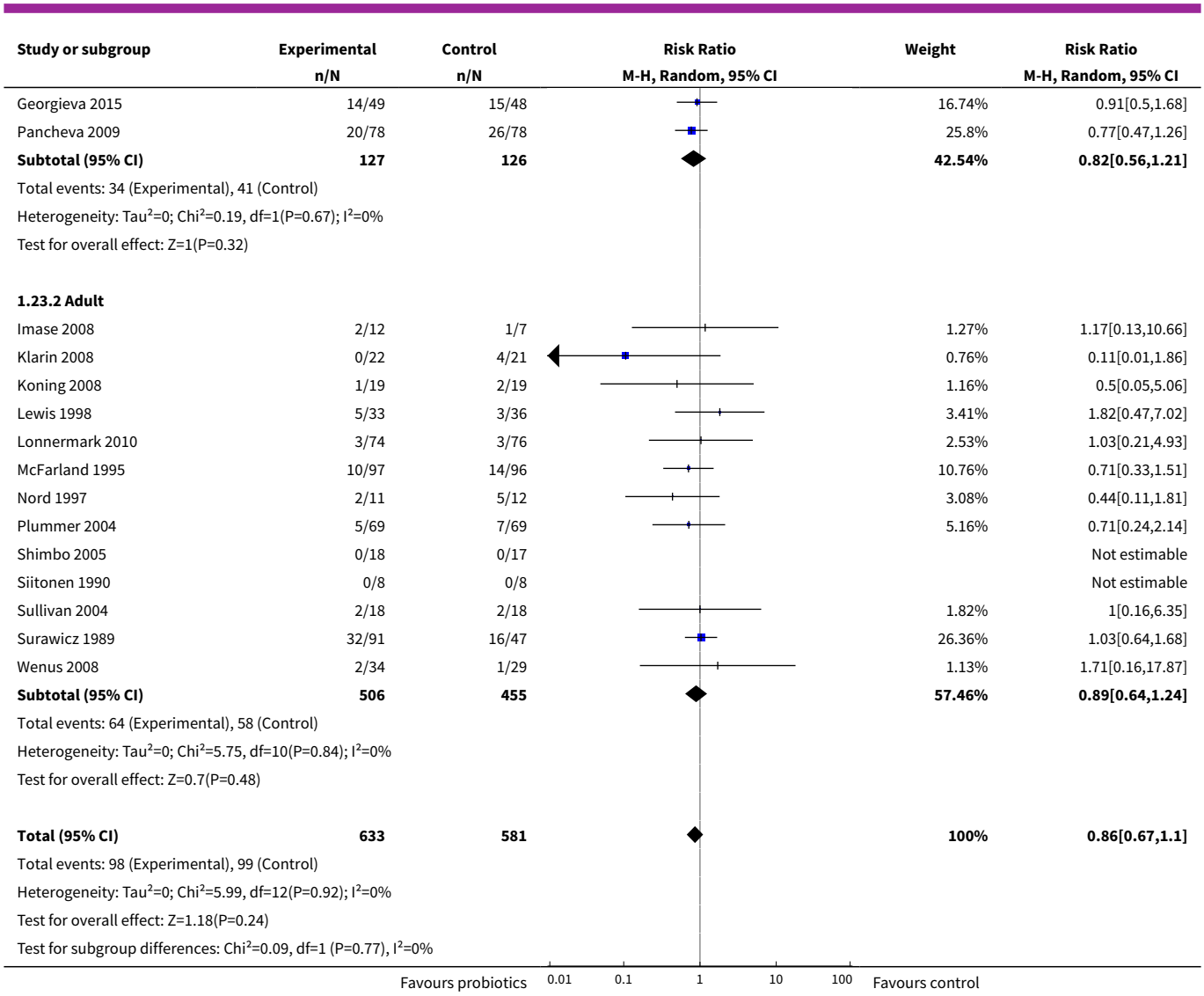


Analysis 1.22. Comparison 1 Probiotics versus control, Outcome 22 Incidence of infection: Subgroup: Species: LGG versus SB.

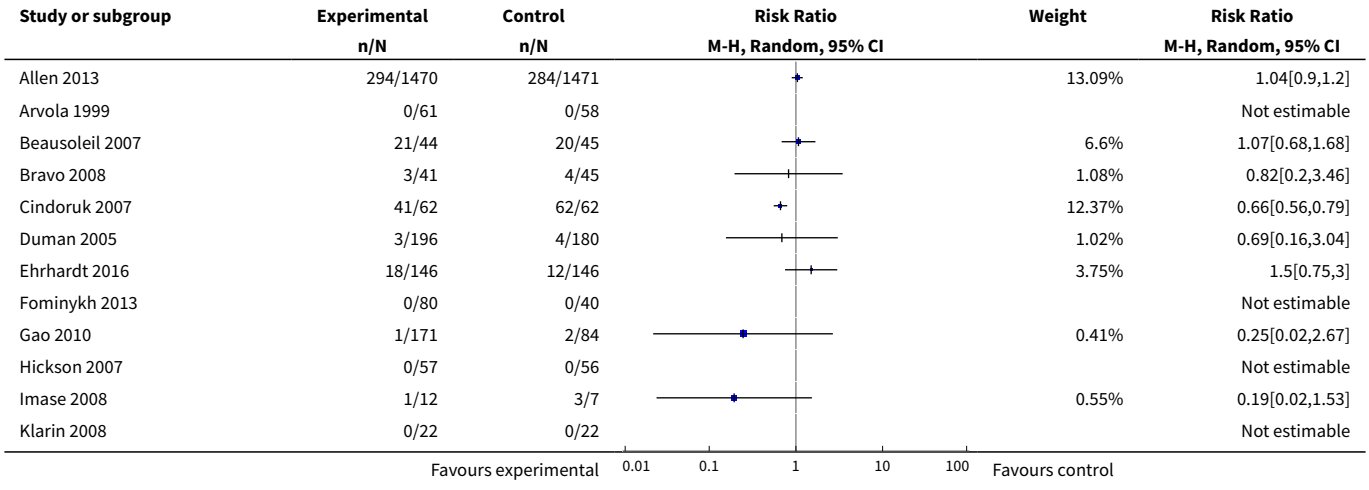


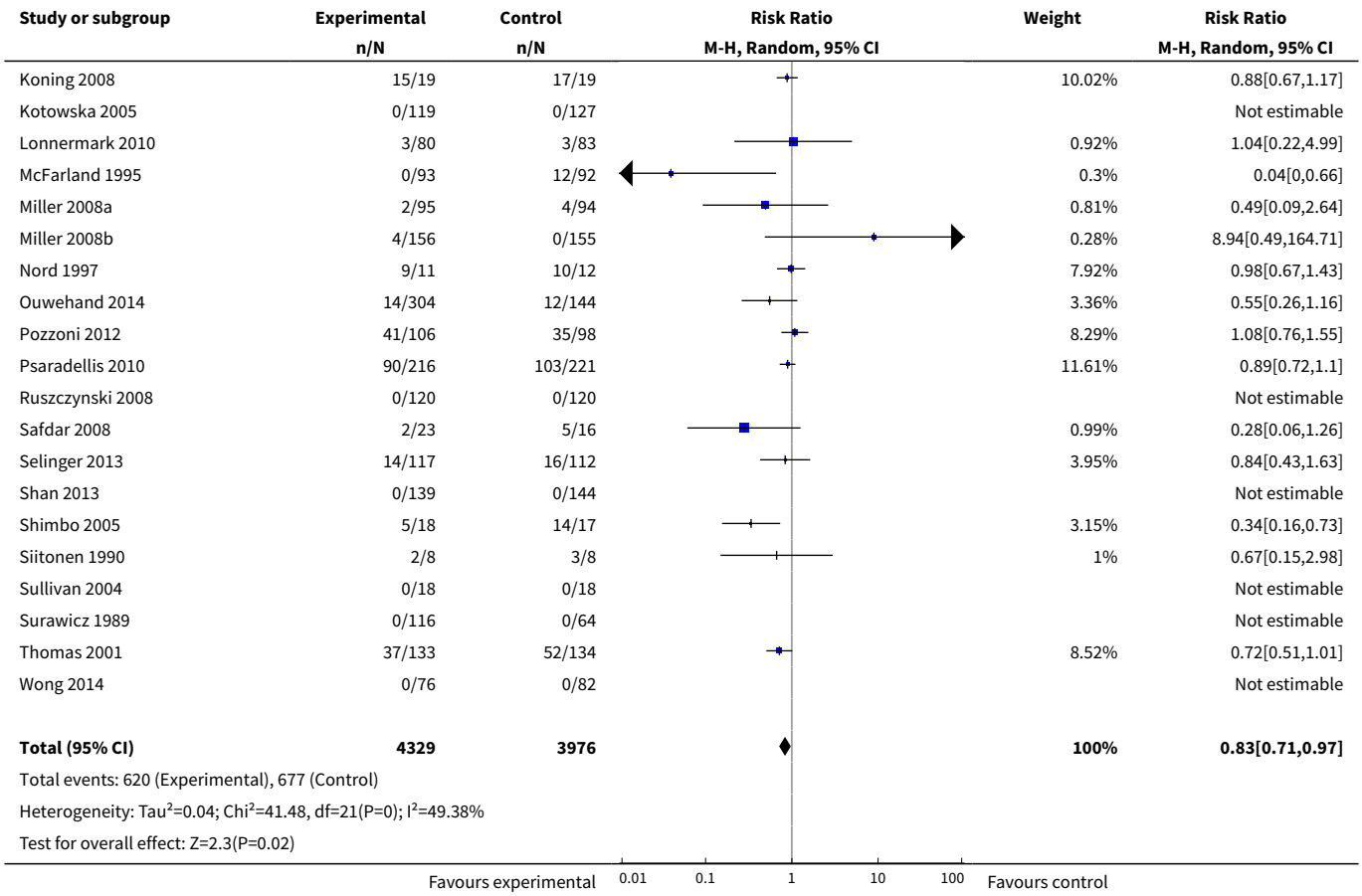
Analysis 1.23. Comparison 1 Probiotics versus control, Outcome 23 Incidence of infection: Adult versus child.



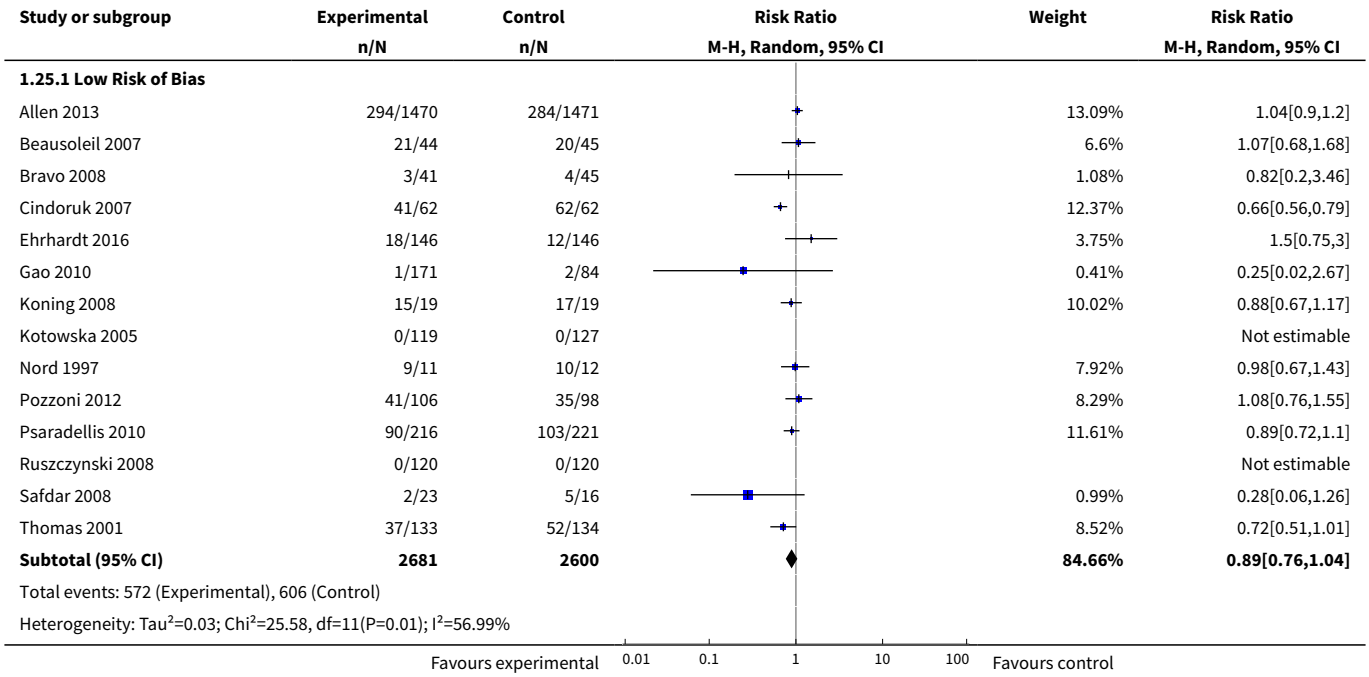


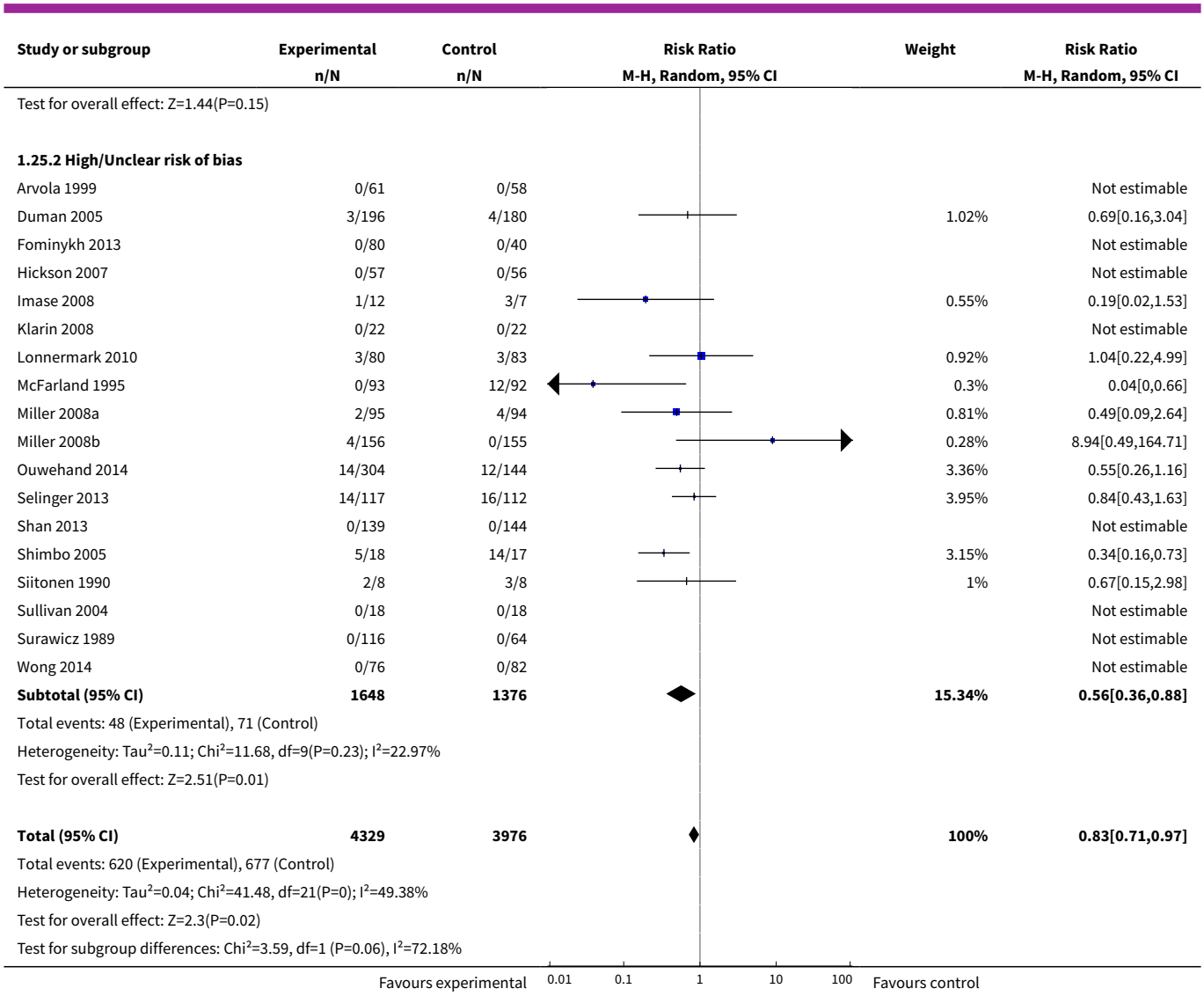
Analysis 1.24. Comparison 1 Probiotics versus control, Outcome 24 Adverse Events: complete case.



