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TITLE PAGE

Title: Systematic Review with Meta-analysis: Efficacy of Faecal Microbiota Transplantation for the Treatment of Irritable Bowel Syndrome.

Short "running" title: Meta-analysis of FMT for IBS.

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Abbreviations:

CI	confidence interval
FMT	faecal microbiota transplantation
IBS	irritable bowel syndrome
IBS-C	IBS with constipation
IBS-D	IBS with diarrhoea
IBS-M	mixed stool pattern IBS
NNH	number needed to harm

NNT	number needed to treat
RCT	randomised controlled trial
RR	relative risk

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SUMMARY

Background: Increasing evidence supports the role of the gut microbiota in the aetiology of irritable bowel syndrome (IBS). Faecal microbiota transplantation (FMT) is a highly effective treatment against recurrent *Clostridioides difficile* infection in randomised controlled trials (RCTs), and may be beneficial in ulcerative colitis. However, its efficacy in IBS is uncertain.

Aims: To perform a systematic review and meta-analysis to examine this issue.

Methods: We searched MEDLINE, EMBASE, EMBASE Classic, the Cochrane Central Register of Controlled Trials, and clinicaltrials.gov through to March 2019. RCTs recruiting adults with IBS, which compared FMT with placebo, were eligible. Dichotomous symptom data were pooled to obtain a relative risk (RR) of remaining symptomatic after therapy, with a 95% confidence interval (CI).

Results: The search strategy identified 322 citations. Five RCTs were eligible for inclusion, containing 267 patients. Overall, 92.2% of included patients had IBS-D or IBS-M, and only 7.8% IBS-C. When data were pooled for all patients, irrespective of stool type, the RR of IBS symptoms not improving was 0.98 (95% CI 0.58-1.66). Placebo capsules administered orally were superior to capsules containing donor stool in two pooled trials (RR = 1.96; 95% CI 1.19-3.20). FMT from donor stool delivered via colonoscopy was superior to autologous stool in two pooled RCTs (RR = 0.63; 95% CI 0.43-0.93). FMT from donor stool via nasojejunal tube showed a trend towards a benefit over autologous stool in one trial (RR = 0.69; 95% CI 0.46-1.02).

Conclusions: Fresh or frozen donor stool delivered via colonoscopy or nasojejunal tube may be beneficial in IBS. Larger, more rigorously conducted trials of FMT in IBS are needed.

INTRODUCTION

Irritable bowel syndrome (IBS) is a chronic functional bowel disorder, with a prevalence of 10% globally. [1] The diagnosis is made based on the presence of recurrent abdominal pain related to defaecation, in association with an alteration in either stool form or stool frequency. [2] Although the pathophysiology of IBS is still poorly understood, known proposed aetiologies that could contribute to the development of the disease include genetics, low-grade inflammation, increased gut permeability, abnormal biliary and serotonin metabolism, central neurologic dysfunction, altered gastrointestinal motility, visceral hypersensitivity, and changes in the composition of the gut microbiota. [3]

The hypothesis that the gut microbiota is involved in the pathophysiology of IBS is supported by a wealth of clinical data. Epidemiological surveys demonstrate, consistently, that a considerable proportion of patients develop IBS following an acute episode of infectious gastroenteritis, so-called "post-infection" IBS. [4-6] Moreover, some investigators have demonstrated that patients with suspected IBS may have evidence of small intestinal bacterial overgrowth on hydrogen breath testing, [7-10] and antibiotic therapy appears to improve symptoms in some of these patients. [8, 11]

Additionally, therapeutic modulators of the gut microbiota have beneficial effects in unselected patients with IBS. Rifaximin, a minimally absorbable antibiotic, has been shown to be effective in randomised controlled trials (RCTs) in both IBS with diarrhoea (IBS-D) and IBS with mixed stool pattern (IBS-M), [12-15] for both global symptoms and bloating. Numerous different probiotic mixtures and strains have also been evaluated in patients with IBS over the last 15 years. A recent meta-analysis reported a beneficial effect of *Lactobacillus plantarum* DSM 9843, *Escherichia coli* DSM1752, *Streptococcus faecium*, and specific multi-strain probiotic formulations, although the evidence was not robust enough to

make any conclusive recommendations as to which individual species or strain was most effective. [13]

