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TITLE PAGE

Title: Systematic Review with Meta-analysis: Efficacy of Prebiotics, Probiotics, Synbiotics, and Antibiotics in Irritable Bowel Syndrome.

Short "running" title: Prebiotics, Probiotics, Synbiotics, and Antibiotics in IBS.

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Abbreviations: ACG American College of Gastroenterology

CI	confidence interval
GI	gastrointestinal
IBS	irritable bowel syndrome
IBS-C	irritable bowel syndrome with constipation
IBS-D	irritable bowel syndrome with diarrhoea
IBS-M	mixed stool pattern irritable bowel syndrome
MeSH	medical subject headings
NNH	number needed to harm
NNT	number needed to treat
RCT	randomised controlled trial
RR	relative risk
SD	standard deviation
SMD	standardised mean difference
SIBO	small intestinal bacterial overgrowth

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ABSTRACT

Background: Irritable bowel syndrome (IBS) is a chronic functional bowel disorder. Disturbances in the gastrointestinal microbiome may be involved in its aetiology.

Aims: To perform a systematic review and meta-analysis to examine the efficacy of prebiotics, probiotics, synbiotics, and antibiotics in IBS.

Methods: MEDLINE, EMBASE, and the Cochrane Controlled Trials Register were searched (up to July 2017). Randomised controlled trials (RCTs) recruiting adults with IBS, comparing prebiotics, probiotics, synbiotics, or antibiotics with placebo or no therapy were eligible. Dichotomous symptom data were pooled to obtain a relative risk (RR) of remaining symptomatic after therapy, with a 95% confidence interval (CI). Continuous data were pooled using a standardised mean differences with a 95% CI.

Results: The search identified 4017 citations. Data for prebiotics and synbiotics were sparse. Fifty-three RCTs of probiotics, involving 5545 patients, were eligible. Particular combinations of probiotics, or specific species and strains, appeared to have beneficial effects on global IBS symptoms and abdominal pain, but it was not possible to draw definitive conclusions about their efficacy. There were five trials of similar design that used rifaximin in non-constipated IBS patients, which was more effective than placebo (RR of symptoms persisting = 0.84; 95% CI 0.79-0.90). Adverse events were no more common with probiotics or antibiotics.

Conclusions: Which particular combination, species, or strain of probiotics are effective for IBS remains, for the most part, unclear. Rifaximin has modest efficacy in improving symptoms in non-constipated IBS.

INTRODUCTION

Irritable bowel syndrome (IBS) is a functional bowel disorder with a relapsing and remitting natural history. [1-3] The global prevalence of the condition in the community is approximately 10%, depending on the criteria used to define its presence, [4] although using the latest Rome IV criteria it is lower, estimated at 6%. [5] Despite being common, only a minority of people who report symptoms suggestive of IBS will consult a physician. [3] Because the pathophysiology of the disorder remains incompletely understood, medical treatment is empirical and is usually based on targeting the predominant symptom reported by the patient. [6] This leads to unsatisfactory control of symptoms for many patients and, therefore, alternative approaches are needed.

The concept that alterations in the gut microbiome might be relevant to IBS arose from observations that symptoms of IBS often developed after an infection, known as post-infectious IBS. [7, 8] Furthermore, small intestinal bacterial overgrowth (SIBO) may cause symptoms indistinguishable from IBS, [9] and data suggest that the colonic microbiome is altered in patients with IBS, when compared with healthy controls. [10-13] In addition, some IBS symptoms, such as bloating, slowed gastrointestinal (GI) transit, and early satiety have been associated with specific gut microbiome profiles. [14, 15]

Data from studies such as these suggest that alterations in the gut microbiome may induce IBS symptoms de novo or exacerbate existing symptoms. This then raises the obvious question of whether antibiotics, or other related interventions, can be used to modulate the gut microbiome and thus improve IBS symptoms. Prebiotics are substrates that are selectively utilised by host microorganisms, conferring a health benefit. [16] Probiotics have been defined as “live microorganisms that, when

administered in adequate amounts, confer a health benefit on the host". [17] Synbiotics, which are also food or dietary supplements, are a mixture of probiotics and prebiotics that act synergistically to promote the growth and survival of beneficial organisms.

The use of antibiotics as a means of treating SIBO, a postulated pathophysiologic mechanism for IBS, remains an area of continuing controversy. This is because the tests commonly used to diagnose SIBO, such as lactulose and glucose hydrogen breath tests and small intestinal aspirates, are fraught with problems such as altered intestinal transit, [18-20] which influence their sensitivity and specificity. Despite the fact that any effect of probiotics in IBS is poorly understood, a recent survey of clinicians demonstrated that most believe probiotics to be a benign therapy and over 90% incorporated probiotics into their clinical practice. [21] Gaining a better understanding of probiotics and their clinical use in IBS remains a challenging task due to variations in study design, strain, species, and dose of probiotic as well as small size of study populations.

Previous systematic reviews by our group, [22, 23] conducted to inform the American College of Gastroenterology's (ACG) monograph on the management of IBS, [24, 25] have examined the role of prebiotics, probiotics, and synbiotics, but not antibiotics, in IBS. In the intervening 4 years since our last meta-analysis, there have been further studies published. We therefore performed an updated systematic review and meta-analysis to examine the efficacy of prebiotics, probiotics, synbiotics, and antibiotics in IBS.

MATERIALS AND METHODS

Search Strategy and Study Selection

We updated our previous systematic review and meta-analysis examining the efficacy of prebiotics, probiotics, and synbiotics in IBS, [23] searching the medical literature using MEDLINE (1946 to July 2017), EMBASE and EMBASE Classic (1947 to July 2017), and the Cochrane central register of controlled trials. Randomised placebo-controlled trials examining the effect of at least 7 days of prebiotics, probiotics, synbiotics, or antibiotics in adult patients (over the age of 16 years) with IBS were eligible for inclusion (Table 1), including the first period of cross-over RCTs, prior to cross-over to the second treatment. The diagnosis of IBS could be based on either a physician's opinion or symptom-based diagnostic criteria, supplemented by the results of investigations to exclude organic disease, where studies deemed this necessary.

Subjects were required to be followed up for at least 1 week, and studies had to report response to therapy as either a dichotomous endpoint or via continuous data. Dichotomous assessment could be in the form of either an assessment of global symptom cure or improvement, or abdominal pain cure or improvement, after completion of therapy. Preferably, this information was reported by the patient, but if this was not recorded then data either as documented by the investigator or via questionnaire were accepted. Continuous data of interest were the effect of therapy on global and individual IBS symptom scores at study end. Where studies did not report these types of dichotomous or continuous data, but were otherwise eligible for inclusion in the systematic review, we attempted to contact the original investigators in order to obtain further information.

The literature search was performed as part of a broader exercise to inform the update of the ACG monograph on the management of IBS. [26] Specifically, studies on IBS were identified with the terms irritable bowel syndrome and functional diseases, colon (both as medical subject heading (MeSH) and free text terms), and IBS, spastic colon, irritable colon, or functional adj5 bowel (as free text terms). These were combined using the set operator AND with studies identified with the terms: Saccharomyces, Lactobacillus, Bifidobacterium, Escherichia coli, probiotics, prebiotics, inulin, fructooligosaccharide, fructo-oligosaccharide, galactooligosaccharide, galacto-oligosaccharide, synbiotics, anti-bacterial agents, penicillins, cephalosporins, rifamycins, quinolones, nitroimidazoles, tetracycline, doxycycline, amoxicillin, ciprofloxacin, metronidazole, or tinidazole (both as MeSH and free text terms), or the following free text terms: antibiotic, or rifaximin.