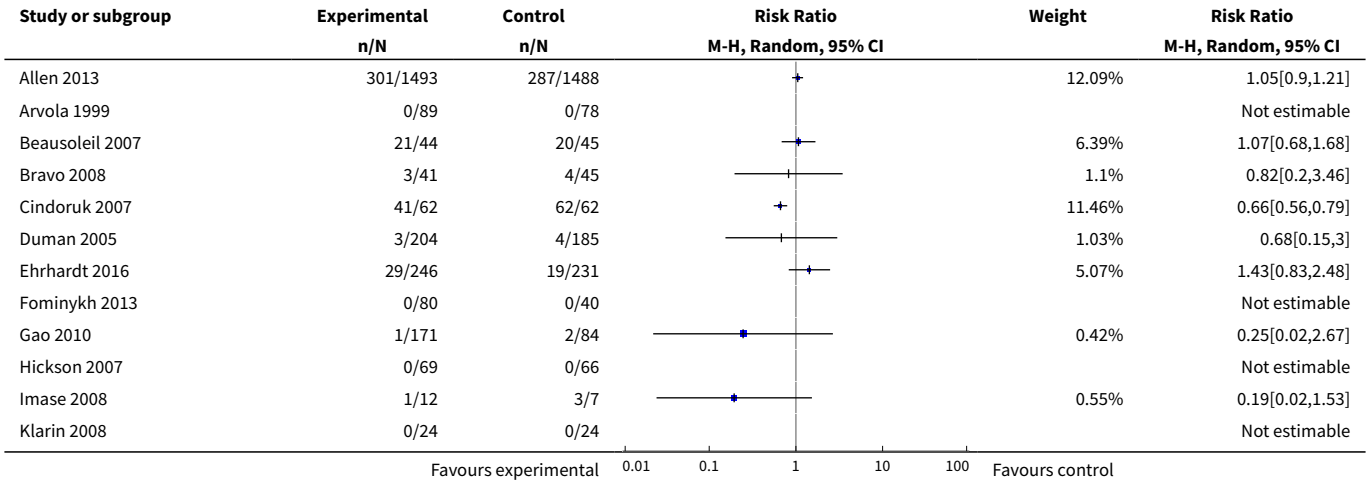


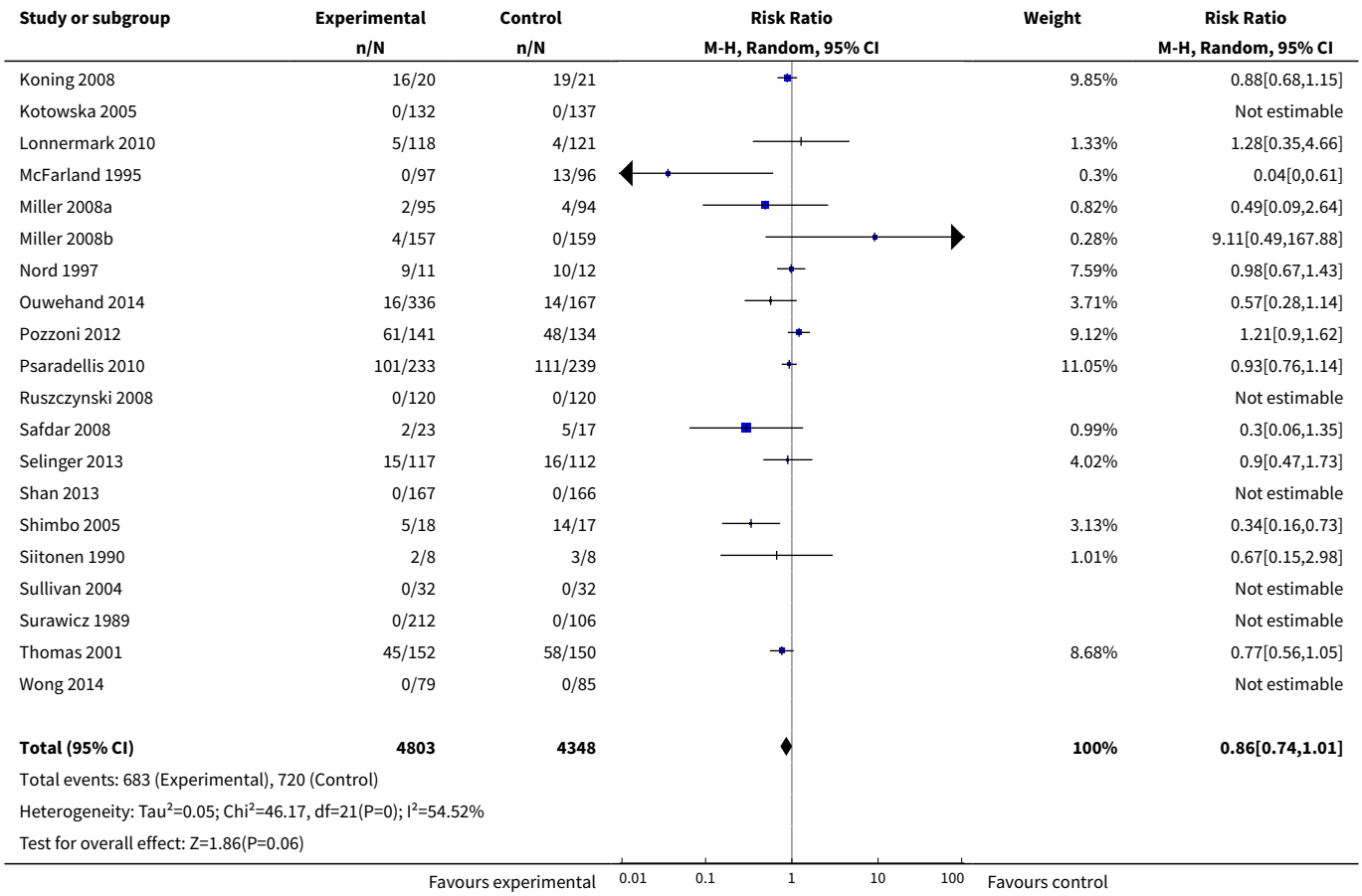
Analysis 1.25. Comparison 1 Probiotics versus control, Outcome 25 Adverse Events: Subgroup: Risk of Bias.



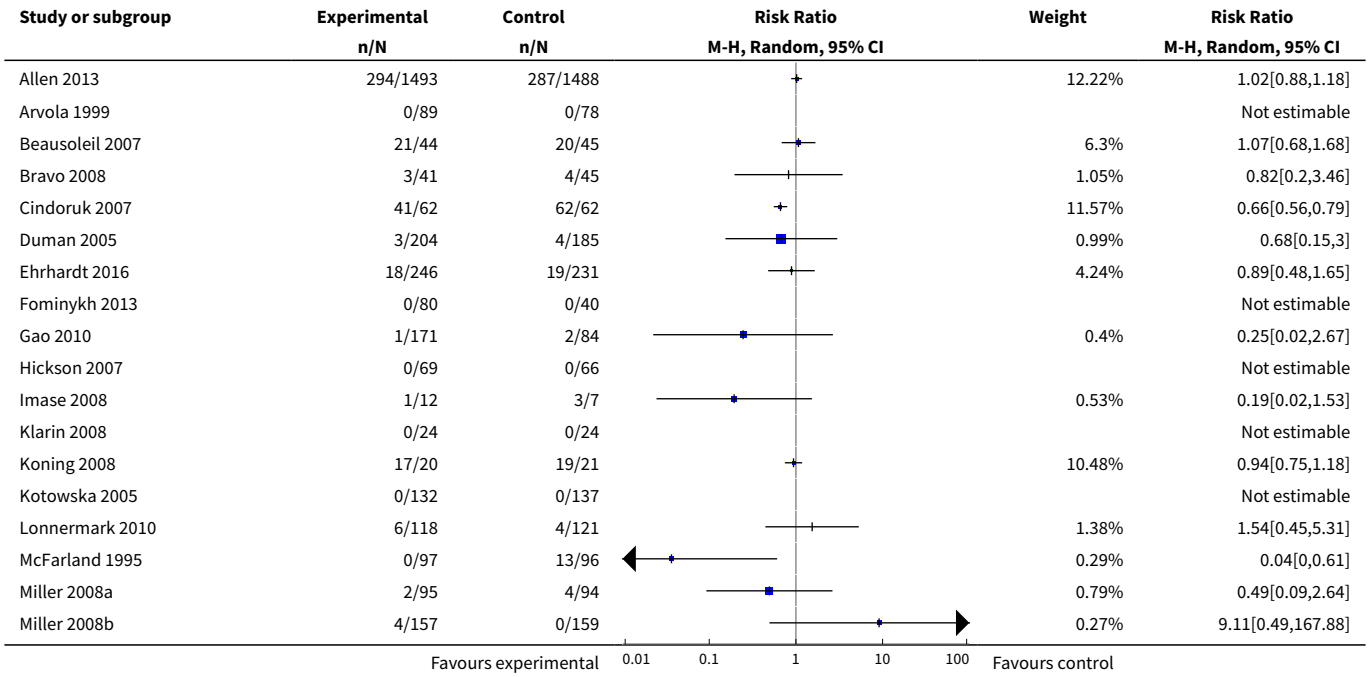


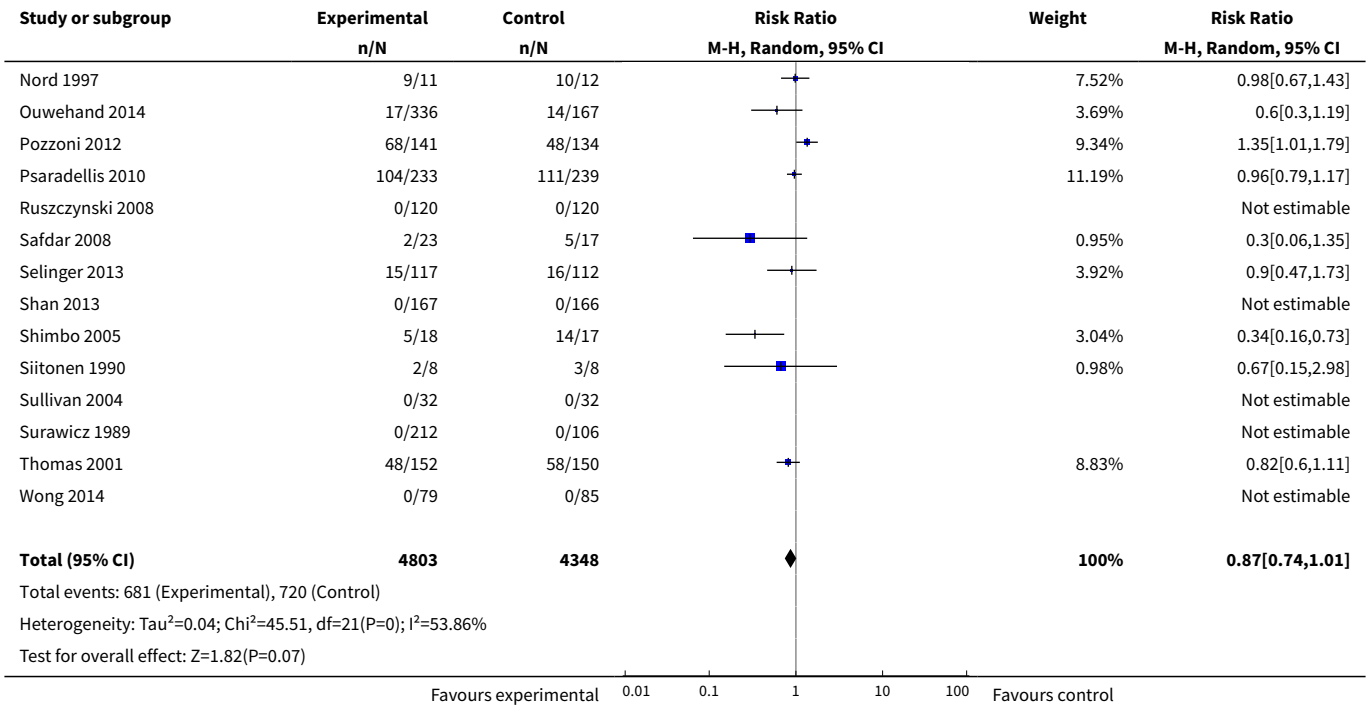
Analysis 1.26. Comparison 1 Probiotics versus control, Outcome 26 Adverse Events: Sensitivity 1.5:1.



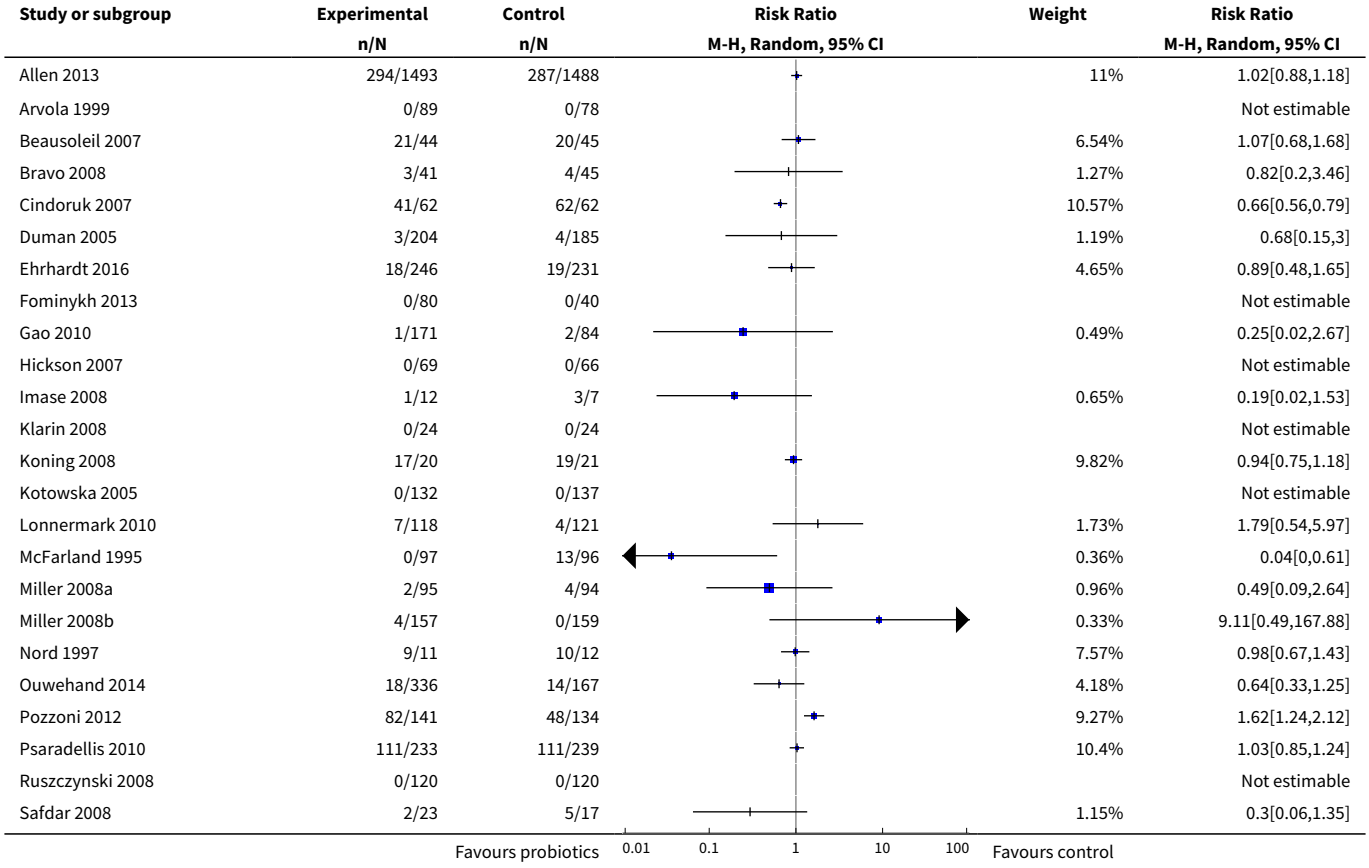


Analysis 1.27. Comparison 1 Probiotics versus control, Outcome 27 Adverse Events: Sensitivity 2:1.





Analysis 1.28. Comparison 1 Probiotics versus control, Outcome 28 Adverse Events: Sensitivity 3:1.



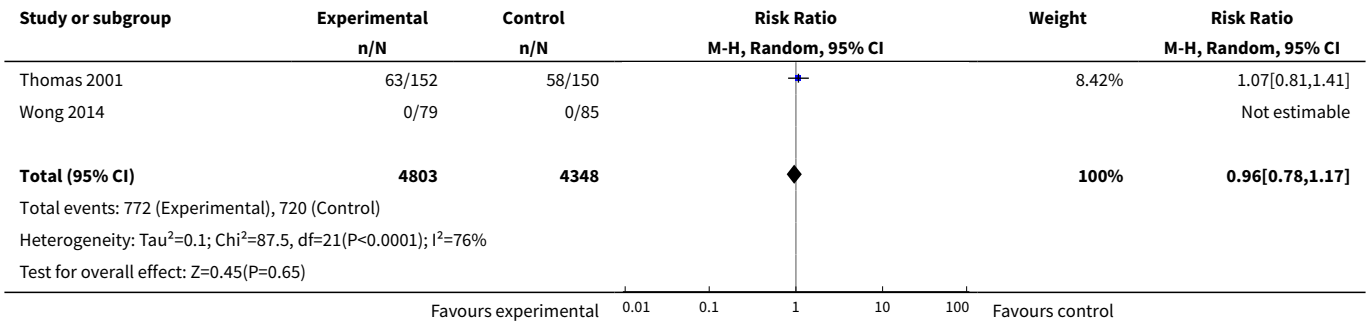
Study or subgroup	Experimental n/N	Control n/N	Risk Ratio M-H, Random, 95% CI	Weight	Risk Ratio M-H, Random, 95% CI
Selinger 2013	16/117	16/112		4.44%	0.96[0.5,1.82]
Shan 2013	0/167	0/166			Not estimable
Shimbo 2005	5/18	14/17		3.44%	0.34[0.16,0.73]
Siitonen 1990	2/8	3/8		1.17%	0.67[0.15,2.98]
Sullivan 2004	0/32	0/32			Not estimable
Surawicz 1989	0/212	0/106			Not estimable
Thomas 2001	53/152	58/150		8.8%	0.9[0.67,1.21]
Wong 2014	0/79	0/85			Not estimable
Total (95% CI)	**4803**	**4348**		**100%**	**0.89[0.75,1.06]**
Total events: 710 (Experimental), 720 (Control)					
Heterogeneity: Tau^2=0.06; Chi^2=56.87, df=21(P<0.0001); I^2=63.08%					
Test for overall effect: Z=1.3(P=0.19)					

Favours probiotics — Favours control

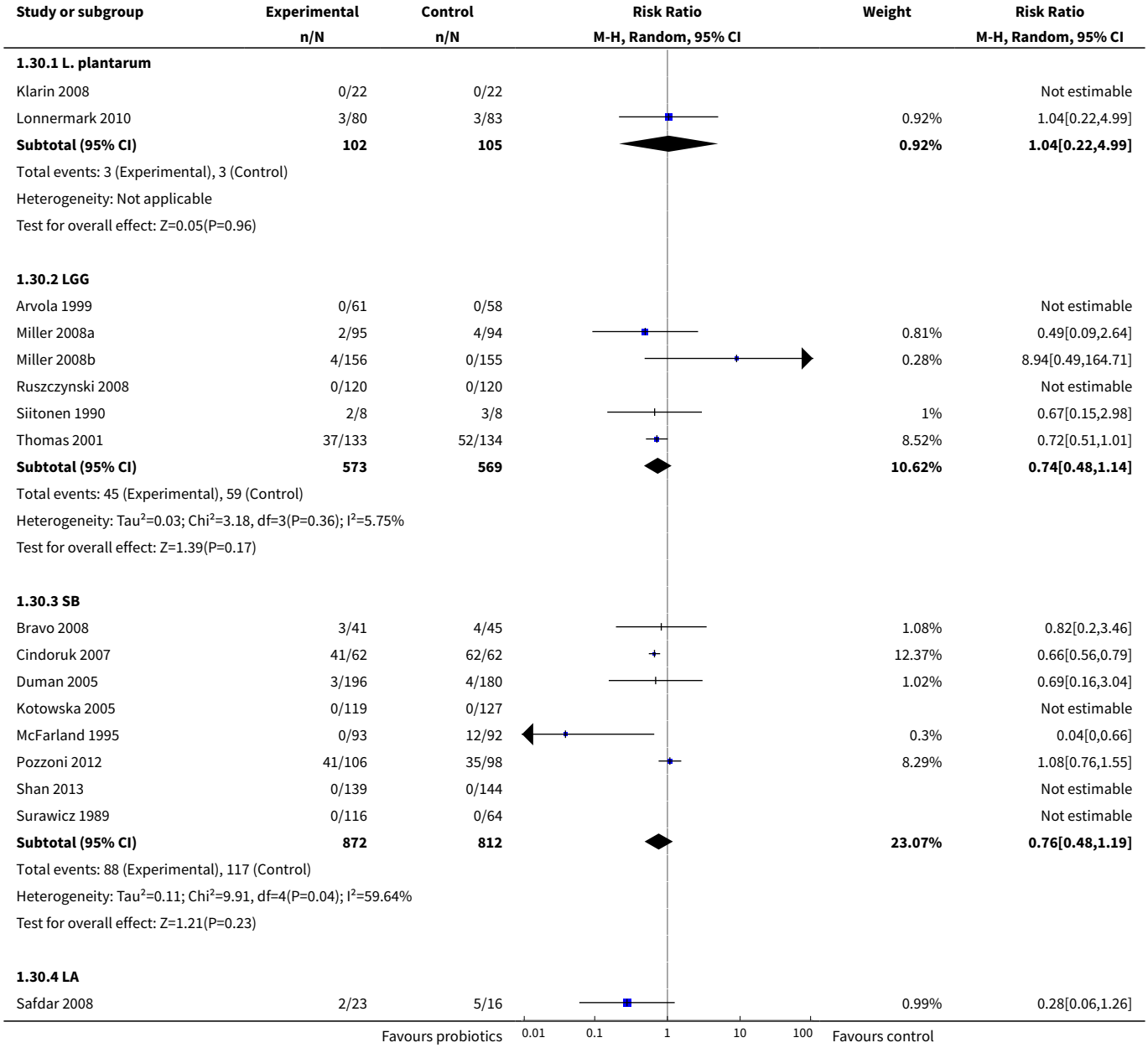
Analysis 1.29. Comparison 1 Probiotics versus control, Outcome 29 Adverse Events: Sensitivity 5:1.