This clinical evidence for a role of the gut microbiota in IBS is reinforced by available microbiological data. Firstly, several studies have demonstrated that the gut microbiota are altered in patients with IBS, compared with healthy subjects. [16-20] Secondly, and more recently, one group of investigators has demonstrated a specific microbiota profile that appears to be associated with the severity of IBS symptoms. [21] This profile was also shown to predict clinical response to a diet low in fermentable oligo-, di-, and mono-saccharides, and polyols in a RCT. [22] Finally, preliminary data suggest that rifaximin, apart from having clinical efficacy in IBS, is able to influence the level of beneficial microbes. In one study, abundance of the short-chain fatty acid producer *Faecalibacterium prausnitzii* was increased in patients with IBS-D or IBS-M following rifaximin, [23] suggesting that manipulation of the gut microbiota could be a therapeutic approach to managing IBS symptoms.

Among other potential strategies to modulate the gut microbiota, faecal microbiota transplantation (FMT) has been shown to be a highly effective treatment against recurrent *Clostridioides difficile* infection, [24, 25] and its efficacy has also been investigated for the treatment of non-infectious gastrointestinal disorders in which there may be a role of the gut microbiota, including ulcerative colitis. [26-29] Some RCTs of FMT in IBS have been published in very recent years, [30-32] so we performed a systematic review and meta-analysis to summarise all the evidence for its efficacy in IBS.

MATERIALS AND METHODS

Search Strategy and Study Selection

A literature search was performed using EMBASE and EMBASE Classic (1947 until March 2019), and MEDLINE (1946 until March 2019), and the Cochrane Central Register of Controlled Trials. We also searched clinicaltrials.gov for unpublished trials, or supplementary data for potentially eligible studies. RCTs comparing the effect of FMT with placebo in adult patients (≥18 years) with IBS were eligible for inclusion, including the first period of cross-over RCTs, prior to cross-over to the second treatment.

Eligibility criteria, which were defined prospectively, are provided in Table 1. The diagnosis of IBS could be based on either a physician's opinion or accepted symptom-based diagnostic criteria, supplemented by investigations to exclude organic disease, where deemed necessary. Subjects were required to be followed up for ≥1 week, and studies had to report a global assessment of IBS symptom cure or improvement after completion of therapy. Preferably this was patient-reported, but if this was not recorded then as documented by the investigator or via questionnaire data. Where studies did not report these types of dichotomous 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 medical literature was searched using the following terms: *irritable bowel syndrome* and *functional diseases, colon* (both as medical subject heading 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: *faecal microbiota transplantation*, *fecal microbiota transplantation*, *faecal* adj5 *transplant*, *fecal* adj5 *transplant*, *faecal* adj5 *therapy*, *fecal* adj5 *therapy*, *faecal microbiota transfer*, *fecal microbiota transfer*, *faecal microbial transplant*, *fecal microbial transplant*, *stool* adj 5

transplant, *stool* adj5 *transfer*, *stool* adj5 *transplantation*, and *stool* adj5 *therapy* (as free text terms).

We did not restrict eligibility to studies published only in English. All titles and abstracts from the search were screened for potential eligibility by two investigators, and those that appeared relevant were retrieved and examined in more detail. Foreign language papers were translated, where necessary. In order to identify potentially eligible studies published only in abstract form, conference proceedings (Digestive Diseases Week, American College of Gastroenterology, United European Gastroenterology Week, and the Asian Pacific Digestive Week) between 2001 and 2018 were also hand-searched. Finally, a recursive search was performed, using the bibliographies of all obtained articles. Eligibility assessment was performed independently by two reviewers (GI and LHE), using pre-designed eligibility forms, with any disagreements resolved by the opinion of a third reviewer (ACF).