There were no language restrictions and abstracts of the papers identified by the initial search were evaluated by the lead reviewer for appropriateness to the study question. All potentially relevant papers were obtained and evaluated in detail, and foreign language papers were translated where necessary. We hand-searched abstract books of conference proceedings (Digestive Diseases Week, American College of Gastroenterology, and United European Gastroenterology Week) between 2001 and 2017 in order to identify potentially eligible studies published only in abstract form. We then used the bibliographies of all identified relevant studies to perform a recursive search of the literature. Two reviewers assessed all identified articles independently, using pre-designed eligibility forms, according to the prospectively defined eligibility criteria, with any disagreements resolved by consensus. The systematic review was not registered a priori with PROSPERO.

Outcome Assessment

The primary outcomes assessed were the effects of prebiotics, probiotics, synbiotics, or antibiotics compared with placebo on global IBS symptoms or abdominal pain after cessation of therapy. Secondary outcomes included their effects on global IBS symptom scores and individual IBS symptom scores at study end, including abdominal pain, bloating, urgency, or flatulence. We also examined numbers of adverse events as a result of prebiotics, probiotics, synbiotics, or antibiotics.

Data Extraction

Two reviewers extracted all data independently on to a Microsoft Excel spreadsheet (XP professional edition; Microsoft Corp, Redmond, WA, USA) as dichotomous outcomes (global IBS symptoms persistent or unimproved, or abdominal pain persistent or unimproved) (Table 2), or mean symptom scores at study end, along with a standard deviation (SD). In addition, the following clinical data were extracted for each trial: setting (primary, secondary, or tertiary care-based), number of centres, country of origin, prebiotic, probiotic, synbiotic, or antibiotic used (including strain and species where applicable), duration of therapy, total number of adverse events reported, criteria used to define IBS, primary outcome measure used to define symptom improvement or cure following therapy, proportion of female patients, and proportion of patients according to predominant stool pattern (IBS with constipation (IBS-C), diarrhoea (IBS-D), or mixed stool pattern (IBS-M)). Data were extracted as intention-to-treat analyses, with all drop-outs assumed to be treatment failures, wherever trial reporting allowed this.

Assessment of Risk of Bias

Two reviewers assessed the risk of bias of each study independently, with disagreements resolved by consensus. Risk of bias was assessed as described in the Cochrane handbook, [27] by recording the method used to generate the randomisation schedule and conceal allocation, whether blinding was implemented for participants, personnel, and outcomes assessment, whether there was evidence of incomplete outcomes data, and whether there was evidence of selective reporting of outcomes.

Data Synthesis and Statistical Analysis

Data were pooled using a random effects model, [28] to give a more conservative estimate of the range of effects of prebiotics, probiotics, synbiotics, or antibiotics, if there was heterogeneity between studies. The impact of prebiotics, probiotics, synbiotics, or antibiotics was expressed as a relative risk (RR) of global IBS symptoms or abdominal pain persisting with intervention compared with control, with 95% confidence intervals (CI), or a standardised mean difference (SMD) in global or individual IBS symptom scores at study end, with 95% CIs. Where possible, we performed subgroup analyses based on particular combinations, species, and strains of probiotic, or type of antibiotic, used as well as a sensitivity analysis including only trials at low risk of bias. Adverse events data were also summarised with RRs. The number needed to treat (NNT) and the number needed to harm (NNH), with 95% CIs, were calculated using the formula NNT or NNH = 1 / (control event rate x (1 – RR)).

Heterogeneity, which is variation between individual study results that has not occurred due to chance, was assessed using both the I^2 statistic with a cut off of ≥ 50%, and the chi-squared test with a P value < 0.10, used to define a significant degree of heterogeneity. [29] Review Manager version 5.3.5 (RevMan for Windows

2014, the Nordic Cochrane Centre, Copenhagen, Denmark) and StatsDirect version 2.7.7 (StatsDirect Ltd, Sale, Cheshire, England) were used to generate Forest plots of pooled RRs and SMDs for primary and secondary outcomes with 95% CIs, as well as funnel plots. The latter were assessed for evidence of asymmetry, and therefore possible publication bias or other small study effects, using the Egger test, [30] if there were sufficient (≥10) eligible studies included in the meta-analysis, in line with recent recommendations, [31] with a P value < 0.10 used to define presence of possible publication bias or other small study effects.

RESULTS

The search strategy generated a total of 4017 citations, of which 111 published articles appeared to be relevant, and were retrieved for further assessment (Figure 1). Of these, 45 were excluded for various reasons, leaving 66 eligible articles, reporting 67 separate RCTs. Agreement between reviewers for assessment of trial eligibility was excellent (kappa statistic = 0.85). Eighteen of the RCTs of probiotics in IBS were identified since our last systematic review. [32-49]

Efficacy and Safety of Prebiotics in IBS

Our previous systematic review identified no trials of prebiotics in IBS. The updated search identified three eligible RCTs. [50-52] We also identified a placebo-controlled trial, where the active intervention was a mixture of 750mg of vegetable oligo- and polysaccharides, but this was not eligible as the prebiotic was combined with 250mg of reticulated protein, so the effects of the two could not be assessed separately.

The first of the three eligible RCTs recruited 98 patients with IBS, according to the Manning criteria, and randomised them to receive either 20g of fructooligosaccharide powder, or placebo, for 12 weeks. [50] This double-blind trial was at low risk of bias. Patients' assessment of treatment response was recorded at the end of therapy, with 58.0% of patients assigned to fructooligosaccharide reporting some improvement in symptoms, compared with 65.2% of those allocated to placebo. This difference was not statistically significant. Mean change in total symptom scores at 12 weeks was also not significantly different between the two arms of the trial (-1.82 with fructooligosaccharide vs. -2.35 with placebo). Adverse events rates in each arm were similar.

The second recruited 79 patients with Rome III defined IBS, and randomised them to a 2.5g sachet of either short-chain fructooligosaccharides or placebo for 4 weeks. [51] This trial was double-blind, but was at unclear risk of bias, as the method used to conceal treatment allocation was not reported. Mean global symptom scores improved in both groups, compared with baseline, but there was no difference in the mean change in global symptoms scores between treatment arms (-122.3 with short-chain fructooligosaccharide vs. -38.1 with placebo, P = 0.13) which, given the magnitude of the difference, is likely due to the trial being underpowered for this endpoint. Again, adverse events rates in each arm were similar.

The third study was a cross-over trial and recruited 60 patients with Rome II-defined IBS. [52] All participants were randomised to placebo for 4 weeks and then, following a wash-out period of 2 weeks, were re-randomised to 4 weeks of low-dose prebiotic (3.5g of trans-galactooligosaccharide), high-dose prebiotic (7g of trans-galactooligosaccharide), or placebo. This study was at unclear risk of bias as the method of randomisation was stated, but not the method of concealment of allocation, and only patients were blinded to treatment allocation. After the second 4 weeks of treatment, patients in both the low- and high-dose prebiotic arms experienced a significant reduction in mean global symptom scores, compared with those at the end of the 2-week washout, but there was no effect on mean abdominal pain scores. Adverse events were similar between all three treatment arms.

Efficacy and Safety of Probiotics in IBS

The 53 RCTs of probiotics in IBS involved 5545 patients. [32-49, 53-87] The proportion of women in trials ranged between 9% and 100%. Twenty-six trials were at low risk of bias, [32, 33, 36-39, 41, 42, 45, 47-49, 56, 58, 63, 65, 67, 68, 72, 74, 76, 77, 79, 83, 85, 86] with the

remainder being unclear. Twenty-nine trials used a combination of probiotics, 11 Lactobacillus, five Saccharomyces, four Bifidobacterium, two E. coli, one Streptococcus, and one either Lactobacillus or Bifidobacterium. Detailed characteristics of included RCTs are provided in Supplementary Table 1.