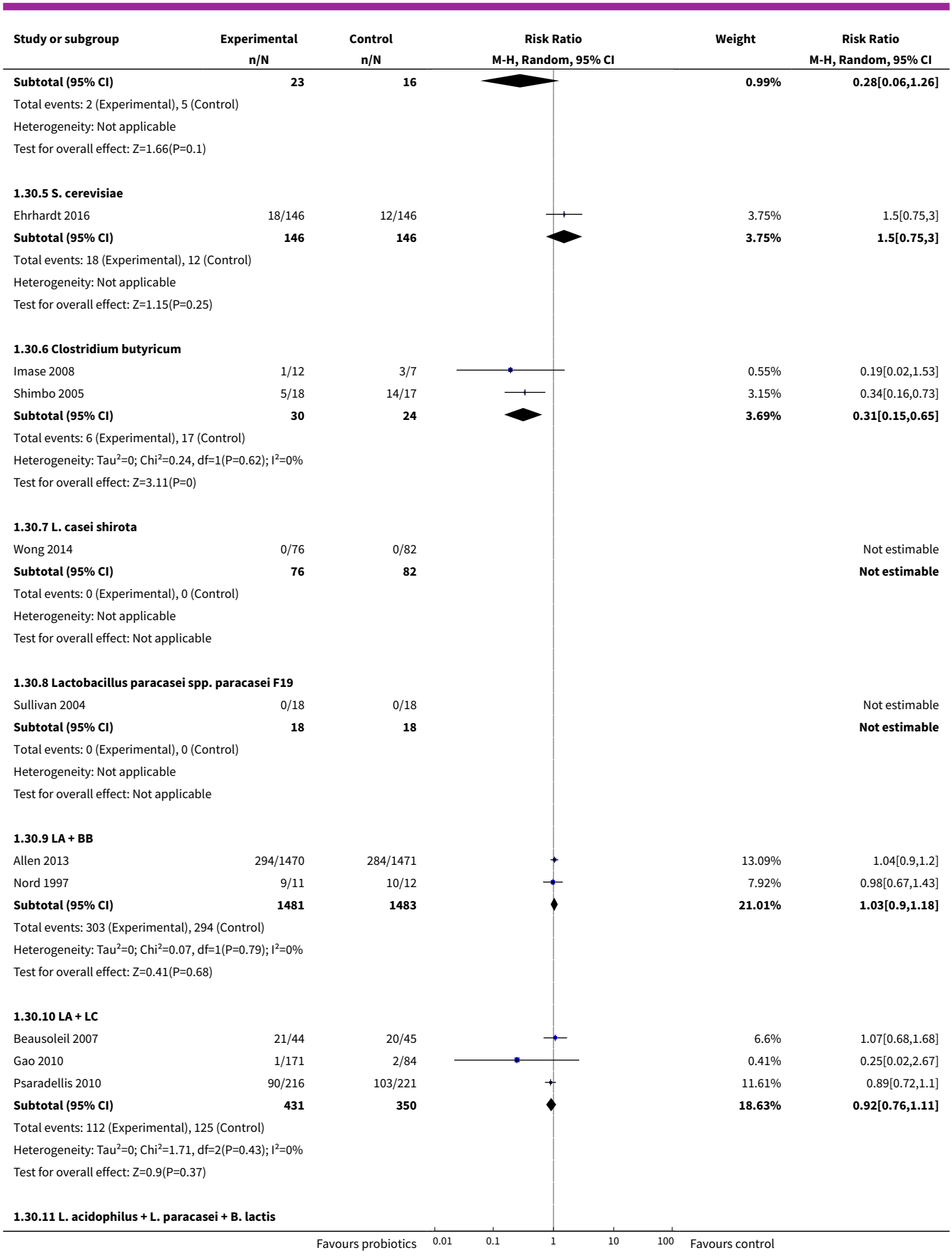
Study or subgroup	Experimental n/N	Control n/N	Risk Ratio M-H, Random, 95% CI	Weight	Risk Ratio M-H, Random, 95% CI
Allen 2013	294/1493	287/1488		9.53%	1.02[0.88,1.18]
Arvola 1999	0/89	0/78			Not estimable
Beausoleil 2007	21/44	20/45		6.64%	1.07[0.68,1.68]
Bravo 2008	3/41	4/45		1.62%	0.82[0.2,3.46]
Cindoruk 2007	41/62	62/62		9.29%	0.66[0.56,0.79]
Duman 2005	4/204	4/185		1.74%	0.91[0.23,3.57]
Ehrhardt 2016	18/246	19/231		5.1%	0.89[0.48,1.65]
Fominykh 2013	0/80	0/40			Not estimable
Gao 2010	1/171	2/84		0.65%	0.25[0.02,2.67]
Hickson 2007	0/69	0/66			Not estimable
Imase 2008	1/12	3/7		0.85%	0.19[0.02,1.53]
Klarin 2008	0/24	0/24			Not estimable
Koning 2008	19/20	19/21		9.35%	1.05[0.88,1.25]
Kotowska 2005	0/132	0/137			Not estimable
Lonnermark 2010	10/118	4/121		2.37%	2.56[0.83,7.95]
McFarland 1995	0/97	13/96		0.48%	0.04[0,0.61]
Miller 2008a	2/95	4/94		1.24%	0.49[0.09,2.64]
Miller 2008b	4/157	0/159		0.45%	9.11[0.49,167.88]
Nord 1997	9/11	10/12		7.39%	0.98[0.67,1.43]
Ouwehand 2014	21/336	14/167		4.85%	0.75[0.39,1.43]
Pozzoni 2012	109/141	48/134		8.73%	2.16[1.69,2.75]
Psaradellis 2010	125/233	111/239		9.27%	1.16[0.96,1.38]
Ruszczynski 2008	0/120	0/120			Not estimable
Safdar 2008	2/23	5/17		1.48%	0.3[0.06,1.35]
Selinger 2013	18/117	16/112		5.08%	1.08[0.58,2.01]
Shan 2013	0/167	0/166			Not estimable
Shimbo 2005	5/18	14/17		3.98%	0.34[0.16,0.73]
Siitonen 1990	2/8	3/8		1.51%	0.67[0.15,2.98]
Sullivan 2004	0/32	0/32			Not estimable
Surawicz 1989	0/212	0/106			Not estimable

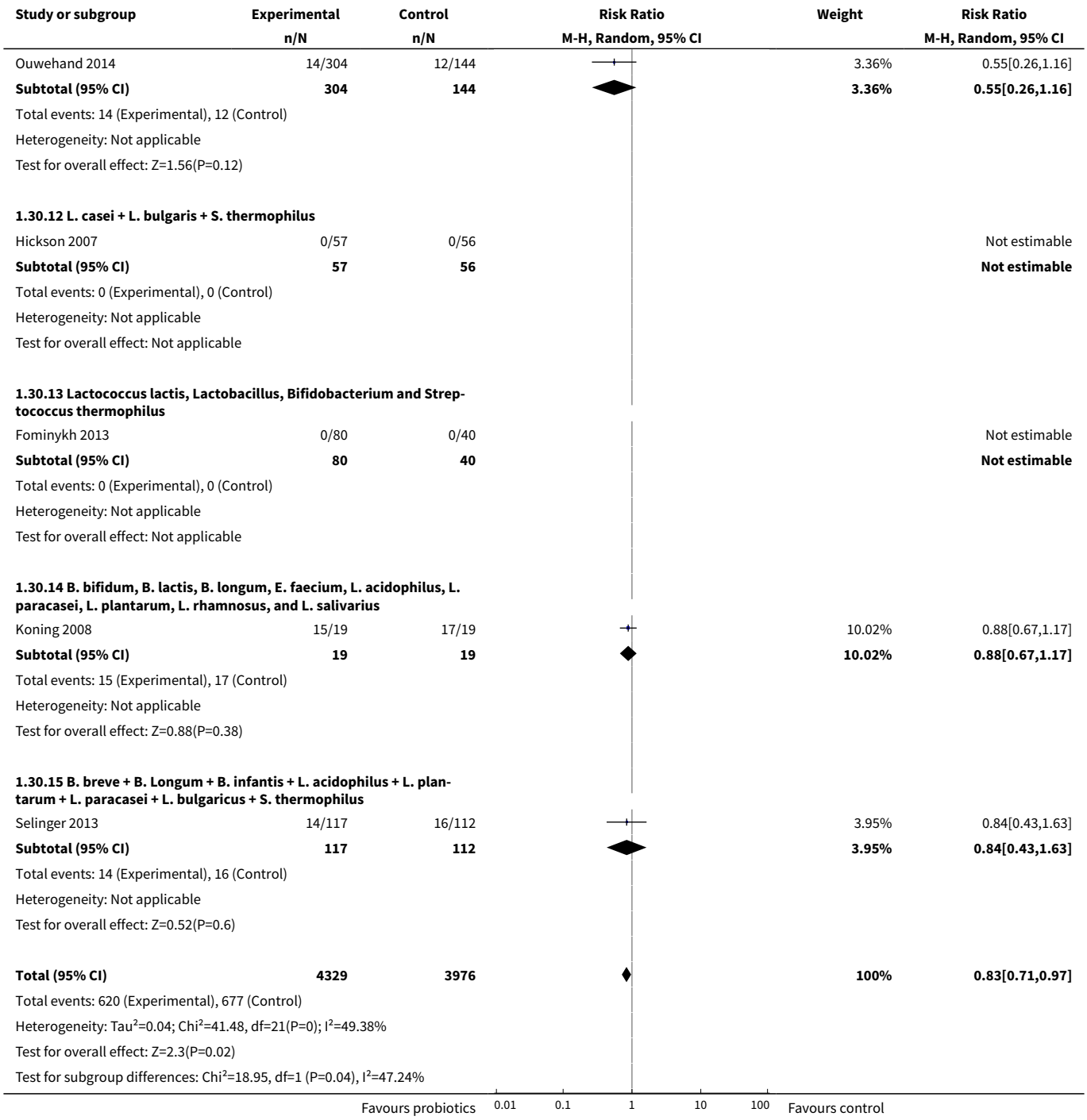
Favours experimental — Favours control



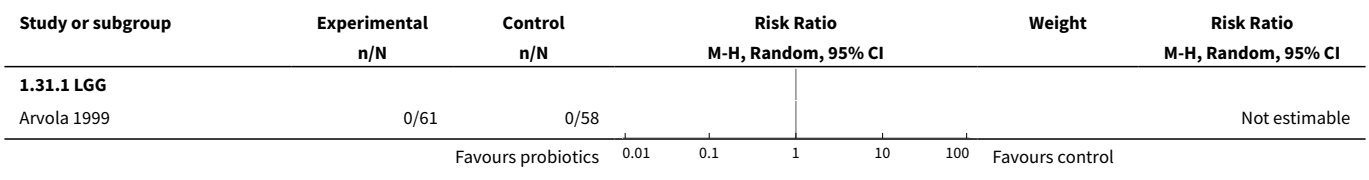
Analysis 1.30. Comparison 1 Probiotics versus control, Outcome 30 Adverse Events: Species: all.

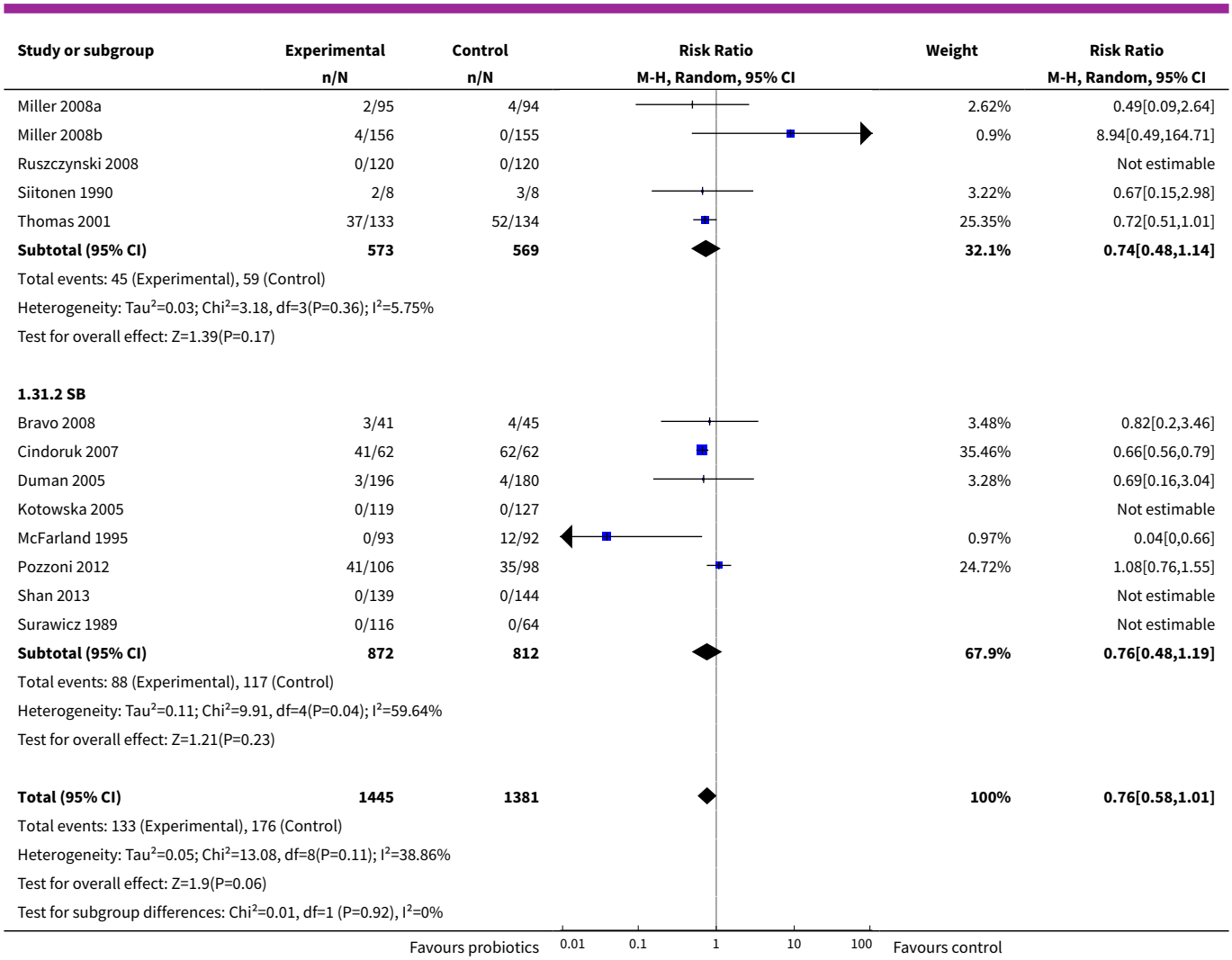




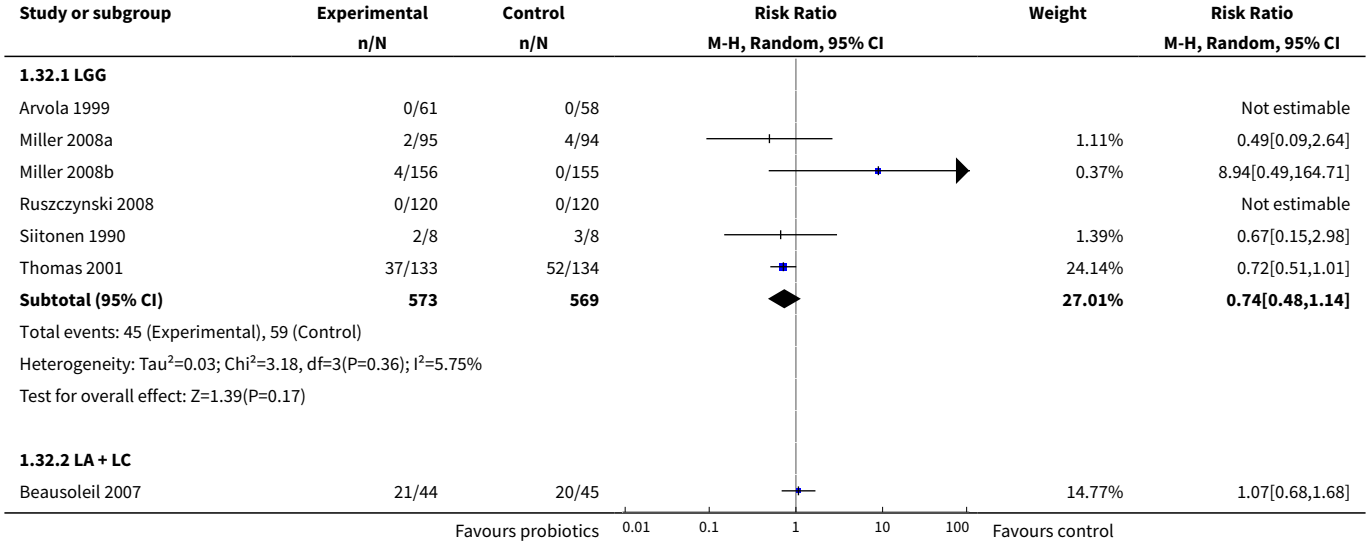


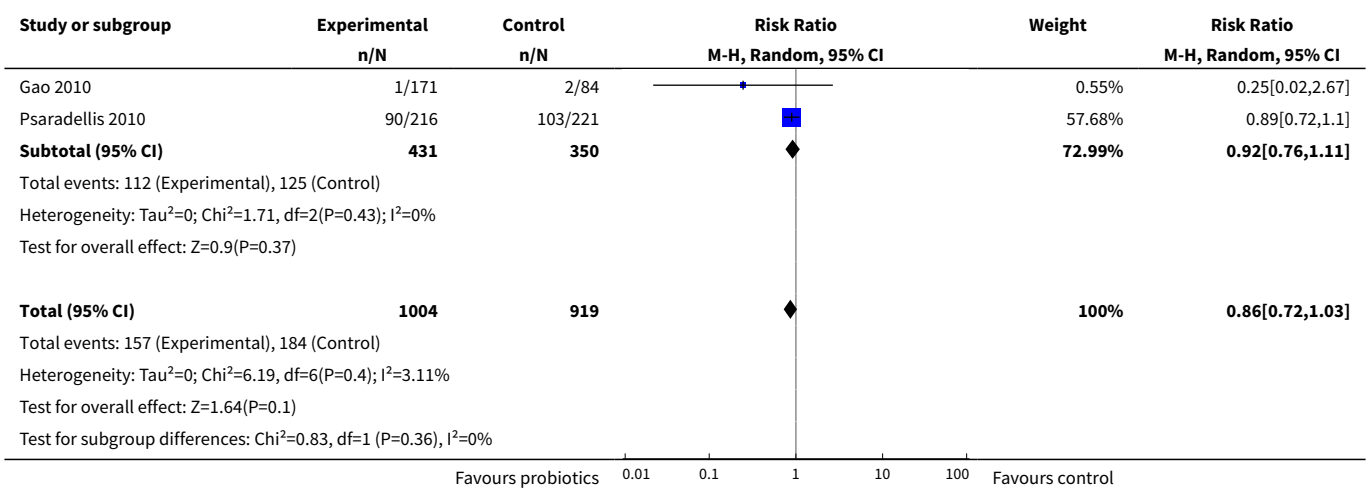
Analysis 1.31. Comparison 1 Probiotics versus control, Outcome 31 Adverse Events: Species: LGG versus SB.