Efficacy of Probiotics in the Treatment of IBS: Effect on Persistence of Symptoms

There were 37 RCTs comparing probiotics with placebo for the treatment of IBS, [33, 35-38, 40, 41, 43-49, 53-57, 63, 65, 66, 68, 71, 72, 74, 76, 78-87] evaluating 4403 patients, which gave outcomes as a dichotomous variable (Figure 2). Combination probiotics were assessed in 21 RCTs, [33, 35-38, 40, 43, 46, 49, 56, 57, 65, 66, 72, 74, 78-81, 86, 87] containing 1931 patients, with a significant effect on symptoms (RR = 0.79; 95% CI 0.68 to 0.91) (Figure 2), but with significant heterogeneity between studies (I^2 = 72%, P < 0.001). There was statistically significant asymmetry detected in the funnel plot (Egger test, P = 0.06), suggesting publication bias or other small study effects. The NNT with combination probiotics was 7 (95% CI 5 to 19).

In terms of the different combinations tested, three trials used the same combination of Lactobacillus paracasei ssp paracasei F19, Lactobacillus acidophilus La5, and Bifidobacterium lactis Bb12 in 269 patients, [74, 79, 86] with no benefit over placebo (RR = 0.92; 95% CI 0.76 to 1.11), two RCTs used a combination of Bifidobacterium longum, B. bifidum, B. lactis, Lactobacillus acidophilus, L. rhamnosus, and Streptococcus thermophiles, known as LacClean Gold, in 130 patients (RR = 0.59; 95% CI 0.37 to 0.93), [38, 43] two RCTs used VSL#3 in 78 patients (RR = 0.82; 95% CI 0.52 to 1.30), [49, 56] and two trials a seven-strain combination of three Bifidobacterium, three Lactobacillus, and one Streptococcus, in 78 patients (RR = 0.48; 95% CI 0.24 to 0.94). [33, 80]

Lactobacillus was used in eight trials (893 patients), [44, 48, 54, 55, 68, 82-84] with no clear benefit detected over placebo (RR = 0.82; 95% CI 0.63 to 1.06), again with significant heterogeneity between studies (I^2 = 83%, P < 0.001). However, when only the three RCTs that used Lactobacillus plantarum DSM 9843 were considered in the analysis, [54, 55, 83] which contained 314 subjects, the RR of symptoms persisting was significantly lower with active therapy (0.67; 95% CI 0.51 to 0.87) (NNT = 3; 95% CI 2 to 8), although the significant heterogeneity observed persisted (I^2 = 63%, P = 0.07). Bifidobacterium was studied in three RCTs (528 patients), [47, 63, 76] with a trend towards a benefit over placebo (RR = 0.70; 95% CI 0.48 to 1.01, P = 0.06). Saccharomyces cerevisiae was used in two RCTs, [41, 45] containing 579 patients, but was not superior to placebo (RR = 0.92; 95% CI 0.82 to 1.03). Escherichia was assessed in two trials (418 patients), [71, 85] with a benefit detected compared with placebo (RR = 0.86; 95% CI 0.79 to 0.93), although only significantly so in the trial of Escherichia coli DSM17252. [71] Finally, Streptococcus faecium was used in one trial recruiting 54 patients, and appeared to be superior to placebo (RR = 0.72; 95% CI 0.53 to 0.99). [53]

Efficacy of Probiotics in the Treatment of IBS: Effect on Global IBS or Abdominal Pain Scores

There were 33 separate trials, [32-35, 38, 39, 41, 42, 48, 54, 56-65, 67, 69, 70, 73-77, 79, 80, 83, 84, 86] making 35 comparisons, containing 3073 patients that reported effect of probiotics on global IBS or abdominal pain scores (Figure 3). There were eight trials (868 patients) that evaluated Lactobacillus, [34, 48, 54, 59, 60, 62, 83, 84] and three trials (501 patients) that investigated Bifidobacterium, [60, 63, 76] and neither were statistically significantly more efficacious than placebo (Figure 3), although there was a trend towards a benefit for

the latter (SMD -0.46; 95% CI -0.92 to 0, P = 0.05). When only the three trials that used Lactobacillus plantarum DSM 9843 were considered in the analysis there was no benefit in 314 patients (SMD = – 0.18; 95% CI – 0.60 to 0.25). [54, 62, 83] Similarly, when only the two trials that used Bifidobacterium infantis 35624 were included in the analysis there was no benefit in 379 patients (SMD = – 0.33; 95% CI – 0.90 to 0.24). [60, 63]

There were 19 trials, [33, 35, 38, 42, 56-58, 61, 64, 65, 67, 69, 70, 73, 74, 77, 79, 80, 86] evaluating 1341 patients, using combinations of probiotics that did suggest a significant improvement in IBS symptoms score with active treatment (SMD – 0.31; 95% CI -0.44 to -0.17) (Figure 3), with no significant heterogeneity between study results (I^2 = 24%, P = 0.17), but evidence of funnel plot asymmetry (Egger test, P = 0.06). When specific combinations were studied, four trials used VSL#3 in 135 patients, with a trend towards a benefit over placebo (SMD – 0.57; 95% CI – 1.14 to 0.00, P = 0.05), [42, 56, 58, 77] three trials used a combination of Lactobacillus paracasei ssp paracasei F19, Lactobacillus acidophilus La5, and Bifidobacterium lactis Bb12 in 217 patients with no benefit over placebo (SMD = – 0.07; 95% CI – 0.34 to 0.20), [74, 79, 86] and two trials used a combination of Bifidobacterium lactis DN-173 010, Streptococcus thermophilus, and Lactobacillus bulgaricus in 299 patients, again with no significant benefit over placebo (SMD = – 0.41; 95% CI – 1.12 to 0.30). [64, 70]

Efficacy of Probiotics in the Treatment of IBS: Effect on Individual Symptom Scores

There were 24 separate trials, [32, 33, 35, 38, 39, 42, 48, 56-58, 60, 61, 63, 64, 69, 70, 73-77, 79, 80, 86] making 26 comparisons, and containing 2256 patients, which reported the effect of probiotics on bloating symptom scores (Figure 4). There was a trend towards a reduction in bloating scores with combination probiotics (SMD = -0.135; 95% CI -

0.34 to -0.01, P = 0.07), but no evidence of any benefit of Bifidobacterium, Saccharomyces, or Lactobacillus.

Eleven trials reported continuous data for the effect of probiotics on flatulence symptom scores in 767 patients (Figure 5). [33, 54, 56-58, 61, 63, 69, 70, 75, 80] Flatulence scores were significantly reduced with combinations of probiotics (SMD = -0.29; 95% CI -0.51 to -0.07), but not with any of the other probiotics studied.

Finally, eight RCTs reported the effect of probiotics on urgency symptom scores in 733 patients. [33, 39, 56, 58, 63, 75, 76, 80] There was no apparent benefit detected for any probiotic, in terms of effect on symptoms of urgency.

Adverse Events with Probiotics

Total adverse events were reported by 36 RCTs, [34-36, 38-42, 44-46, 48, 53-59, 64, 66-69, 71-77, 80, 82, 83, 85, 86] containing 4183 patients. Overall, 433 (19.4%) of 2228 patients allocated to probiotics experienced any adverse event, compared with 332 (17.0%) of 1955 assigned to placebo. The RR of experiencing any adverse event was not significantly higher with probiotics (1.09; 95% CI 0.91 to 1.29), but there was significant heterogeneity between studies (I^2 = 36%, P = 0.05), and evidence of funnel plot asymmetry (Egger test, P = 0.08).

Efficacy and Safety of Synbiotics in IBS

The two RCTs of synbiotics in IBS recruited a total of 198 patients. [88, 89] The first was a single-blind RCT conducted in Italy, [88] using a combination of Lactobacillus acidophilus and helveticus, with Bifidobacterium species, in a vitamin and phytoextract-enriched medium in 68 patients with Rome II-defined IBS for 12 weeks, which did not report the subtypes of IBS recruited. The second, conducted in

South Korea, [89] used Bifidobacterium lactis in combination with acacia fibre in 130 patients who met the Rome III criteria for IBS for 8 weeks. Of these patients, 35.0% had IBS-C, 29.9% IBS-D, and 8.5% IBS-M. This double-blind trial was at unclear risk of bias due to failure to report the method used to conceal treatment allocation. Only one trial reported dichotomous data, [88] and there were 7 (20.6%) of 34 patients assigned to synbiotics with persistent symptoms, compared with 30 (88.2%) of 34 assigned to control ($P < 0.01$). Both trials assessed IBS symptoms on a continuous scale in 185 patients. There was no statistically significant effect of synbiotics in reducing symptoms, even though both trials were individually positive, due to significant heterogeneity between studies (SMD = -1.73; 95% CI -3.73 to 0.27, I^2 = 96%, P = 0.09). Adverse events were reported in both studies, there were none of any significance in either treatment arm.

Efficacy and Safety of Antibiotics in IBS

We identified nine trials, reported in eight separate papers, [90-97] which evaluated antibiotic therapy in 2845 patients with IBS (Figure 6). Detailed trial characteristics are provided in Table 3. One trial evaluated neomycin in 111 patients, [93] with a significant effect in favour of neomycin (RR = 0.73; 95% CI 0.56 to 0.96), with a NNT of 5 (95% CI 3 to 33). Another trial evaluated norfloxacin in 80 patients, [90] again with a significant effect in favour of the antibiotic (RR = 0.63; 95% CI 0.49 to 0.80) with a NNT of 3 (95% CI 2 to 5).

Five RCTs, reported in four articles, [94-97] used the minimally absorbed antibiotic rifaximin in 1805 non-constipated IBS patients (predominantly IBS with diarrhoea). There was a statistically significant benefit in favour of rifaximin (RR = 0.84; 95% CI 0.79 to 0.90) with no significant heterogeneity noted between the

studies (I^2 = 0%, P = 0.74). The NNT was 9 (95% CI 7 to 15). A sixth trial, [91] which randomised 636 patients with IBS-D, who had responded to open-label rifaximin and then experience symptomatic relapse, to two repeat courses of treatment showed a trend towards a benefit of rifaximin (RR = 0.90; 95% CI 0.81 to 1.01, P = 0.08). Finally, there was a seventh trial, [92] recruiting 213 patients with IBS, which was excluded as patients also had lactose intolerance and bacterial overgrowth on breath testing, and therefore represented a highly selected group of IBS patients. When both these trials were pooled in the analysis, rifaximin remained an effective treatment (RR = 0.82; 95% CI 0.72 to 0.95), but with significant heterogeneity between studies (I^2 = 77%, P < 0.001). The NNT was 8 (95% CI 5 to 29). There were four low risk of bias rifaximin trials, assessing 1966 patients. [91, 94, 97] There remained a significant effect in favour of active therapy when only these RCTs were considered in the analysis (RR = 0.87; 95% CI 0.82 to 0.93) with no significant heterogeneity (I^2 = 0%, P = 0.81) and a NNT of 11 (95% CI 8 to 21).

Adverse Events with Antibiotics

One paper pooled adverse events from two RCTs, meaning that these data were not extractable. [97] As a result, only three RCTs reported adverse events in 817 patients. [91, 93, 94] However, one of the RCTs reported no adverse events, [94] and one reported a single adverse event in the placebo arm, [93] meaning there were insufficient data to pool. A post hoc pooled analysis from the phase 2b and phase 3 rifaximin RCTs revealed no difference in adverse events (52% in both rifaximin and placebo arms) or serious adverse events (approximately 1.5% and 2.2% in each arm) between rifaximin and placebo. [98]

There has been concern surrounding the risk of developing Clostridium difficile infection with antibiotics for IBS. A pooled analysis of the phase 2b study and two of the phase 3 studies found C. difficile in one patient at study entry who subsequently was removed from the study [98]. There was a zero incidence of C. difficile colitis that developed de novo. In the TARGET 3 trial, a further case of C. difficile colitis was reported among the 328 patients randomised to re-treatment with rifaximin [91].

DISCUSSION

This systematic review and meta-analysis has demonstrated that particular combinations of probiotics, or specific species and strains, appear to have beneficial effects in IBS in terms of effect on global IBS symptoms and abdominal pain, but it is not possible to draw definitive conclusions about their efficacy. However, there was significant heterogeneity between studies, and evidence of publication bias or other small study effects, in some analyses. We found evidence to support the use of combinations of probiotics as a group, and for particular combinations, although in small numbers of RCTs. In terms of individual probiotics, Lactobacillus plantarum DSM 9843, E. coli DSM1752, and Streptococcus faecium, also appeared beneficial, although the latter two were only used in one RCT each. There was also a trend towards a beneficial effect of Bifidobacterium, in terms of improvement of global IBS symptoms and pain scores, although which particular strain or species may be of benefit remains unclear. The largest trial was a dose-ranging study of Bifidobacterium infantis 35624, and demonstrated efficacy, in terms of global symptoms and abdominal pain, at a dose of $1x10^8$ CFU. [63] Overall, rifaximin was also superior to placebo for the treatment of non-constipated IBS, with a NNT of 9. There was only one trial each of norfloxacin and neomycin, making it difficult to draw any firm conclusions regarding their efficacy. The RR of adverse events was not significantly greater with either probiotics or antibiotics. Data for both prebiotics and synbiotics were sparse, with neither appearing to be of particular benefit in IBS, albeit in only five trials in total.

We used rigorous and reproducible methodology when conducting this systematic review and meta-analysis. We reported our search strategy in full, and performed the assessment of eligibility and data extraction independently, and in

duplicate. We used an intention-to-treat analysis and pooled data with a random effects model, to minimise the likelihood that treatment effect would be overestimated. We also contacted investigators of potentially eligible studies to either obtain dichotomous data and continuous data. This inclusive approach has provided us with access to data for >5500 IBS patients treated with probiotics. Finally, we performed subgroup analyses in an attempt to assess treatment effect according to combinations of, and individual, probiotics used and we extracted and pooled adverse events data, where reported.

This updated meta-analysis identified a further 18 RCTs of probiotics and three trials of prebiotics since the previous iteration 4 years ago, but it is still not possible to draw clear inferences from the data concerning the efficacy and safety of either prebiotics or synbiotics. For probiotics, it remains unclear whether a particular combination of probiotics, or a specific species or strain, is more likely to be effective, or whether there is a particular IBS subtype that is more likely to benefit. Other limitations of this systematic review and meta-analysis arise from the nature of the studies available for synthesis. The risk of bias of many of the trials we identified was unclear, and there was evidence of heterogeneity between RCTs and publication bias in some of our analyses of probiotics. However, there was no heterogeneity between studies when only the five RCTs of rifaximin of similar design conducted in non-constipated IBS were included, although the treatment effect in favour of rifaximin in these studies was modest.

The fact that there have been another 18 RCTs of probiotics conducted since the last version of this meta-analysis, only 4 years ago, underlines the continuing interest in the manipulation of the GI microbiome as a potential therapy for IBS. This systematic review provides support for the use of some probiotics to achieve this, but

there are still insufficient data to recommend a specific species or strain of organism. In addition, there has been a further trial of rifaximin in IBS conducted in the last 2 years, [91] and the drug is now licensed for the treatment of IBS with diarrhoea in the US. This latter RCT studied the efficacy and safety of a further two 14-day courses of rifaximin in IBS with diarrhoea, following 2 weeks of open-label treatment with the drug, demonstrating that repeat treatment led to a durable and reproducible symptom response, which was superior to placebo in the original trial. However, the efficacy was modest after each course of treatment, and the long-term safety of repeated courses of rifaximin, and how many times to re-treat patients whose symptoms recur remains uncertain.