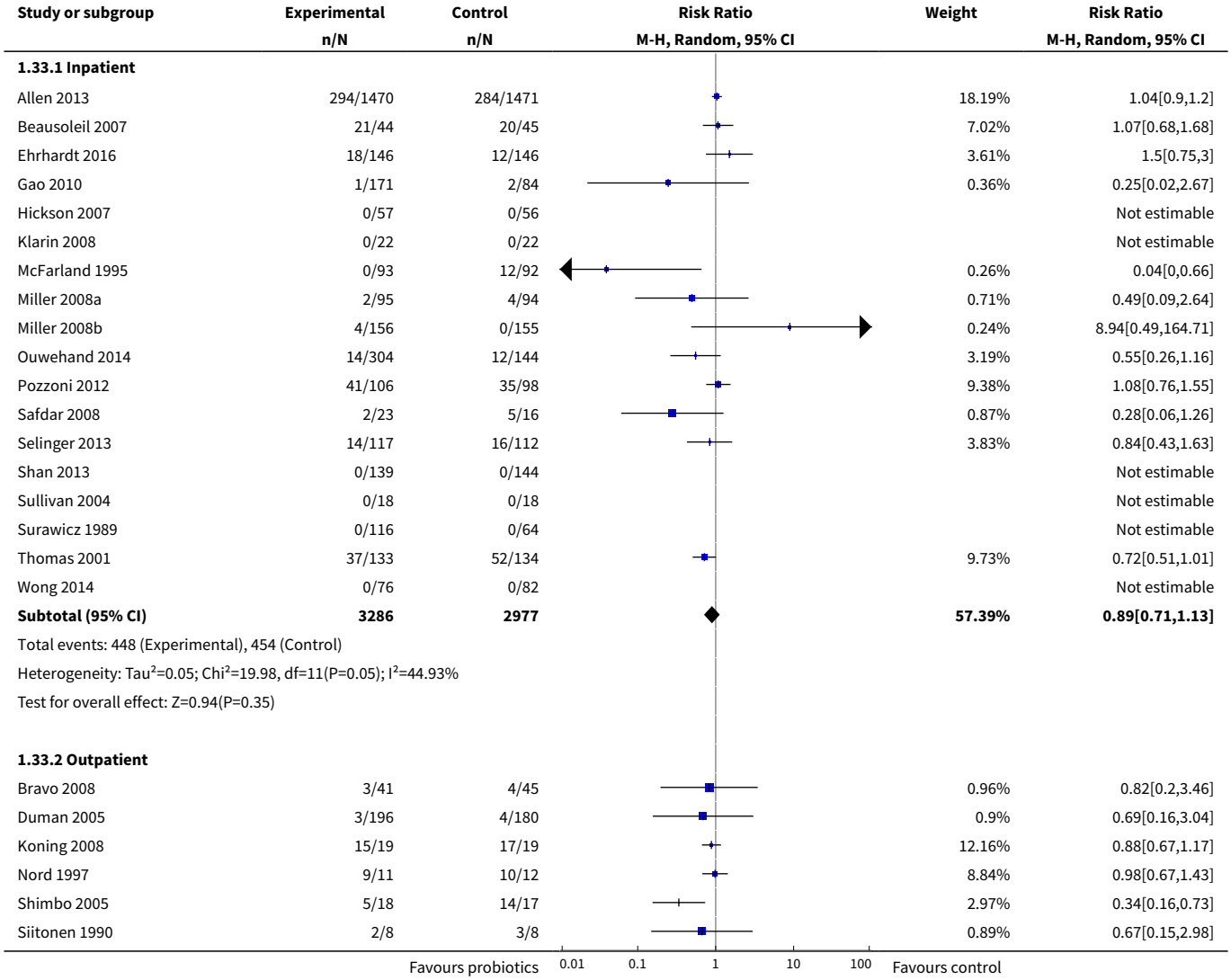


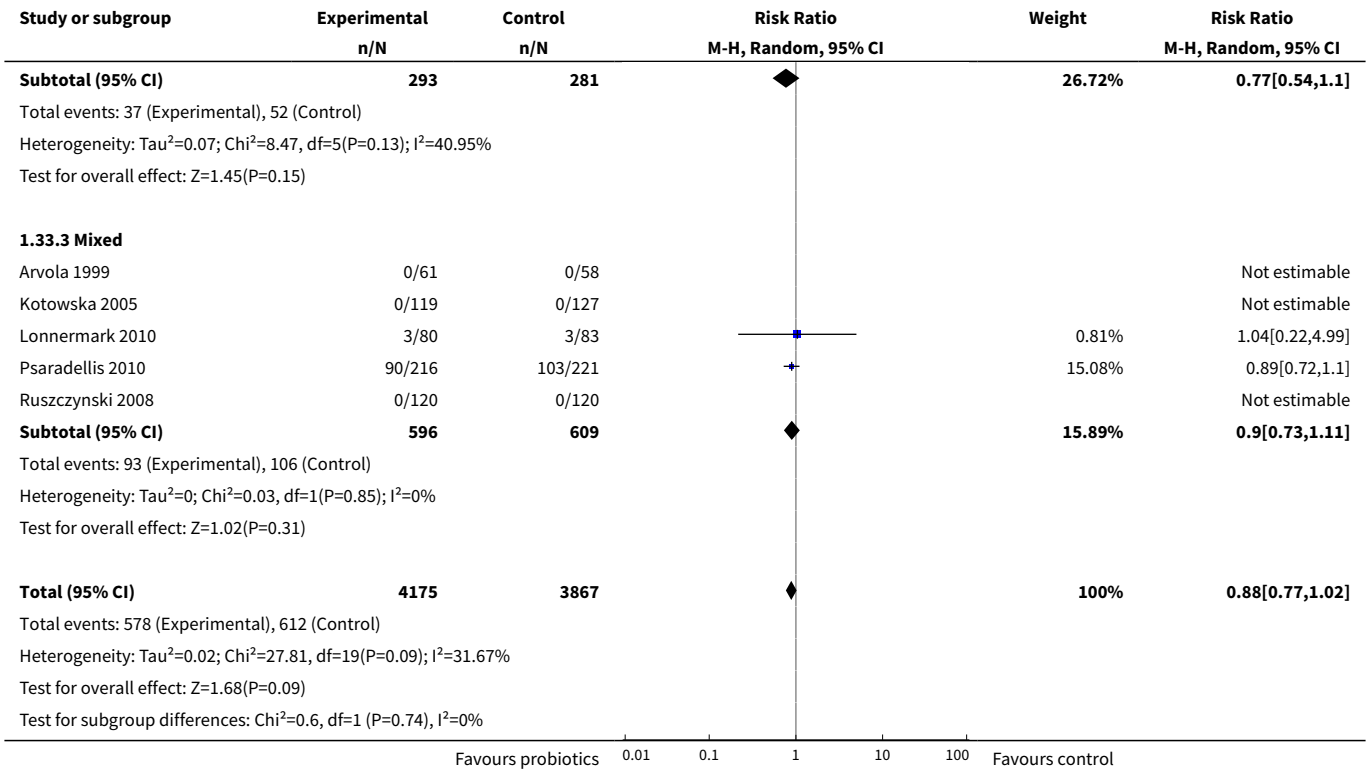
Analysis 1.32. Comparison 1 Probiotics versus control, Outcome 32 Adverse Events: Species: LGG versus LA + LC.



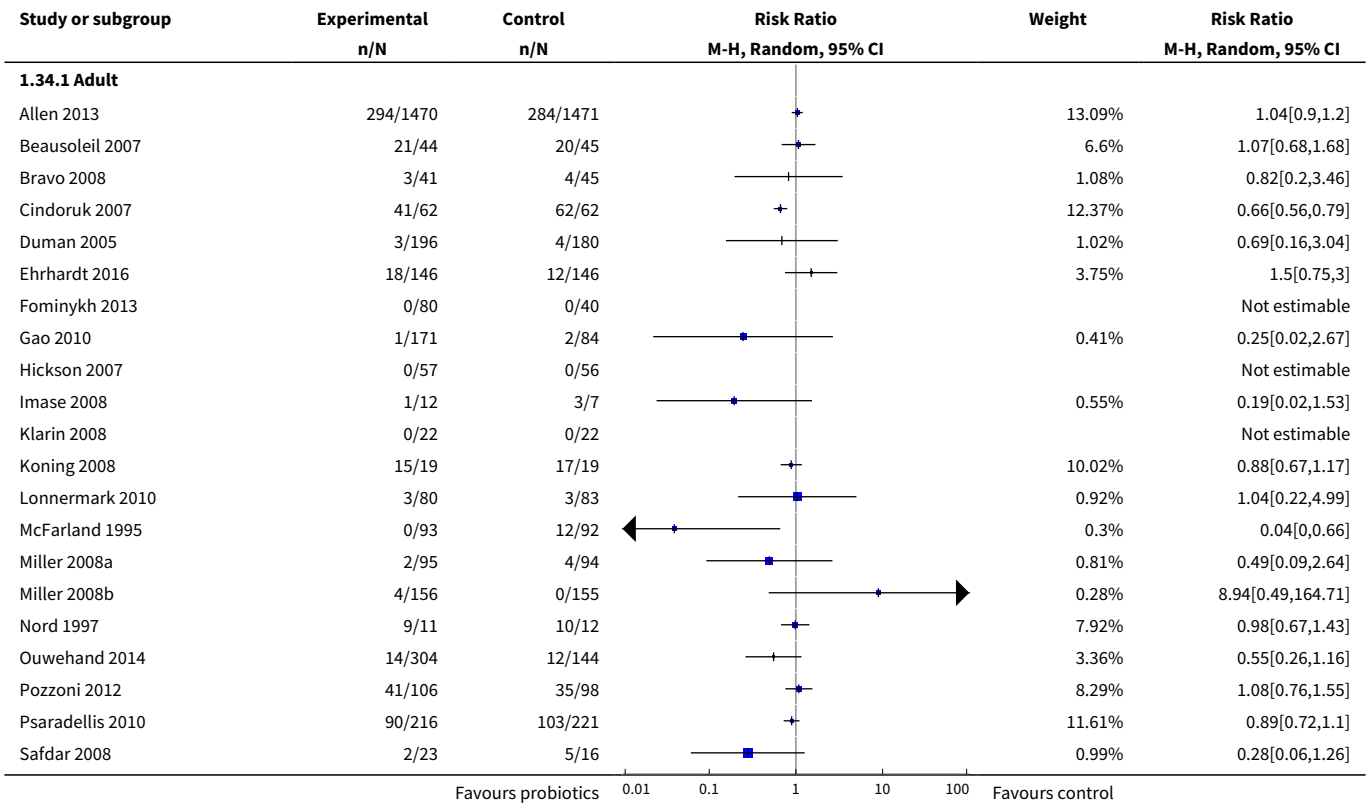


Analysis 1.33. Comparison 1 Probiotics versus control, Outcome 33 Adverse Events: Inpatient versus outpatient.

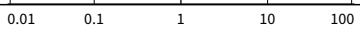




Analysis 1.34. Comparison 1 Probiotics versus control, Outcome 34 Adverse Events: Adult versus child.

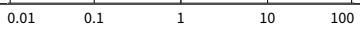


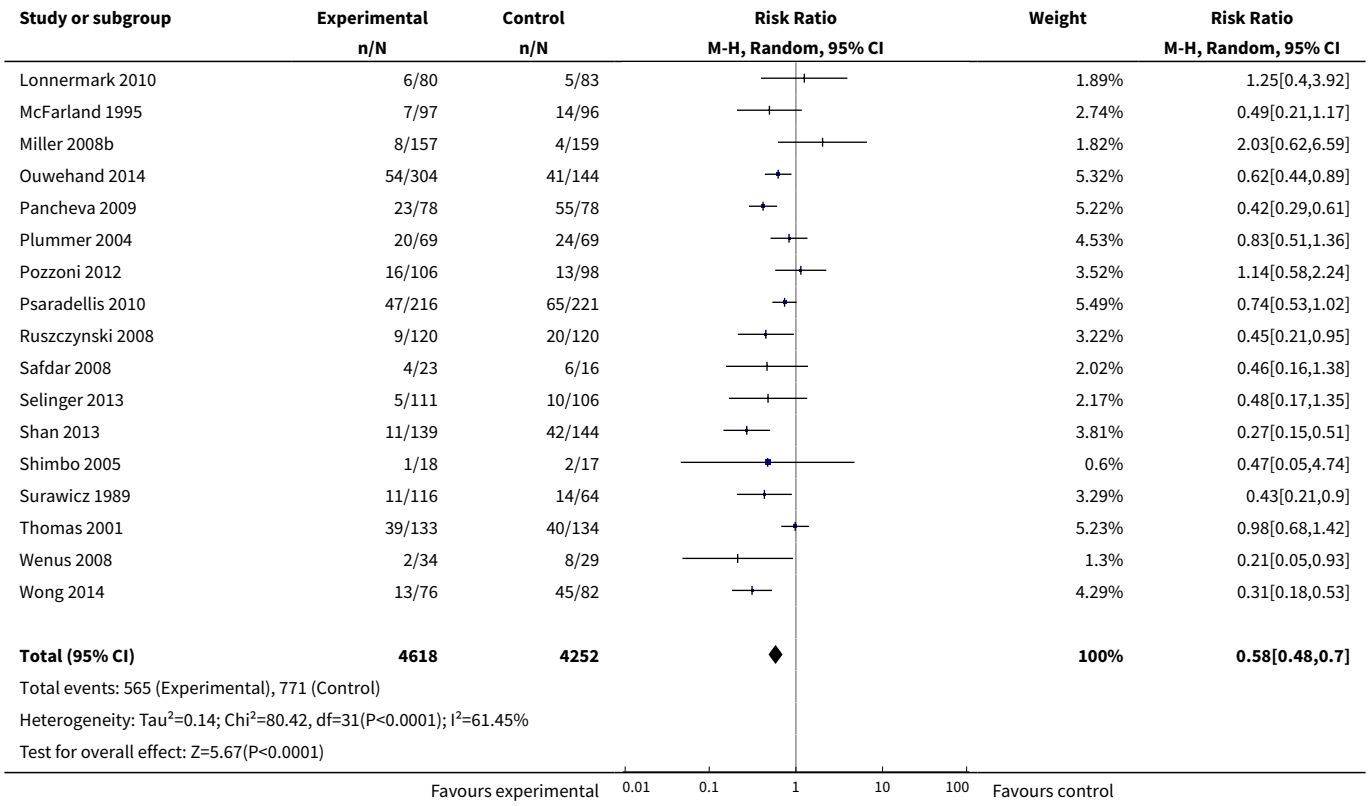
Study or subgroup	Experimental n/N	Control n/N	Risk Ratio M-H, Random, 95% CI	Weight	Risk Ratio M-H, Random, 95% CI
Selinger 2013	14/117	16/112		3.95%	0.84[0.43,1.63]
Shimbo 2005	5/18	14/17		3.15%	0.34[0.16,0.73]
Siitonen 1990	2/8	3/8		1%	0.67[0.15,2.98]
Sullivan 2004	0/18	0/18			Not estimable
Surawicz 1989	0/116	0/64			Not estimable
Thomas 2001	37/133	52/134		8.52%	0.72[0.51,1.01]
Wong 2014	0/76	0/82			Not estimable
Subtotal (95% CI)	**3890**	**3527**		**100%**	**0.83[0.71,0.97]**
Total events: 620 (Experimental), 677 (Control)					
Heterogeneity: Tau^2=0.04; Chi^2=41.48, df=21(P=0); I^2=49.38%					
Test for overall effect: Z=2.3(P=0.02)					
1.34.2 Children					
Arvola 1999	0/61	0/58			Not estimable
Kotowska 2005	0/119	0/127			Not estimable
Ruszczynski 2008	0/120	0/120			Not estimable
Shan 2013	0/139	0/144			Not estimable
Subtotal (95% CI)	**439**	**449**			**Not estimable**
Total events: 0 (Experimental), 0 (Control)					
Heterogeneity: Not applicable					
Test for overall effect: Not applicable					
Total (95% CI)	**4329**	**3976**		**100%**	**0.83[0.71,0.97]**
Total events: 620 (Experimental), 677 (Control)					
Heterogeneity: Tau^2=0.04; Chi^2=41.48, df=21(P=0); I^2=49.38%					
Test for overall effect: Z=2.3(P=0.02)					
Test for subgroup differences: Not applicable					



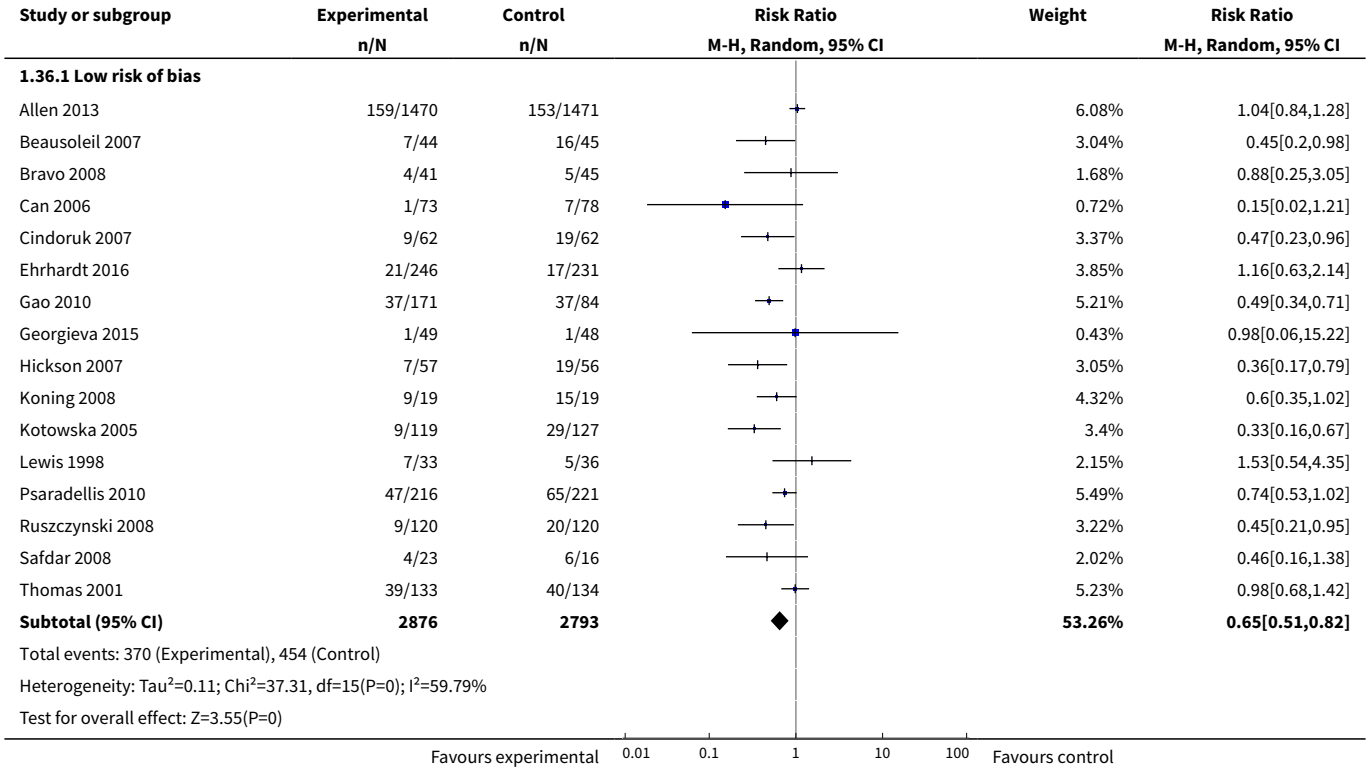
Analysis 1.35. Comparison 1 Probiotics versus control, Outcome 35 Incidence AAD: complete case.

Study or subgroup	Experimental n/N	Control n/N	Risk Ratio M-H, Random, 95% CI	Weight	Risk Ratio M-H, Random, 95% CI
Allen 2013	159/1470	153/1471		6.08%	1.04[0.84,1.28]
Arvola 1999	3/61	9/58		1.66%	0.32[0.09,1.11]
Beausoleil 2007	7/44	16/45		3.04%	0.45[0.2,0.98]
Bravo 2008	4/41	5/45		1.68%	0.88[0.25,3.05]
Can 2006	1/73	7/78		0.72%	0.15[0.02,1.21]
Cindoruk 2007	9/62	19/62		3.37%	0.47[0.23,0.96]
Duman 2005	14/204	28/185		3.87%	0.45[0.25,0.83]
Ehrhardt 2016	21/246	17/231		3.85%	1.16[0.63,2.14]
Fominykh 2013	0/80	0/40			Not estimable
Gao 2010	37/171	37/84		5.21%	0.49[0.34,0.71]
Georgieva 2015	1/49	1/48		0.43%	0.98[0.06,15.22]
Hickson 2007	7/57	19/56		3.05%	0.36[0.17,0.79]
Imase 2008	1/12	3/7		0.73%	0.19[0.02,1.53]
Koning 2008	9/19	15/19		4.32%	0.6[0.35,1.02]
Kotowska 2005	9/119	29/127		3.4%	0.33[0.16,0.67]
Lewis 1998	7/33	5/36		2.15%	1.53[0.54,4.35]

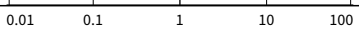




Analysis 1.36. Comparison 1 Probiotics versus control, Outcome 36 Incidence AAD: Subgroup: Risk of Bias.



Study or subgroup	Experimental n/N	Control n/N	Risk Ratio M-H, Random, 95% CI	Weight	Risk Ratio M-H, Random, 95% CI
1.36.2 High or Unclear risk of bias					
Arvola 1999	3/61	9/58		1.66%	0.32[0.09,1.11]
Duman 2005	14/204	28/185		3.87%	0.45[0.25,0.83]
Fominykh 2013	0/80	0/40			Not estimable
Imase 2008	1/12	3/7		0.73%	0.19[0.02,1.53]
Lonnermark 2010	6/80	5/83		1.89%	1.25[0.4,3.92]
McFarland 1995	7/97	14/96		2.74%	0.49[0.21,1.17]
Miller 2008b	8/157	4/159		1.82%	2.03[0.62,6.59]
Ouwehand 2014	54/304	41/144		5.32%	0.62[0.44,0.89]
Pancheva 2009	23/78	55/78		5.22%	0.42[0.29,0.61]
Plummer 2004	20/69	24/69		4.53%	0.83[0.51,1.36]
Pozzoni 2012	16/106	13/98		3.52%	1.14[0.58,2.24]
Selinger 2013	5/111	10/106		2.17%	0.48[0.17,1.35]
Shan 2013	11/139	42/144		3.81%	0.27[0.15,0.51]
Shimbo 2005	1/18	2/17		0.6%	0.47[0.05,4.74]
Surawicz 1989	11/116	14/64		3.29%	0.43[0.21,0.9]
Wenus 2008	2/34	8/29		1.3%	0.21[0.05,0.93]
Wong 2014	13/76	45/82		4.29%	0.31[0.18,0.53]
Subtotal (95% CI)	**1742**	**1459**		**46.74%**	**0.52[0.4,0.67]**
Total events: 195 (Experimental), 317 (Control)					
Heterogeneity: Tau^2=0.11; Chi^2=29.5, df=15(P=0.01); I^2=49.15%					
Test for overall effect: Z=4.94(P<0.0001)					
Total (95% CI)	**4618**	**4252**		**100%**	**0.58[0.48,0.7]**
Total events: 565 (Experimental), 771 (Control)					
Heterogeneity: Tau^2=0.14; Chi^2=80.42, df=31(P<0.0001); I^2=61.45%					
Test for overall effect: Z=5.67(P<0.0001)					
Test for subgroup differences: Chi^2=1.54, df=1 (P=0.21), I^2=35.01%					

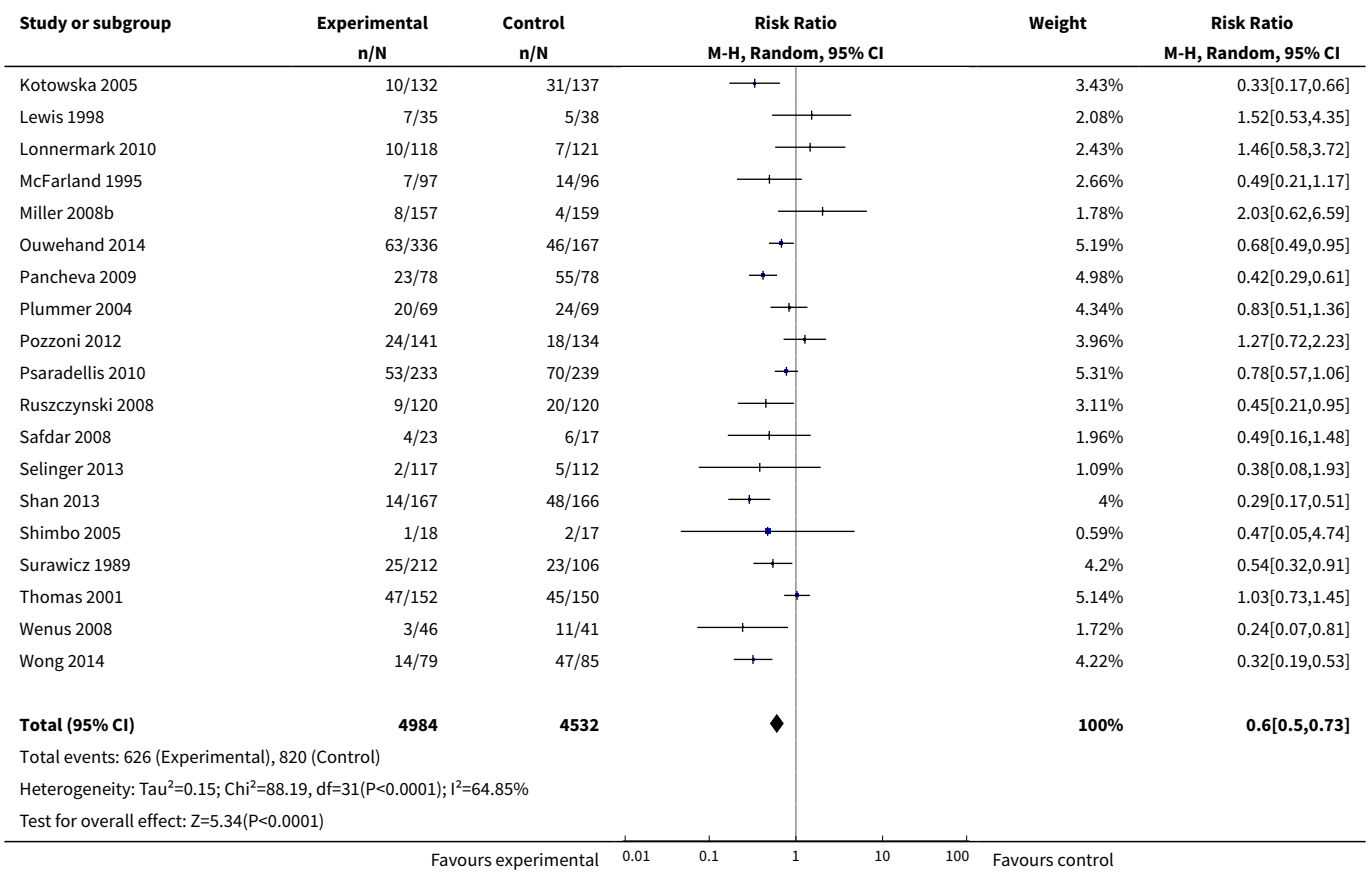


Favours experimental | Favours control

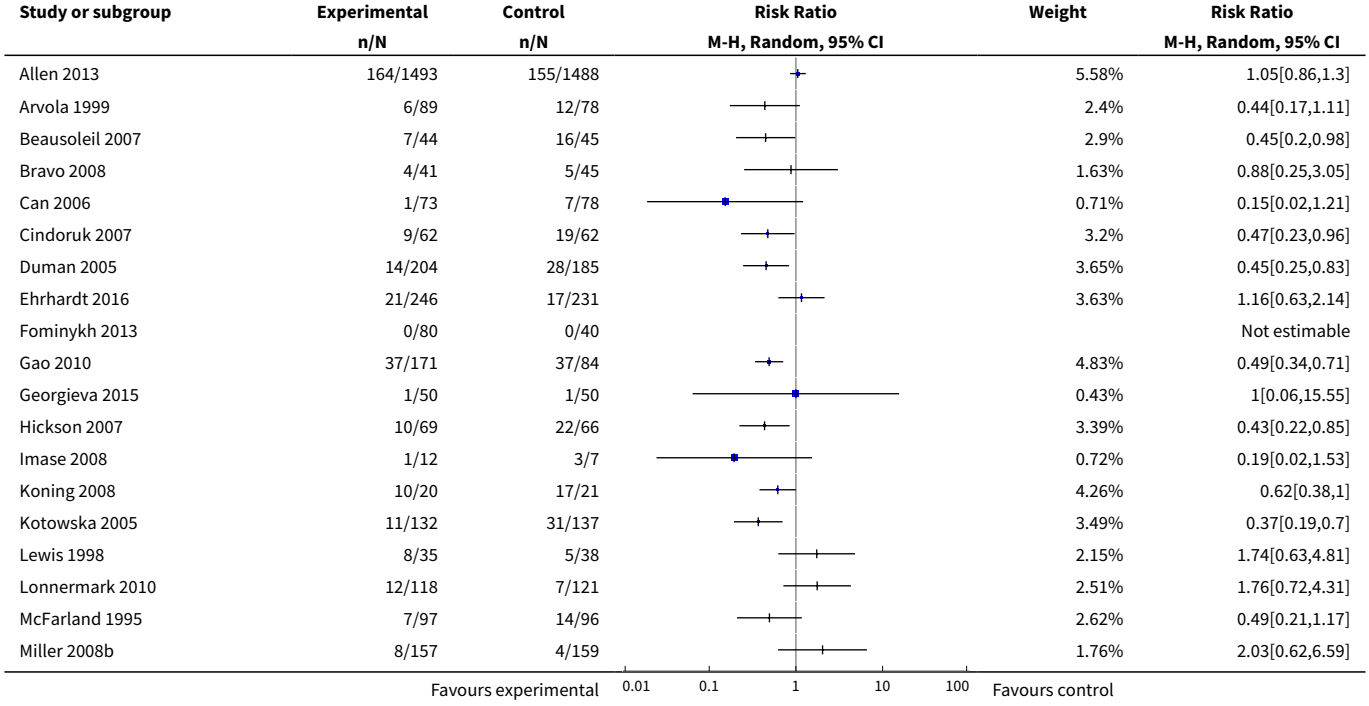
Analysis 1.37. Comparison 1 Probiotics versus control, Outcome 37 Incidence AAD: sensitivity (1.5:1).

Study or subgroup	Experimental n/N	Control n/N	Risk Ratio M-H, Random, 95% CI	Weight	Risk Ratio M-H, Random, 95% CI
Allen 2013	163/1493	155/1488		5.76%	1.05[0.85,1.29]
Arvola 1999	5/89	12/78		2.23%	0.37[0.13,0.99]
Beausoleil 2007	7/44	16/45		2.95%	0.45[0.2,0.98]
Bravo 2008	4/41	5/45		1.64%	0.88[0.25,3.05]
Can 2006	1/73	7/78		0.72%	0.15[0.02,1.21]
Cindoruk 2007	9/62	19/62		3.26%	0.47[0.23,0.96]
Duman 2005	14/204	28/185		3.73%	0.45[0.25,0.83]
Ehrhardt 2016	21/246	17/231		3.71%	1.16[0.63,2.14]
Fominykh 2013	0/80	0/40			Not estimable
Gao 2010	37/171	37/84		4.97%	0.49[0.34,0.71]
Georgieva 2015	1/50	1/50		0.43%	1[0.06,15.55]
Hickson 2007	9/69	22/66		3.31%	0.39[0.19,0.79]
Imase 2008	1/12	3/7		0.72%	0.19[0.02,1.53]
Koning 2008	10/20	17/21		4.37%	0.62[0.38,1]

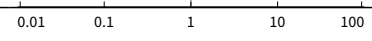
Favours experimental | Favours control



Analysis 1.38. Comparison 1 Probiotics versus control, Outcome 38 Incidence AAD: sensitivity (2:1).



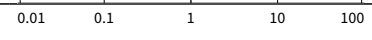
Study or subgroup	Experimental n/N	Control n/N	Risk Ratio M-H, Random, 95% CI	Weight	Risk Ratio M-H, Random, 95% CI
Ouwehand 2014	65/336	46/167		5.05%	0.7[0.51,0.98]
Pancheva 2009	23/78	55/78		4.84%	0.42[0.29,0.61]
Plummer 2004	20/69	24/69		4.23%	0.83[0.51,1.36]
Pozzoni 2012	27/141	18/134		3.95%	1.43[0.82,2.46]
Psaradellis 2010	54/233	70/239		5.16%	0.79[0.58,1.07]
Ruszczynski 2008	9/120	20/120		3.06%	0.45[0.21,0.95]
Safdar 2008	4/23	6/17		1.94%	0.49[0.16,1.48]
Selinger 2013	6/117	11/112		2.31%	0.52[0.2,1.36]
Shan 2013	15/167	48/166		3.99%	0.31[0.18,0.53]
Shimbo 2005	1/18	2/17		0.59%	0.47[0.05,4.74]
Surawicz 1989	29/212	23/106		4.21%	0.63[0.38,1.03]
Thomas 2001	50/152	45/150		5.02%	1.1[0.79,1.53]
Wenus 2008	3/46	11/41		1.71%	0.24[0.07,0.81]
Wong 2014	14/79	47/85		4.12%	0.32[0.19,0.53]
Total (95% CI)	**4984**	**4532**		**100%**	**0.63[0.52,0.75]**
Total events: 651 (Experimental), 826 (Control)					
Heterogeneity: Tau^2=0.15; Chi^2=91.91, df=31(P<0.0001); I^2=66.27%					
Test for overall effect: Z=4.93(P<0.0001)					



Favours experimental — Favours control

Analysis 1.39. Comparison 1 Probiotics versus control, Outcome 39 Incidence AAD: sensitivity (3:1).