The rationale for the use of antibiotics in patients with IBS was based on diagnostic confusion between IBS and SIBO, with patients in the initial studies undergoing hydrogen breath testing to confirm the presence of SIBO prior to enrolment. [93, 99] However, in the pivotal RCTs of rifaximin breath testing was only undertaken in a subset of individuals, and the results were not reported in full. [91, 97] In addition, the mechanism of action of rifaximin in IBS remains unclear. A small mechanistic trial found no difference in terms of the faecal microbiome, intestinal permeability, or faecal bile acid levels between individuals with IBS randomised to rifaximin or placebo, [100] but demonstrated an acceleration in ascending colon emptying times among those allocated to rifaximin. Given the drugs beneficial effects in patients with IBS with diarrhoea, this would seem paradoxical. Studies that have evaluated the effect of rifaximin on the microbiome, show that any changes are limited, and are not sustained. [100-102] Although the limited research regarding rates of C. difficile infection and microbial resistance are reassuring, continued monitoring of patients receiving repeated courses of the drug will be required. Additionally,

advances in molecular techniques may provide further insight into the faecal microbiome of patients with IBS, which may in turn improve the understanding of the role of antibiotic therapy in the treatment of this complex disorder.

The mechanism of action of individual probiotics in improving symptoms in IBS also remains speculative. There have been previous studies conducted that have suggested that some probiotics, such as Lactobacillus acidophilus NCFM, have the ability to modify the expression of pain receptors in the gut in both mice and humans. [103, 104] In addition, in one of the trials we identified, Bifidobacterium infantis 35624 had the ability to normalise interleukin levels in patients with IBS. [60] More recently, the probiotic Bifidobacterium longum NCC3001 has been demonstrated to have a beneficial effect on depression scores among patients with IBS in a RCT. [47] Brain activation to fearful stimuli, seen on functional magnetic resonance imaging, was also reduced among patients allocated to the probiotic in this study. Interestingly, both this effect and the improvement in depression scores appeared to be most pronounced among those with adequate relief of their IBS symptoms. However, it is unlikely that these are class effects of probiotics, and further research in humans is required to identify species and strains of probiotics that are consistently beneficial, as well as to elucidate how these benefits are achieved.

In summary, this meta-analysis has demonstrated little evidence for the use of prebiotics or synbiotics in IBS. Amongst combination probiotics, LacClean Gold and the seven-strain combination of three Bifidobacterium, three Lactobacillus, and one Streptococcus were associated with significant improvements in global symptoms, and there was a trend towards an improvement in global symptom scores or abdominal pain scores with VSL#3. Among individual probiotics, Lactobacillus plantarum DSM 9843, Escherichia coli DSM17252, and Streptococcus faecium also

had beneficial effects on global symptoms. We could not show any evidence of benefit for any particular combination, strain, or species of probiotics for the other endpoints of interest. Overall, therefore, it remains unclear which combination, species, or strain should be preferred in the individual patient. Five trials of similar design that used rifaximin demonstrated a consistent, although modest, benefit in IBS with a NNT of 9. Both probiotics and antibiotics appeared to be safe in IBS, but the longer terms effects of repeated treatment with the latter on the microbiome, and the safety of this approach, remains unclear.

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CONFLICTS OF INTEREST/STUDY SUPPORT

Guarantor of the article: ACF is guarantor.

Specific author contributions: ACF, LAH, BEL, EMMQ, and PM conceived the study. ACF and PM collected all data. ACF and PM analysed and interpreted the data. ACF drafted the manuscript. All authors commented on drafts of the paper. All authors have approved the final draft of the manuscript.

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FIGURE LEGENDS

Figure 1. Flow Diagram of Assessment of Studies Identified in the Updated Systematic Review and Meta-analysis.

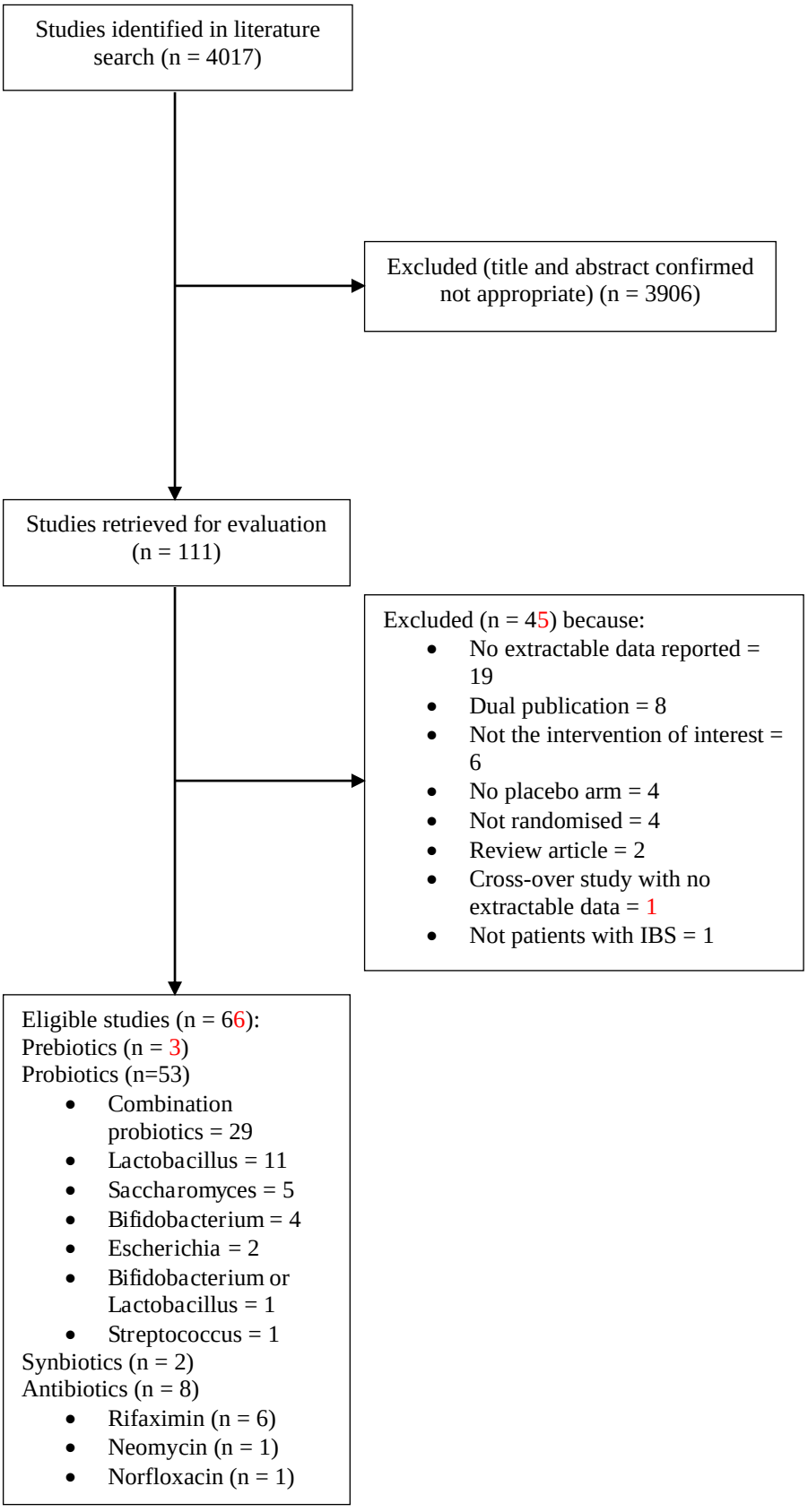

Figure 2. Forest Plot of Randomised Controlled Trials of Probiotics Versus Placebo in Irritable Bowel Syndrome: Effect on Persistence of Symptoms.