Study or subgroup	Experimental n/N	Control n/N	Risk Ratio M-H, Random, 95% CI	Weight	Risk Ratio M-H, Random, 95% CI
Allen 2013	166/1493	155/1488		5.39%	1.07[0.87,1.31]
Arvola 1999	7/89	12/78		2.55%	0.51[0.21,1.23]
Beausoleil 2007	7/44	16/45		2.89%	0.45[0.2,0.98]
Bravo 2008	4/41	5/45		1.64%	0.88[0.25,3.05]
Can 2006	1/73	7/78		0.73%	0.15[0.02,1.21]
Cindoruk 2007	9/62	19/62		3.17%	0.47[0.23,0.96]
Duman 2005	14/204	28/185		3.6%	0.45[0.25,0.83]
Ehrhardt 2016	21/246	17/231		3.58%	1.16[0.63,2.14]
Fominykh 2013	0/80	0/40			Not estimable
Gao 2010	37/171	37/84		4.7%	0.49[0.34,0.71]
Georgieva 2015	1/50	1/50		0.44%	1[0.06,15.55]
Hickson 2007	11/69	22/66		3.47%	0.48[0.25,0.91]
Imase 2008	1/12	3/7		0.73%	0.19[0.02,1.53]
Koning 2008	10/20	17/21		4.18%	0.62[0.38,1]
Kotowska 2005	12/132	31/137		3.54%	0.4[0.22,0.75]
Lewis 1998	8/35	5/38		2.15%	1.74[0.63,4.81]
Lonnermark 2010	15/118	7/121		2.62%	2.2[0.93,5.2]
McFarland 1995	7/97	14/96		2.62%	0.49[0.21,1.17]
Miller 2008b	8/157	4/159		1.77%	2.03[0.62,6.59]
Ouwehand 2014	71/336	46/167		4.94%	0.77[0.56,1.06]
Pancheva 2009	23/78	55/78		4.71%	0.42[0.29,0.61]
Plummer 2004	20/69	24/69		4.15%	0.83[0.51,1.36]
Pozzoni 2012	32/141	18/134		3.98%	1.69[1,2.86]
Psaradellis 2010	58/233	70/239		5.04%	0.85[0.63,1.14]



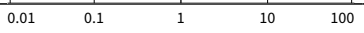
Favours experimental — Favours control

Study or subgroup	Experimental n/N	Control n/N	Risk Ratio M-H, Random, 95% CI	Weight	Risk Ratio M-H, Random, 95% CI
Ruszczynski 2008	9/120	20/120		3.04%	0.45[0.21,0.95]
Safdar 2008	4/23	6/17		1.95%	0.49[0.16,1.48]
Selinger 2013	6/117	11/112		2.31%	0.52[0.2,1.36]
Shan 2013	18/167	48/166		4.12%	0.37[0.23,0.61]
Shimbo 2005	1/18	2/17		0.6%	0.47[0.05,4.74]
Surawicz 1989	38/212	23/106		4.28%	0.83[0.52,1.31]
Thomas 2001	56/152	45/150		4.94%	1.23[0.89,1.69]
Wenus 2008	4/46	11/41		2.03%	0.32[0.11,0.94]
Wong 2014	15/79	47/85		4.13%	0.34[0.21,0.56]
Total (95% CI)	**4984**	**4532**		**100%**	**0.66[0.55,0.8]**
Total events: 694 (Experimental), 826 (Control)					
Heterogeneity: Tau^2=0.16; Chi^2=98.6, df=31(P<0.0001); I^2=68.56%					
Test for overall effect: Z=4.25(P<0.0001)					

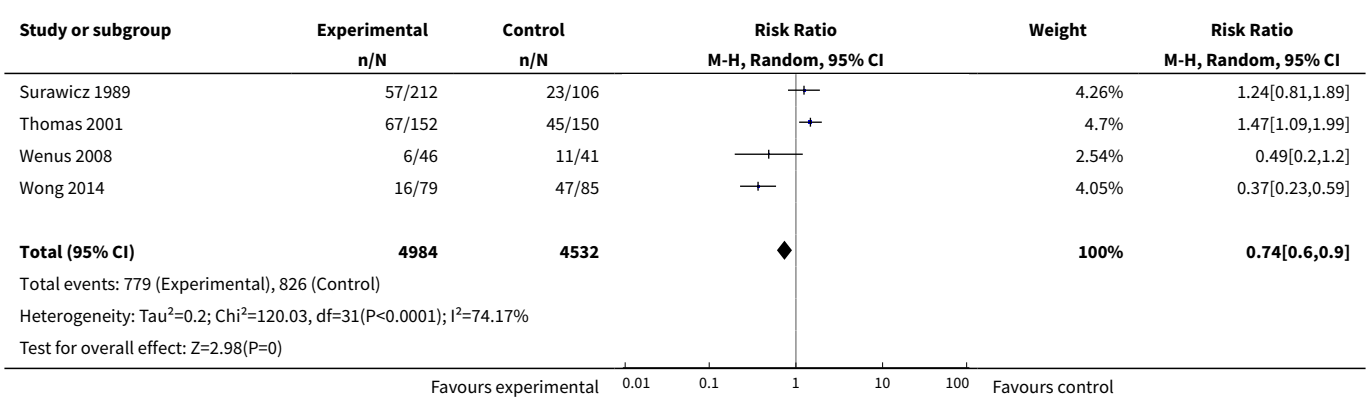
Favours experimental — Favours control

Analysis 1.40. Comparison 1 Probiotics versus control, Outcome 40 Incidence AAD: sensitivity (5:1).

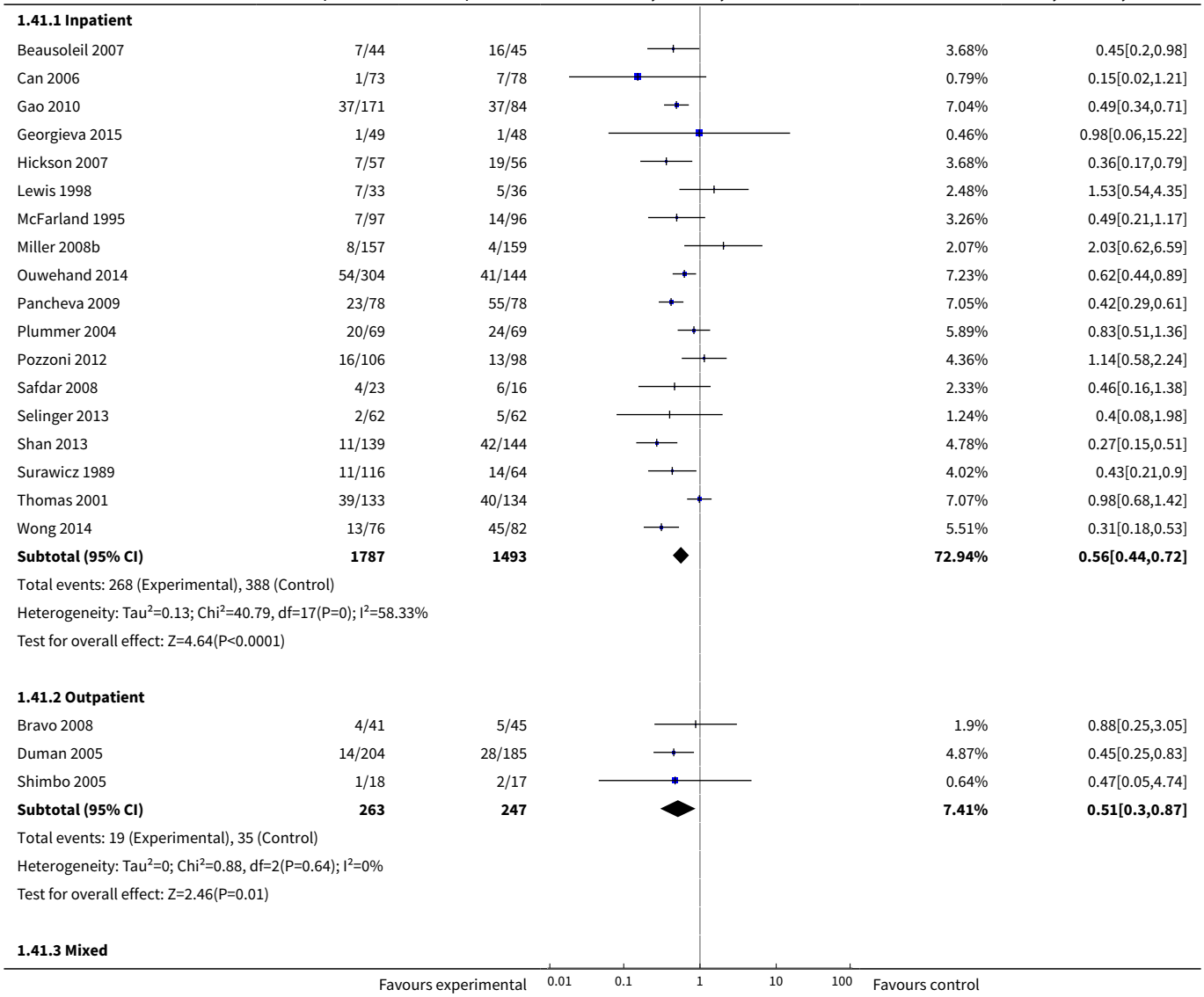
Study or subgroup	Experimental n/N	Control n/N	Risk Ratio M-H, Random, 95% CI	Weight	Risk Ratio M-H, Random, 95% CI
Allen 2013	171/1493	155/1488		4.99%	1.1[0.9,1.35]
Arvola 1999	10/89	12/78		2.92%	0.73[0.33,1.6]
Beausoleil 2007	7/44	16/45		2.91%	0.45[0.2,0.98]
Bravo 2008	4/41	5/45		1.73%	0.88[0.25,3.05]
Can 2006	1/73	7/78		0.79%	0.15[0.02,1.21]
Cindoruk 2007	9/62	19/62		3.16%	0.47[0.23,0.96]
Duman 2005	14/204	28/185		3.54%	0.45[0.25,0.83]
Ehrhardt 2016	21/246	17/231		3.52%	1.16[0.63,2.14]
Fominykh 2013	0/80	0/40			Not estimable
Gao 2010	37/171	37/84		4.45%	0.49[0.34,0.71]
Georgieva 2015	1/50	1/50		0.48%	1[0.06,15.55]
Hickson 2007	14/69	22/66		3.65%	0.61[0.34,1.09]
Imase 2008	1/12	3/7		0.8%	0.19[0.02,1.53]
Koning 2008	11/20	17/21		4.17%	0.68[0.43,1.06]
Kotowska 2005	14/132	31/137		3.63%	0.47[0.26,0.84]
Lewis 1998	9/35	5/38		2.29%	1.95[0.72,5.27]
Lonnermark 2010	20/118	7/121		2.78%	2.93[1.29,6.67]
McFarland 1995	7/97	14/96		2.66%	0.49[0.21,1.17]
Miller 2008b	8/157	4/159		1.86%	2.03[0.62,6.59]
Ouwehand 2014	82/336	46/167		4.68%	0.89[0.65,1.21]
Pancheva 2009	23/78	55/78		4.46%	0.42[0.29,0.61]
Plummer 2004	20/69	24/69		4%	0.83[0.51,1.36]
Pozzoni 2012	42/141	18/134		3.97%	2.22[1.35,3.65]
Psaradellis 2010	65/233	70/239		4.76%	0.95[0.72,1.27]
Ruszczynski 2008	9/120	20/120		3.04%	0.45[0.21,0.95]
Safdar 2008	4/23	6/17		2.03%	0.49[0.16,1.48]
Selinger 2013	6/117	11/112		2.38%	0.52[0.2,1.36]
Shan 2013	22/167	48/166		4.13%	0.46[0.29,0.72]
Shimbo 2005	1/18	2/17		0.66%	0.47[0.05,4.74]

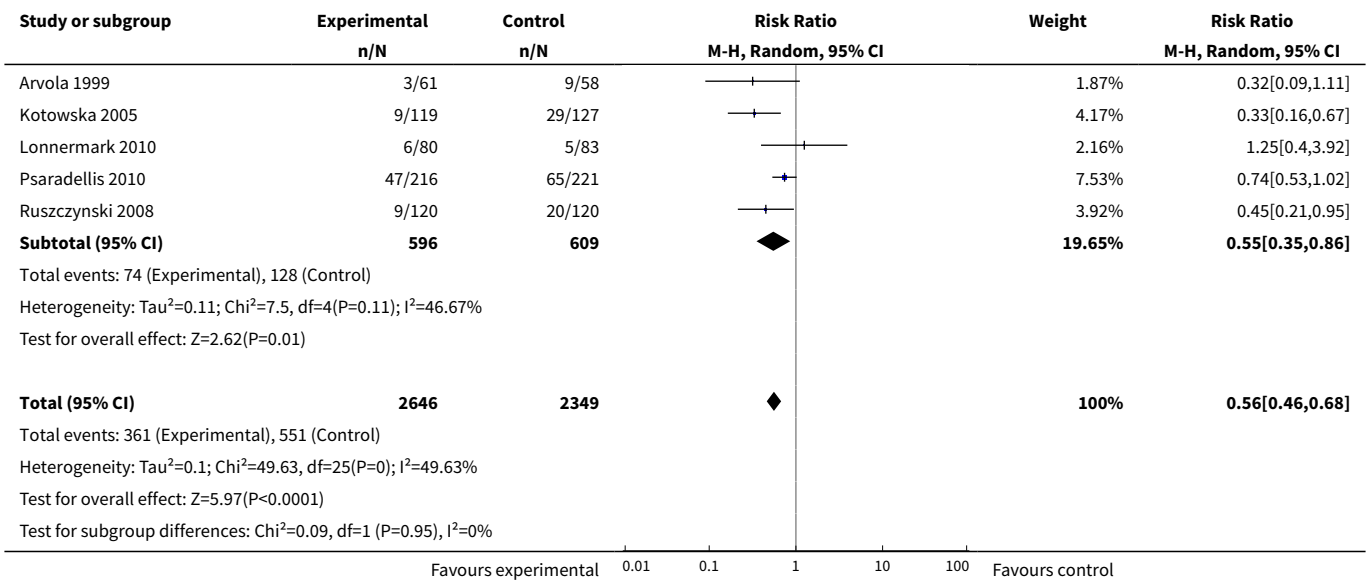


Favours experimental — Favours control

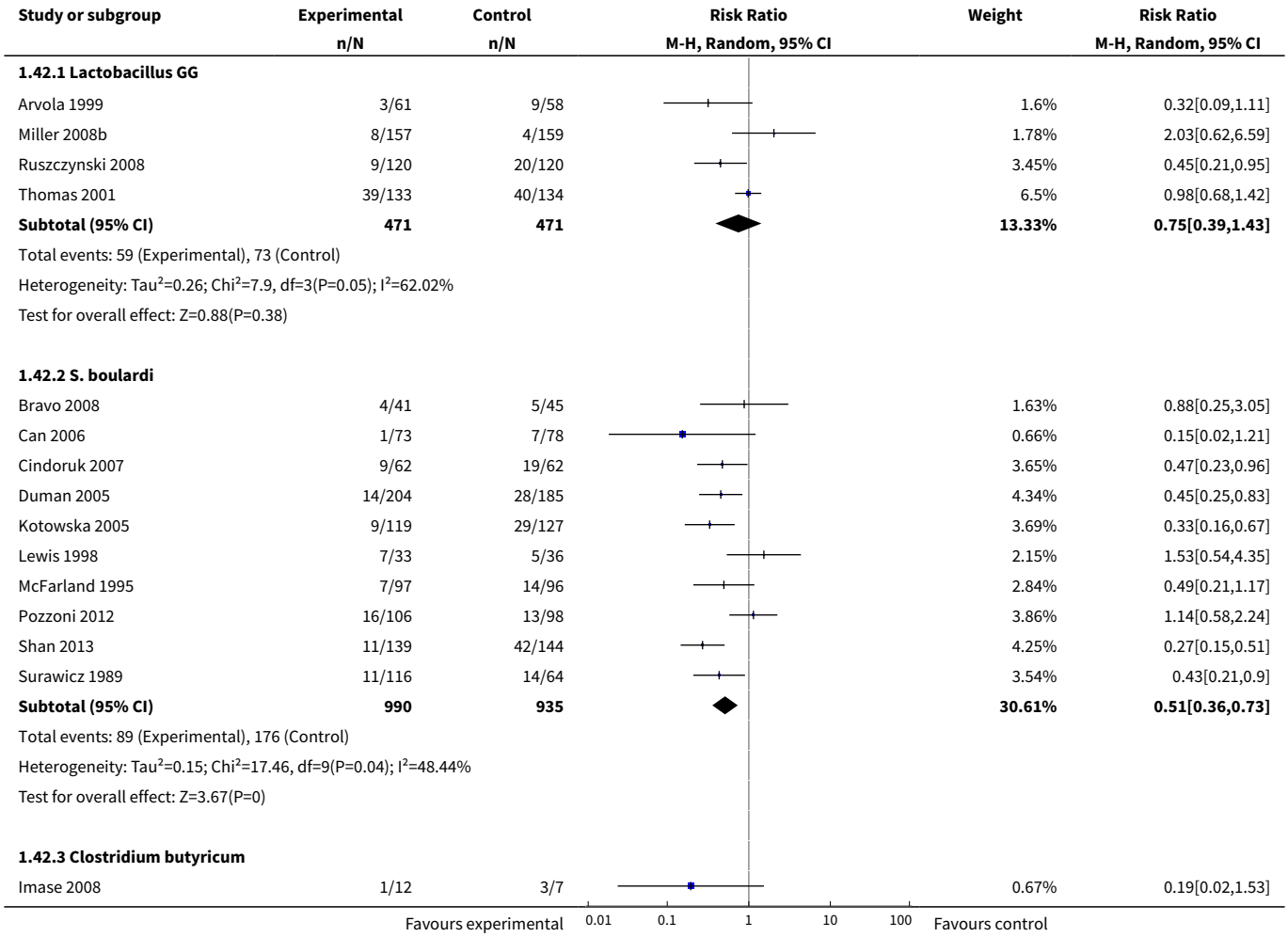


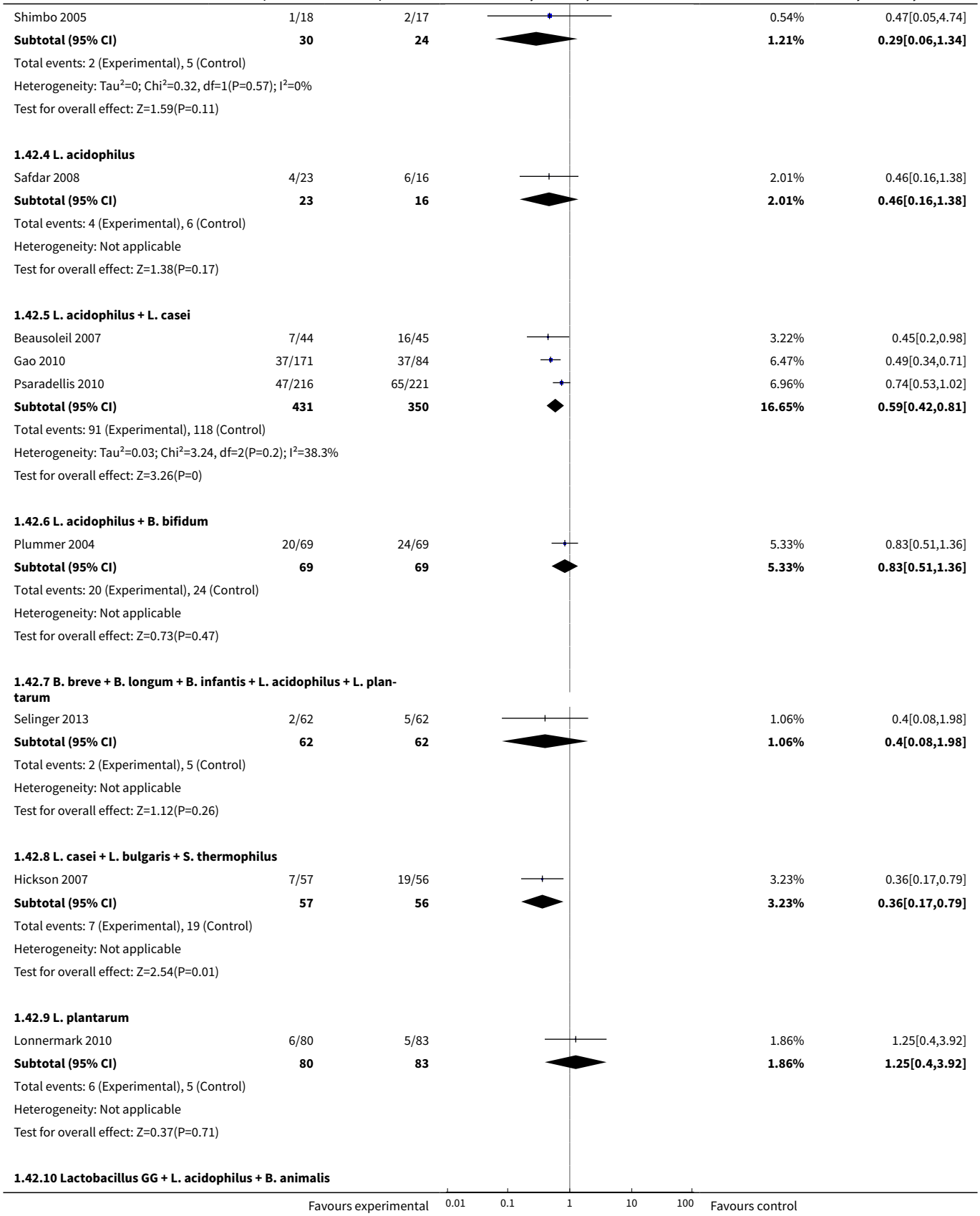
Analysis 1.41. Comparison 1 Probiotics versus control, Outcome 41 Incidence AAD: Patient population.

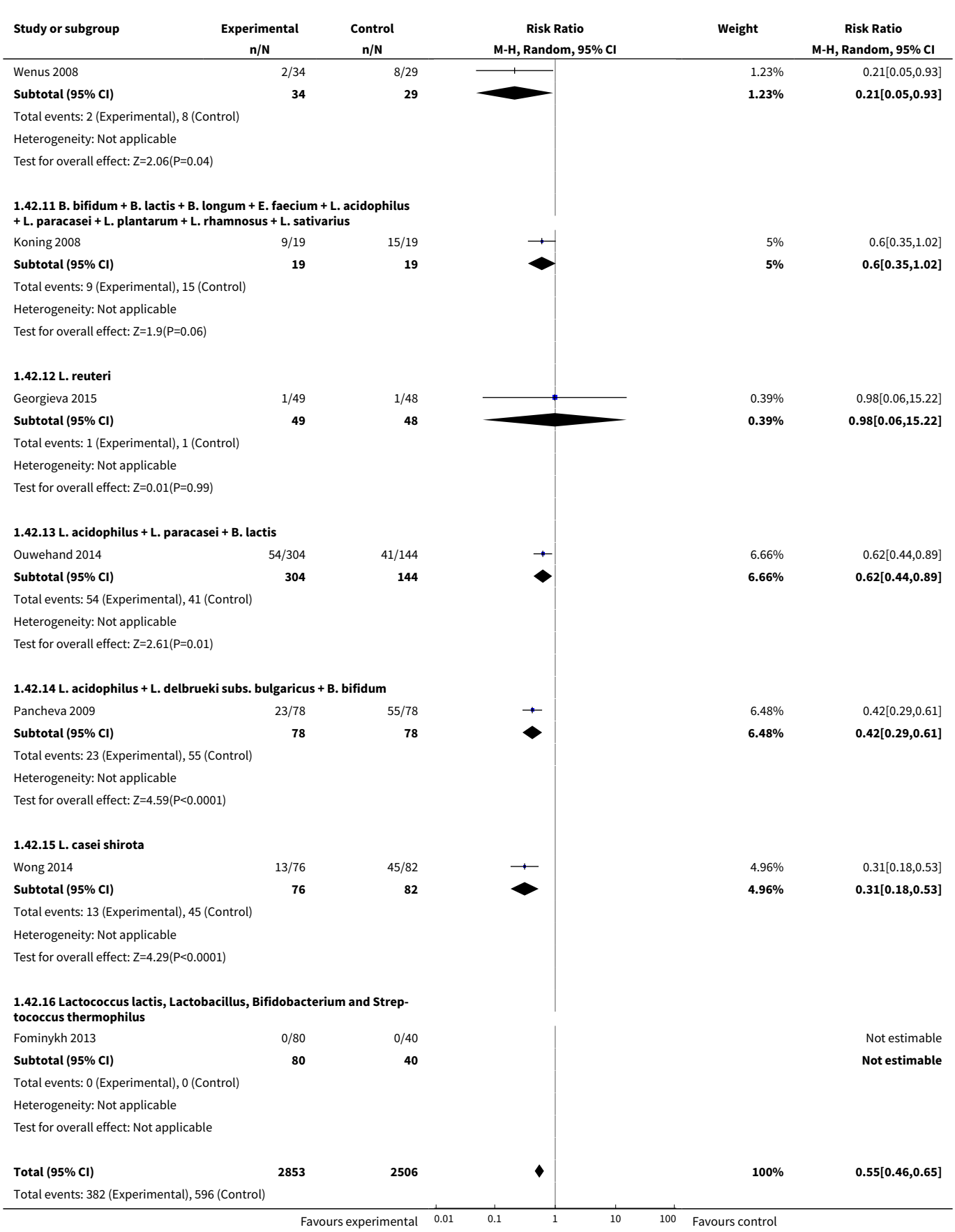




Analysis 1.42. Comparison 1 Probiotics versus control, Outcome 42 Incidence AAD: Species: all.







Study or subgroup	Experimental n/N	Control n/N	Risk Ratio M-H, Random, 95% CI	Weight	Risk Ratio M-H, Random, 95% CI
Heterogeneity: Tau²=0.09; Chi²=52.78, df=29(P=0); I²=45.06%					
Test for overall effect: Z=6.77(P<0.0001)					
Test for subgroup differences: Chi²=16.58, df=1 (P=0.28), I²=15.58%					

Favours experimental 0.01 0.1 1 10 100 Favours control

Analysis 1.43. Comparison 1 Probiotics versus control, Outcome 43 Incidence AAD: Species: LGG versus SB.

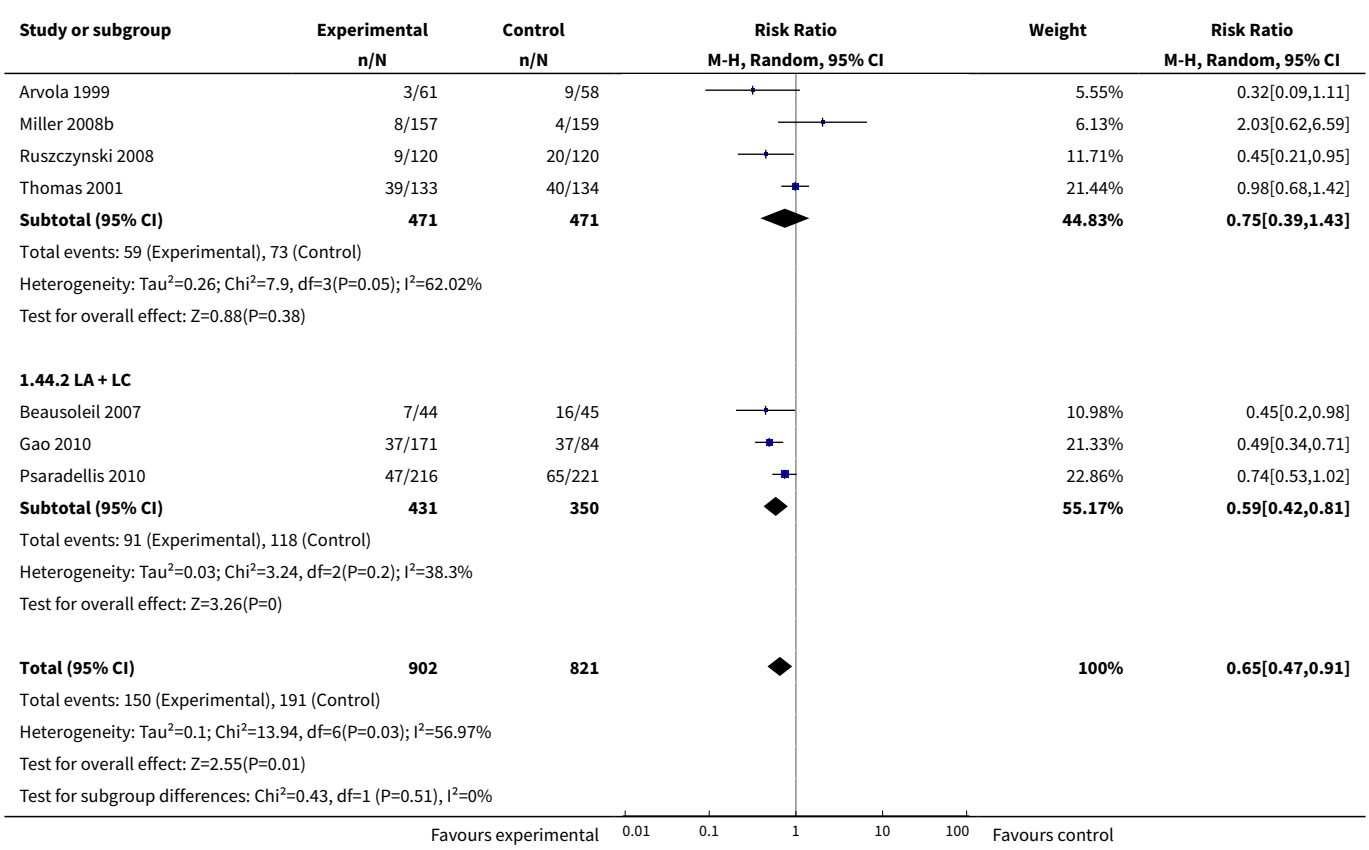
Study or subgroup	Experimental n/N	Control n/N	Risk Ratio M-H, Random, 95% CI	Weight	Risk Ratio M-H, Random, 95% CI
1.43.1 LGG					
Arvola 1999	3/61	9/58		4.58%	0.32[0.09,1.11]
Miller 2008b	8/157	4/159		4.97%	2.03[0.62,6.59]
Ruszczynski 2008	9/120	20/120		7.97%	0.45[0.21,0.95]
Thomas 2001	39/133	40/134		11.42%	0.98[0.68,1.42]
Subtotal (95% CI)	**471**	**471**		**28.94%**	**0.75[0.39,1.43]**
Total events: 59 (Experimental), 73 (Control)					
Heterogeneity: Tau²=0.26; Chi²=7.9, df=3(P=0.05); I²=62.02%					
Test for overall effect: Z=0.88(P=0.38)					
1.43.2 SB					
Bravo 2008	4/41	5/45		4.64%	0.88[0.25,3.05]
Can 2006	1/73	7/78		2.16%	0.15[0.02,1.21]
Cindoruk 2007	9/62	19/62		8.27%	0.47[0.23,0.96]
Duman 2005	14/204	28/185		9.18%	0.45[0.25,0.83]
Kotowska 2005	9/119	29/127		8.32%	0.33[0.16,0.67]
Lewis 1998	7/33	5/36		5.74%	1.53[0.54,4.35]
McFarland 1995	7/97	14/96		7.01%	0.49[0.21,1.17]
Pozzoni 2012	16/106	13/98		8.56%	1.14[0.58,2.24]
Shan 2013	11/139	42/144		9.07%	0.27[0.15,0.51]
Surawicz 1989	11/116	14/64		8.11%	0.43[0.21,0.9]
Subtotal (95% CI)	**990**	**935**		**71.06%**	**0.51[0.36,0.73]**
Total events: 89 (Experimental), 176 (Control)					
Heterogeneity: Tau²=0.15; Chi²=17.46, df=9(P=0.04); I²=48.44%					
Test for overall effect: Z=3.67(P=0)					
Total (95% CI)	**1461**	**1406**		**100%**	**0.57[0.41,0.8]**
Total events: 148 (Experimental), 249 (Control)					
Heterogeneity: Tau²=0.22; Chi²=32.54, df=13(P=0); I²=60.05%					
Test for overall effect: Z=3.28(P=0)					
Test for subgroup differences: Chi²=1, df=1 (P=0.32), I²=0%					

Favours experimental 0.01 0.1 1 10 100 Favours control

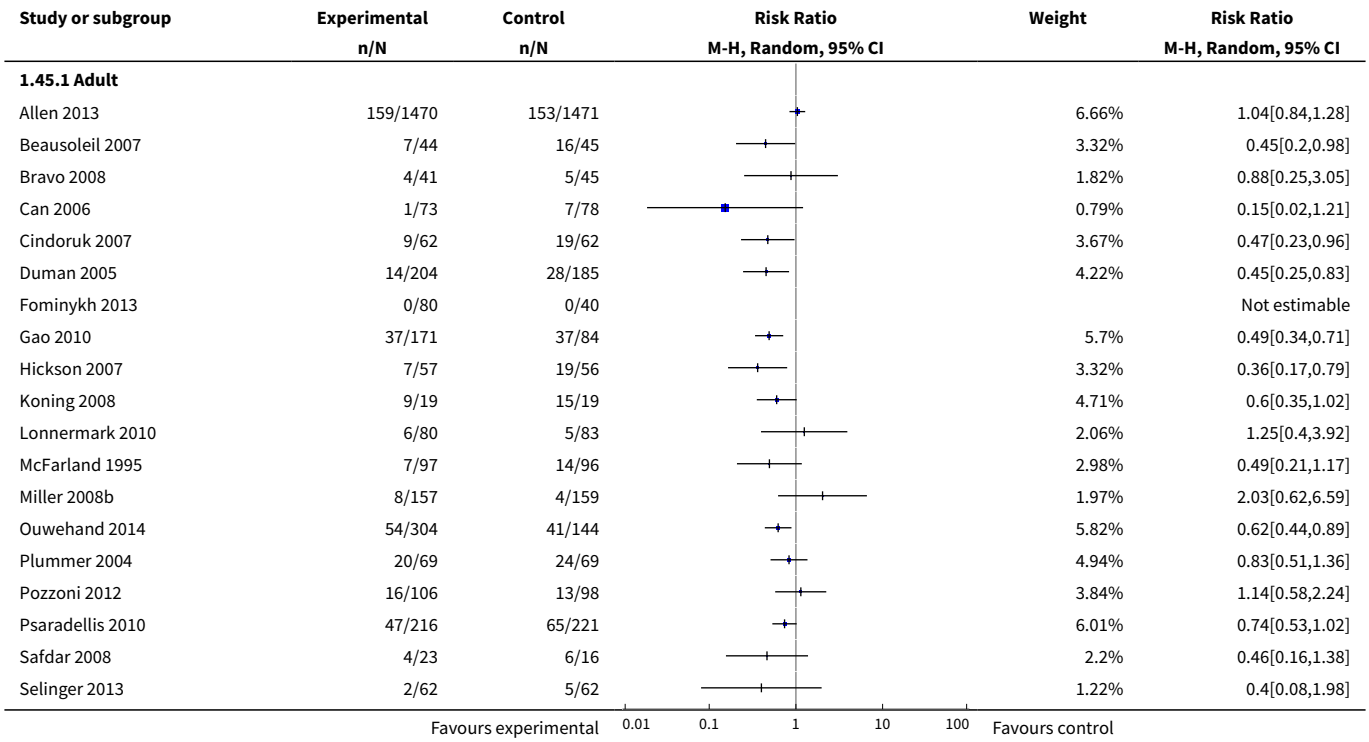
Analysis 1.44. Comparison 1 Probiotics versus control, Outcome 44 Incidence AAD: Species: LGG versus LA + LC.

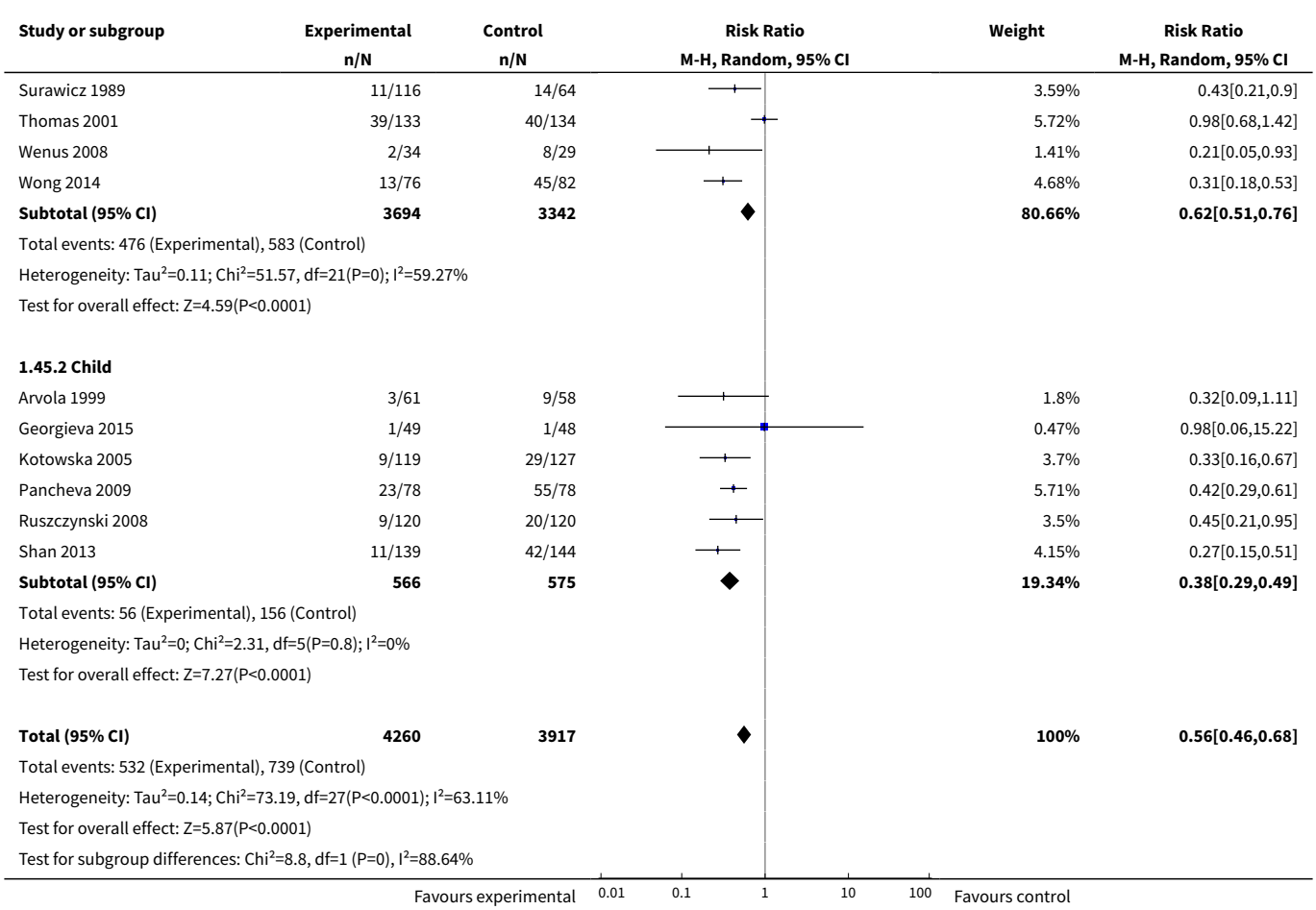
Study or subgroup	Experimental n/N	Control n/N	Risk Ratio M-H, Random, 95% CI	Weight	Risk Ratio M-H, Random, 95% CI
1.44.1 LGG					