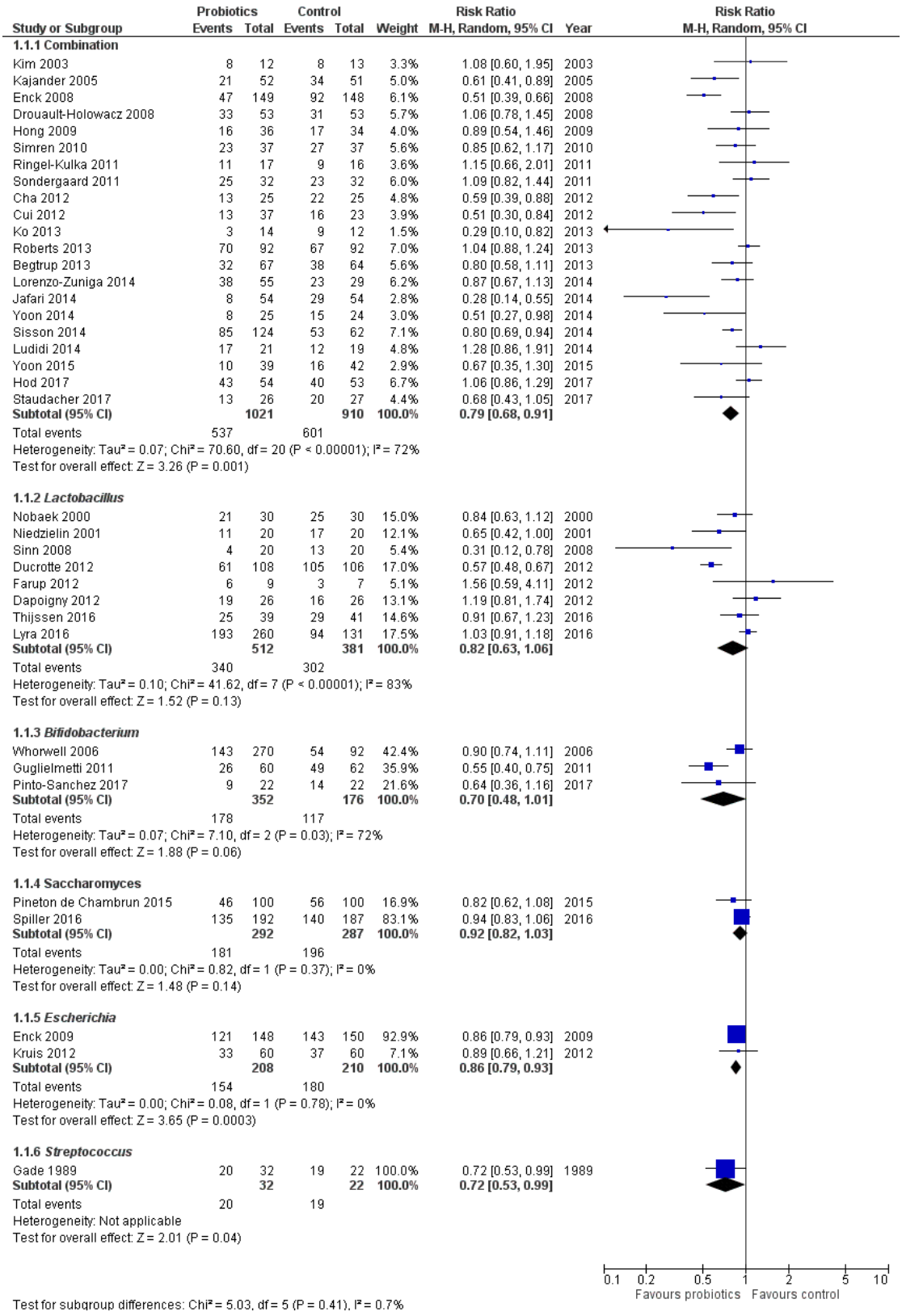

Figure 3. Forest Plot of Randomised Controlled Trials of Probiotics Versus Placebo in Irritable Bowel Syndrome: Effect on Global Symptom or Abdominal Pain Scores.

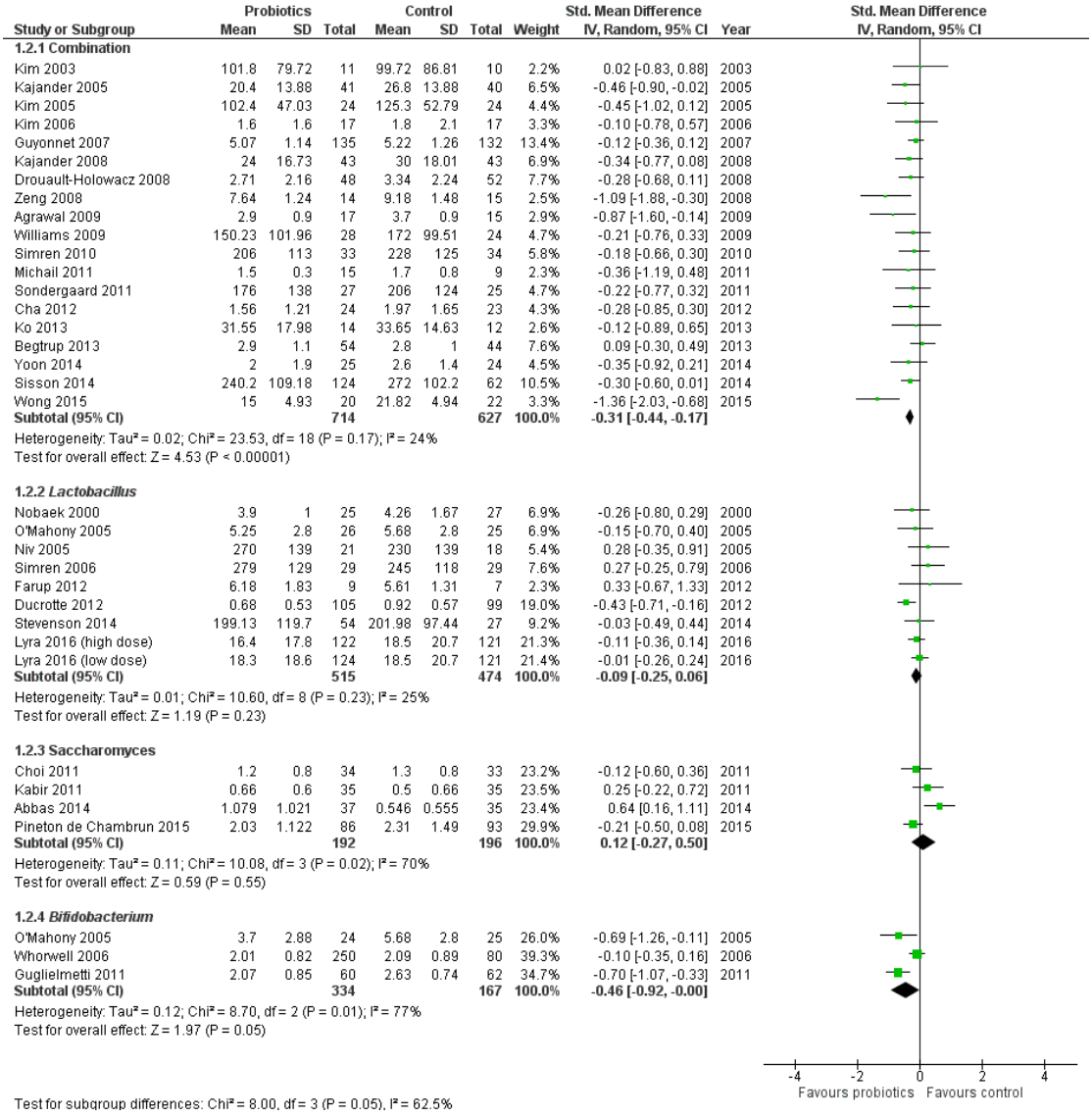

Figure 4. Forest Plot of Randomised Controlled Trials of Probiotics Versus Placebo in Irritable Bowel Syndrome: Effect on Bloating Scores.

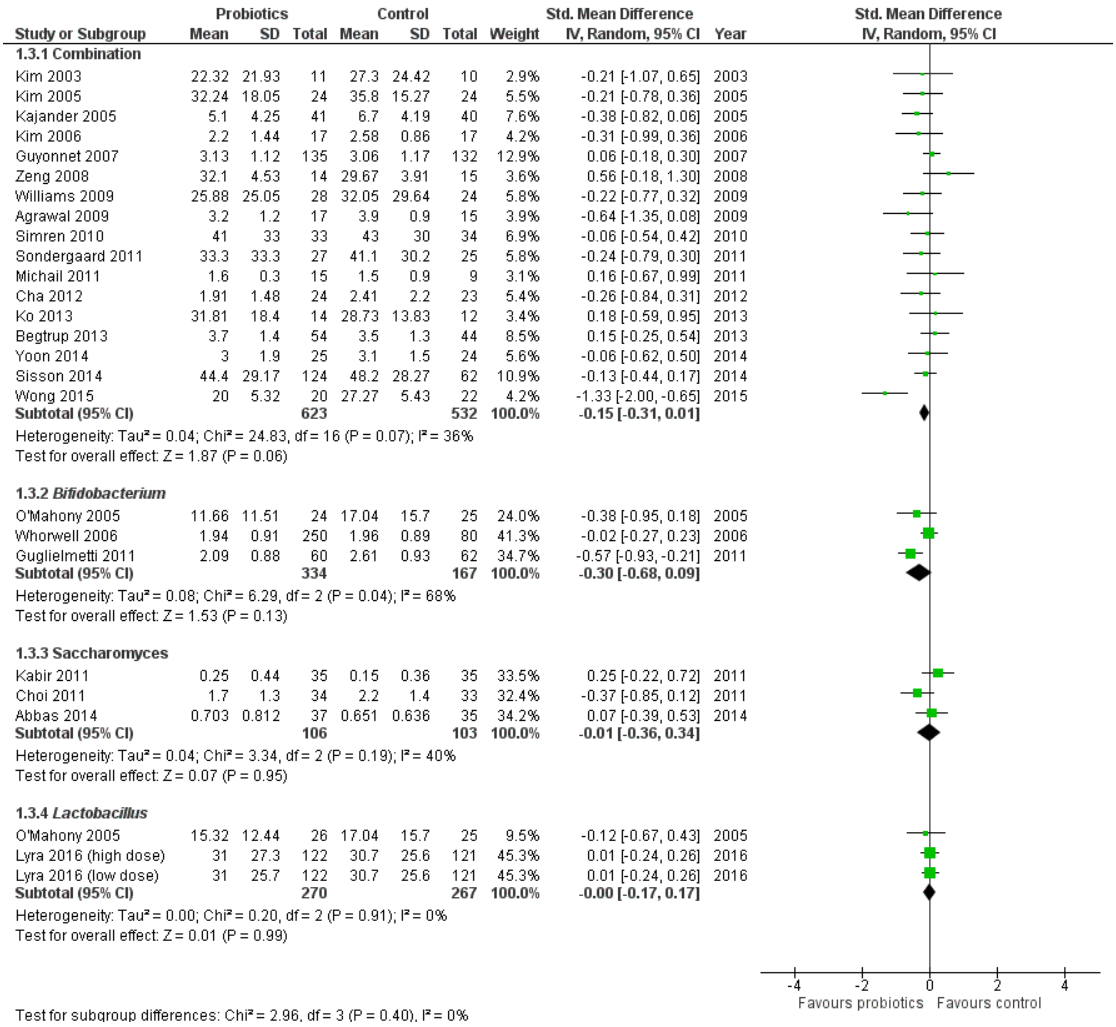

Figure 5. Forest Plot of Randomised Controlled Trials of Probiotics Versus Placebo in Irritable Bowel Syndrome: Effect on Flatulence Scores.

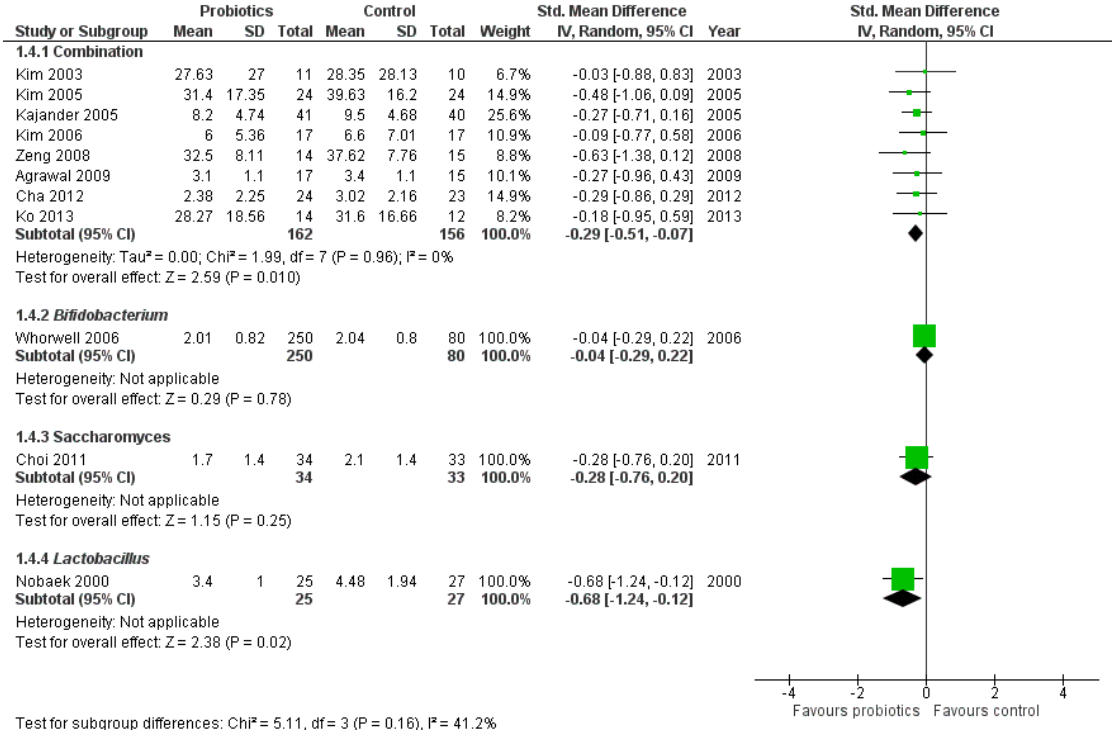

Figure 6. Forest Plot of Randomised Controlled Trials of Antibiotics Versus Placebo in Irritable Bowel Syndrome: Effect on Persistence of Symptoms.