Favours experimental 0.01 0.1 1 10 100 Favours control

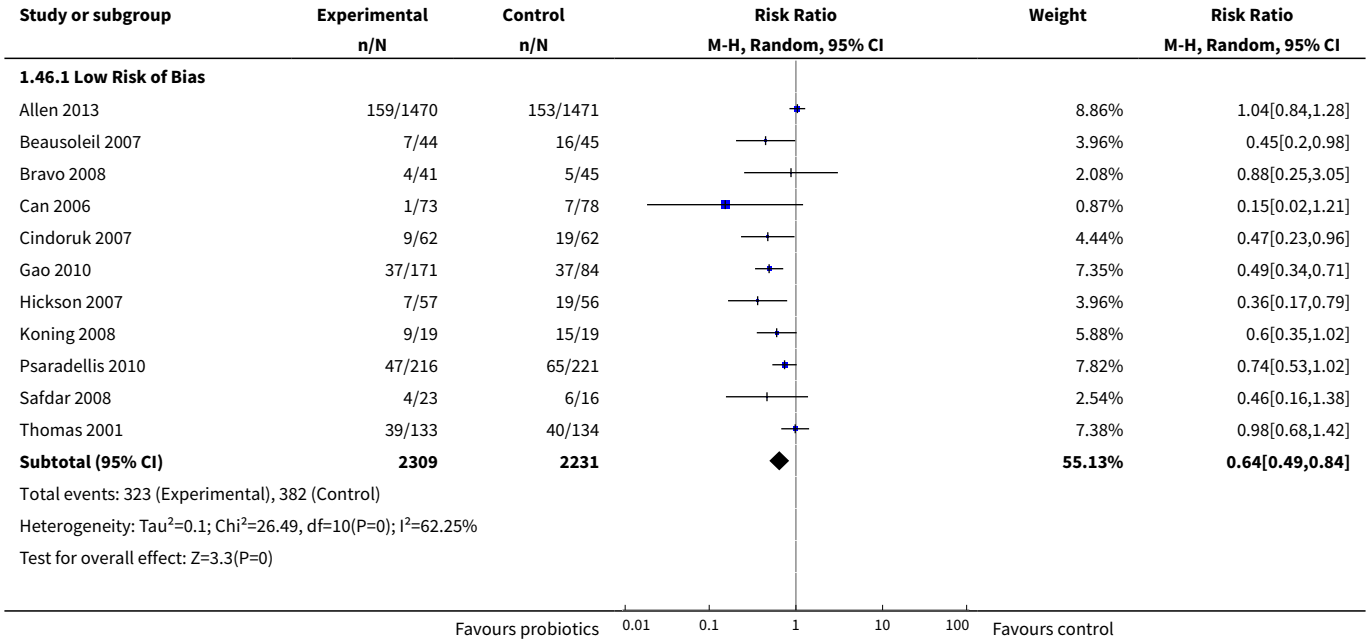


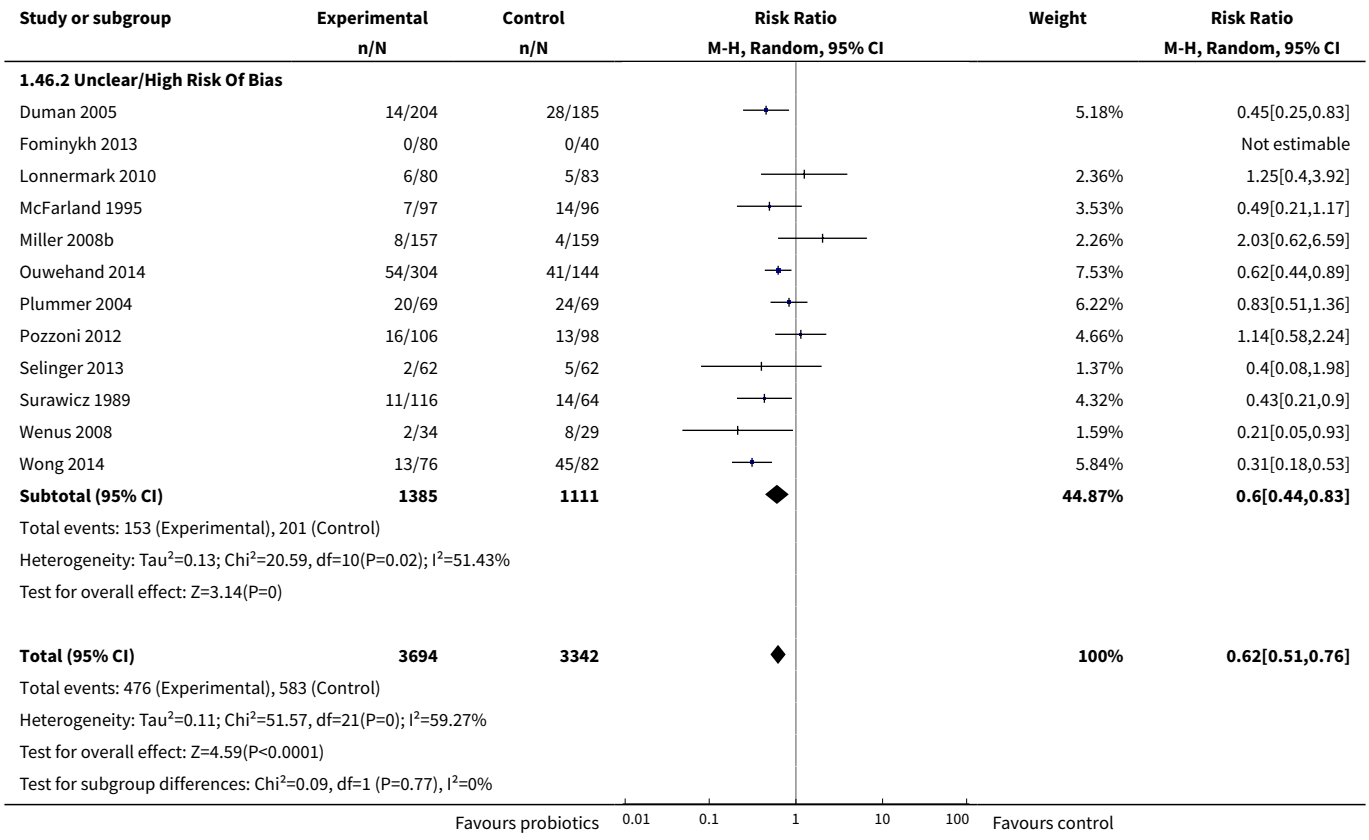
Analysis 1.45. Comparison 1 Probiotics versus control, Outcome 45 Incidence AAD: Adult versus child.



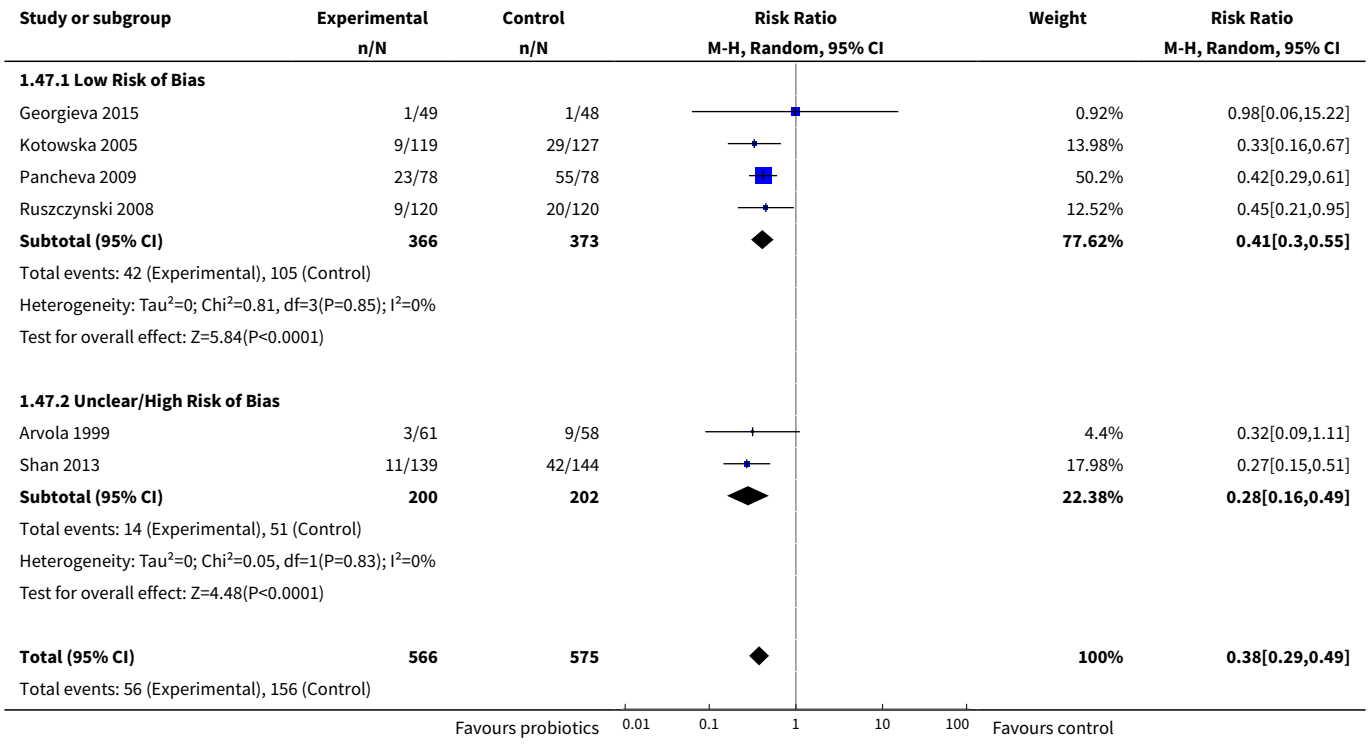


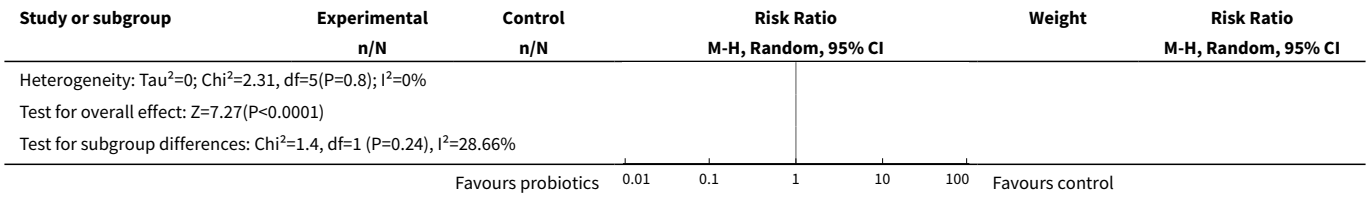
Analysis 1.46. Comparison 1 Probiotics versus control, Outcome 46 Incidence AAD: Adult (RoB).



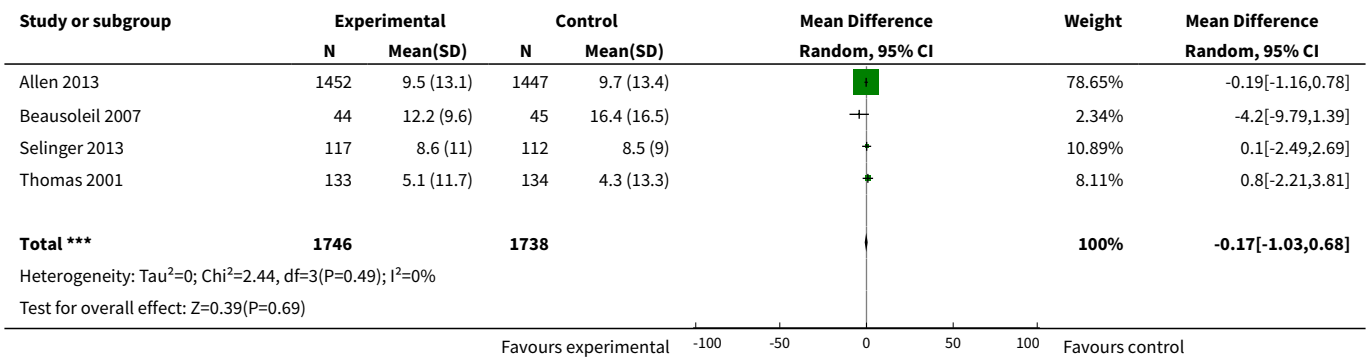


Analysis 1.47. Comparison 1 Probiotics versus control, Outcome 47 Incidence AAD: Child (RoB).





Analysis 1.48. Comparison 1 Probiotics versus control, Outcome 48 Length of Hospital Stay: complete case.



APPENDICES

Appendix 1. Search strategies

EMBASE

1. random$.mp.

2. factorial$.mp.

3. (crossover$ or cross over$ or cross-over$).mp.

4. placebo$.mp.

5. single blind.mp.

6. double blind.mp.

7. triple blind.mp.

8. (singl$ adj blind$).mp.

9. (double$ adj blind$).mp.

10. (tripl$ adj blind$).mp.

11. assign$.mp.

12. allocat$.mp.

13. crossover procedure/

14. double blind procedure/

15. single blind procedure/

16. triple blind procedure/

17. randomized controlled trial/

18. or/1-17

19. (exp animal/ or animal.hw. or nonhuman/) not (exp human/ or human cell/ or (human or humans).ti.)

20. 18 not 19

21. exp Probiotics/

22. exp Synbiotics/

23. probiotic*.mp.

24. synbiotic*.mp.

25. exp Lactobacillus/

26. lactobacill*.mp.

27. exp Bifidobacterium/

28. (bifidus or bifidobacter*).mp.

29. exp Streptococcus thermophilus/

30. streptococcus thermophilus.mp.

31. streptococc*.mp.

32. exp Lactococcus/

33. lactococc*.mp.

34. Bacillus subtilis/

35. bacillus subtilis.mp.

36. exp Enterococcus/

37. enterococcus faec*.mp.

38. exp Saccharomyces/

39. saccharomyc*.mp.

40. leuconostoc.mp.

41. pediococc*.mp.

42. bulgarian bacillus.mp.

43. (beneficial adj3 bacter*).mp.dairy.mp.

44. dairy.mp.

45. yog?urt.mp.

46. kefir.mp.

47. clostridium.mp.

48. or/21-47

49. clostridium difficile.mp.

50. c diff.mp.

51. 49 or 50

52. (diarrhea or diarrhoe or diarhe or diarhoe or dystener* or gastroenteritis).mp.

53. 48 and 51 and 52

54. 53 and 20

PubMed

1. randomized controlled trial.pt.

2. controlled clinical trial.pt.

3. randomized.ab.

4. placebo.ab.

5. clinical trials as topic.sh.

6. randomly.ab.

7. trial.ti.

8. or/1-7

9. exp animals/ not humans.sh.

10. 8 not 9

11. exp Probiotics/

12. exp Synbiotics/

13. probiotic*.mp.

14. synbiotic*.mp.

15. exp Lactobacillus/

16. lactobacill*.mp.

17. exp Bifidobacterium/

18. (bifidus or bifidobacter*).mp.

19. exp Streptococcus thermophilus/

20. streptococcus thermophilus.mp.

21. streptococc*.mp.

22. exp Lactococcus/

23. lactococc*.mp.

24. Bacillus subtilis/

25. bacillus subtilis.mp.

26. exp Enterococcus/

27. enterococcus faec*.mp.

28. exp Saccharomyces/

29. saccharomyc*.mp.

30. leuconostoc.mp.

31. pediococc*.mp.

32. bulgarian bacillus.mp.

33. (beneficial adj3 bacter*).mp.dairy.mp.

34. dairy.mp.

35. yog?urt.mp.

36. kefir.mp.

37. clostridium.mp.

38. or/11-37

39. clostridium difficile.mp.

40. c diff.mp.

41. 39 or 40

42. (diarrhea or diarrhoe or diarhe or diarhoe or dystener* or gastroenteritis).mp.

43. 38 and 41 and 42

44. 43 and 10

CENTRAL

#1) exp Probiotics/

#2) exp Synbiotics/

#3) probiotic*.mp.

#4) synbiotic*.mp.

#5) exp Lactobacillus/

#6) lactobacill*.mp.

#7) exp Bifidobacterium/

#8) (bifidus or bifidobacter*).mp.

#9) exp Streptococcus thermophilus/

#10) streptococcus thermophilus.mp.

#11) streptococc*.mp.

#12) exp Lactococcus/

#13) lactococc*.mp.

#14) Bacillus subtilis/

#15) bacillus subtilis.mp.

#16) exp Enterococcus/

#17) enterococcus faec*.mp.

#18) exp Saccharomyces/

#19) saccharomyc*.mp.

#20) leuconostoc.mp.

#21) pediococc*.mp.

#22) bulgarian bacillus.mp.

#23) (beneficial adj3 bacter*).mp.

#24) dairy.mp.

#25) yog?urt.mp.

#26) kefir.mp.

#27) clostridium.mp.

#28) #1 or #2 or #3 or #4 or #5 or #6 or #7 or #8 or #9 or #10 or #11 or #12 or #13 or #14 or #15 or #16 or #17 or #18 or #19 or #20 or #21 or #22 or #23 or #24 or #25 or #26 or #2739. clostridium difficile.mp.

#29). c diff or c difficile or c. diff or c. diffficile

#30) (diarrhea or diarrhoe or diarhe or diarhoe or dystener* or gastroenteritis

#31) #28 and #29 and #30

Cochrane IBD Group Specialized Register

Title or abstract: probiotic or symbiotic or lactobacillus or Bifidobacterium or streptococcus or lactococcus or bacillus subtilis or enterococcus or saccharomyces or leuconostoc or pediococc or Bulgarian or beneficial bacteria or dairy or yogurt or yoghurt or kefir or clostridium

WHAT'S NEW

Date	Event	Description
21 March 2017	New citation required and conclusions have changed	Updated review with changes to the conclusions and new authors
21 March 2017	New search has been performed	New literature search was performed on 21 March 2017. Eight new studies were added to the review

CONTRIBUTIONS OF AUTHORS

Joshua Goldenberg: Screening, inclusion/exclusion, data extraction, quality assessment, data analysis, manuscript preparation, administrative and technical support, critical revision of article

Christina Yap: Screening, inclusion/exclusion, data extraction

Lyubov Lytvyn: Data extraction, quality assessment, data analysis

Calvin Ka-Fung Lo: Data analysis, manuscript preparation

Jennifer Beardsley: Search strategy, manuscript preparation, critical revision of article

Dominik Mertz: Quality assessment, manuscript preparation, critical revision of article

Bradley C Johnston: Concept, developed review protocol, screening, inclusion/exclusion, data extraction, quality assessment, data analysis, manuscript preparation, critical revision of article

DECLARATIONS OF INTEREST

Joshua Z Goldenberg: None known

Christina Yap: None known

Lyubov Lytvyn: None known

Calvin Ka-Fung Lo: None known

Jennifer Beardsley: None known

Dominik Mertz: None known

Bradley Johnston: None known

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External sources

- No sources of support supplied

DIFFERENCES BETWEEN PROTOCOL AND REVIEW

The secondary outcomes of mortality, need of antibiotics to treat *C. difficile* infection, and recurrence of *C. difficile* infection were not evaluated due to inadequate number of studies with this data. In addition to funnel plots, we used the Harbord linear regression method to detect small study effect in view of recently proposed guidelines. In this latest version, we used recently developed guidelines for heterogeneity evaluation (Gagnier 2013). We changed our language around asymptomatic presence of *C. difficile* from incidence of "C. difficile infection" to "detection of C. difficile" to better align with how the terms are used clinically. Finally, we conducted a post hoc subgroup analysis on baseline risk.

INDEX TERMS

Medical Subject Headings (MeSH)

*Clostridioides difficile; Anti-Bacterial Agents [*adverse effects]; Diarrhea [microbiology] [*prevention & control]; Enterocolitis, Pseudomembranous [*complications] [epidemiology]; Incidence; Probiotics [*therapeutic use]; Randomized Controlled Trials as Topic

MeSH check words

Adult; Child; Humans