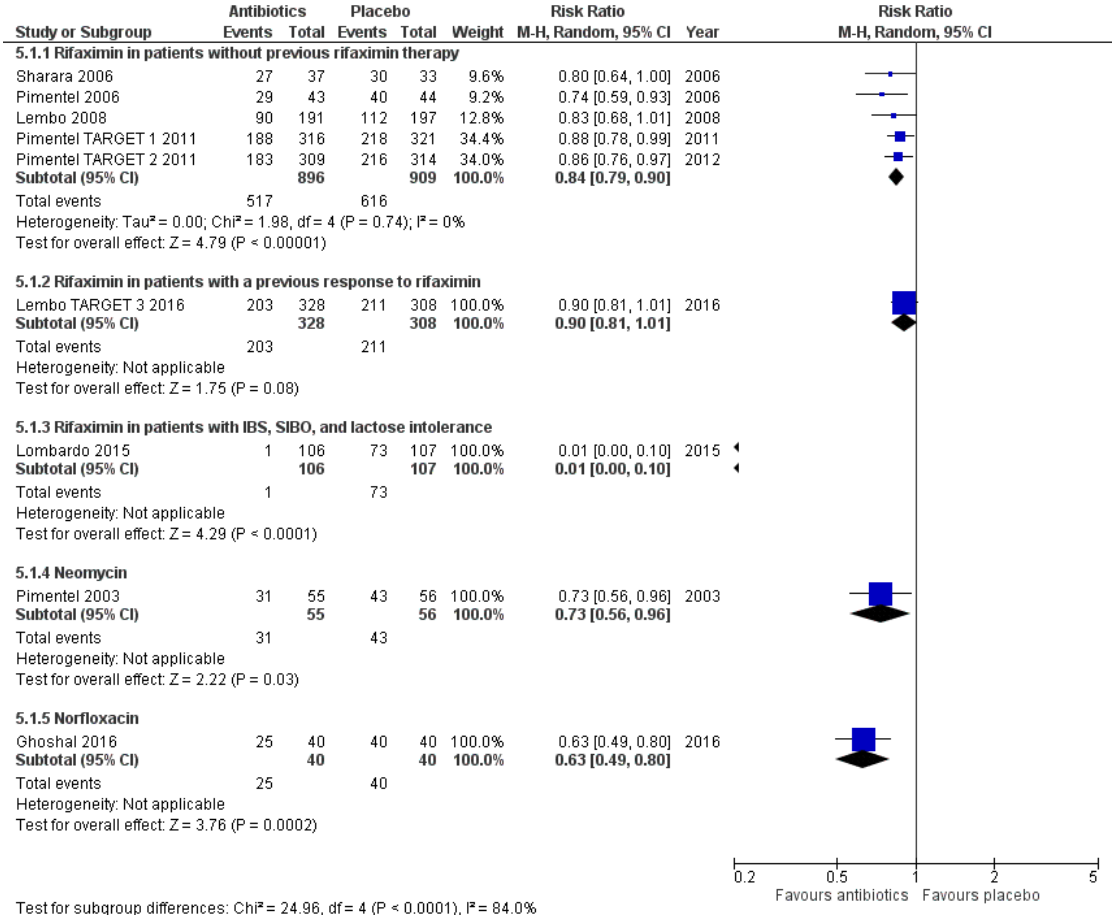

Table 1. Eligibility Criteria.

Randomised controlled trials.
Adults (participants aged >16 years).
Diagnosis of IBS based on either a clinician's opinion, or meeting specific diagnostic criteria*, supplemented by negative investigations where trials deemed this necessary.
Compared prebiotics, probiotics, synbiotics, or antibiotics with placebo.
Minimum treatment duration of 7 days.
Minimum follow-up duration of 7 days.
Dichotomous assessment of response to therapy in terms of effect on global IBS symptoms or abdominal pain following therapy, or continuous data in the form of effect on IBS symptom scores at study end.†

*Manning, Kruis score, Rome I, II, III, or IV.

†Preferably patient-reported, but if this was not available then as assessed by a physician or questionnaire data.

Table 2. Data extraction methodology.

Outcome of interest: improvement in global IBS symptoms preferable, if not reported then improvement in abdominal pain.
Reporting of outcomes: patient-reported preferable, if not available then investigator-reported.
Time of assessment: upon completion of therapy.
Denominator used: true intention-to-treat analysis, if not available then all evaluable patients.
Cut off used for dichotomisation: any improvement in global IBS symptoms or abdominal pain for Likert-type scales.

Table 3. Characteristics of Randomised Controlled Trials of Antibiotics Versus Placebo in Irritable Bowel Syndrome.

Study	Country and recruitment	Criteria used to define symptom improvement following therapy	Sample size (% female) and diagnostic criteria for IBS	Antibiotic used and duration of therapy	Methodology
Pimentel 2003 [93]	USA, advertising	50% improvement in IBS symptom composite score	111 (55), Rome I, 34.2% IBS-C, 41.4% IBS-D	Neomycin 500mg b.d. for 10 days	Method of randomisation not stated. Method of concealment of allocation stated. Double-blind. Unclear if other IBS medications allowed.
Pimentel 2006 [95]	USA, tertiary care	>50% improvement in VAS score for global severity and bloating as compared with run-in baseline severity	87 (67), Rome I, subtype not reported	Rifaximin 400mg t.i.d. for 10 days	Method of randomisation and concealment of allocation not stated. Double-blind. Unclear if other IBS medications allowed.

Sharara 2006 [94]	Lebanon, advertising	Patient stated whether IBS symptoms improved 10 days after end of antibiotic therapy	70 (55), Rome II, 38.3% IBS-C, 20% IBS-D, 41.7% IBS-M	Rifamixin 400mg b.d. for 10 days	Method of randomisation and concealment of allocation stated. Double-blind. Unclear if other IBS medications allowed.
Lembo 2008 [96]	USA, recruitment unclear	Adequate relief of global IBS symptoms	388 (72), Rome II, 100% IBS-D	Rifamixin 550mg b.d. for 2 weeks	Method of randomisation and concealment of allocation not stated. Double-blind. Unclear if other IBS medications allowed.
Pimentel TARGET 1 2011 [97]	USA, recruitment unclear	Adequate relief of global IBS symptoms	623 (73), Rome II, 100% IBS-D or IBS-M	Rifamixin 550mg t.i.d. for 2 weeks	Method of randomisation and concealment of allocation stated. Double-blind. Antidepressant therapy allowed.
Pimentel TARGET 2 2011[97]	USA, recruitment unclear	Adequate relief of global IBS symptoms	637 (71), Rome II, 100% IBS-D or IBS-M	Rifamixin 550mg t.i.d. for 2 weeks	Method of randomisation and concealment of allocation stated. Double-blind. Antidepressant therapy allowed.

Lombardo 2015 [92]	Italy, tertiary care	"Completely" asymptomatic"	213 (not reported), clinical criteria, subtype not stated	Rifaximin 1200mg per day for 2 weeks plus lactose exclusion diet versus lactose exclusion diet alone	Method of randomisation and concealment of allocation not stated. Open-label. Unclear if other IBS medications allowed.
Ghoshal 2016 [90]	India, tertiary care	"Negative for Rome III criteria" at one month	80 (19), Rome III, subtype not stated	Norfloxacin 400mg b.d. for 10 days	Method of randomisation and concealment of allocation stated. Double-blind. Unclear if other IBS medications allowed.
Lembo TARGET 3 2016 [91]	USA, UK, and Germany, recruitment unclear	Decrease in abdominal pain ≥30% from baseline and a decrease in frequency of loose stools of ≥50% from baseline for ≥2 weeks over a 4-week period	636 (69), Rome III, 100% IBS-D	Rifamixin 550mg t.i.d. for 2 weeks	Method of randomisation and concealment of allocation stated. Double-blind. Antidepressant therapy allowed.