Outcome Assessment

The primary outcomes assessed were the effects of FMT compared with placebo on global IBS symptoms at study end. Secondary outcomes included assessing efficacy according to method of administration of the intervention, route of administration of the intervention (upper or lower gastrointestinal tract), type of placebo used (inactive placebo or autologous stool), and IBS subtype, as well as adverse events (overall numbers, as well as individual adverse events, including constipation, diarrhoea, headache, abdominal pain, abdominal distension, or nausea, if sufficient studies reported these data).

Data Extraction

Data were extracted independently by two reviewers (GI and LHE) on to a Microsoft Excel spreadsheet (XP professional edition; Microsoft, Redmond, WA, USA) as dichotomous outcomes (response or no response to therapy), again with any discrepancies resolved by the opinion of a third investigator (ACF). The following data were collected for each study: country of origin; number of centres; FMT modality and number of FMT treatments; 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; duration of follow-up; proportion of female patients; and proportion of patients according to predominant stool pattern (IBS with constipation (IBS-C), IBS-D, or IBS-M). We also recorded the handling of the control arm. Data were extracted as intention-to-treat analyses, with drop-outs assumed to be treatment failures (i.e. no response to therapy), wherever trial reporting allowed. If this was not clear from the original article, we performed an analysis on all patients with reported evaluable data. We obtained further information, if necessary, directly from original investigators.

Quality Assessment and Risk of Bias

Two investigators performed this independently at the individual study level, using the Cochrane risk of bias tool. [33] Any disagreements were resolved by discussion. We recorded the method used to generate the randomisation schedule and conceal treatment 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, [34] to provide a more conservative estimate of the range of effects of FMT in IBS. The impacts of different interventions were expressed as a relative risk (RR) of global IBS symptoms not improving with intervention compared with control, with 95% confidence intervals (CI). RRs were also used to summarise adverse events data. We planned to calculate the number needed to treat (NNT) and the number needed to harm (NNH), with 95% CIs, using the formula NNT or NNH = 1 / (control event rate x (1 – RR)).

Heterogeneity is variation between individual study results arising because of either differences in study participants or methodology. We assessed this using both the I^2 statistic with a cut off ≥50%, and the chi-squared test with a P value <0.10, used to define a significant degree of heterogeneity. [35] Review Manager version 5.3.5 (RevMan for Windows 2014, the Nordic Cochrane Centre, Copenhagen, Denmark) and StatsDirect version 3.1.20 (StatsDirect Ltd, Sale, Cheshire, England) were used to generate Forest plots of pooled RRs for primary and secondary outcomes with 95% CIs, as well as funnel plots. We planned to assess the latter for evidence of asymmetry, and therefore possible publication bias or other small study effects, using the Egger test, [36] if there were sufficient (≥10) eligible studies included in the meta-analysis, in line with published recommendations. [37]

RESULTS

The search strategy identified 322 citations. From these we identified 23 that appeared to be relevant to the study question. There were five articles that fulfilled the eligibility criteria, representing five separate trials containing 267 subjects (Figure 1). [30-32, 38, 39] Overall, 92.2% of included patients had IBS-D or IBS-M, and 7.8% IBS-C. Two trials compared capsules containing donor stool with placebo capsules delivered orally, [30, 38] two trials compared an infusion of donor stool with a placebo of the autologous stool delivered via colonoscopy, [31, 32] and one trial compared an infusion of donor stool with a placebo of the autologous stool delivered via a nasojejunal tube. [39] Agreement between investigators for assessment of study eligibility was perfect (kappa statistic = 1). Detailed characteristics of all included studies are provided in Table 2. No trials were at low risk of bias across all domains, and four did not report a true intention-to-treat analysis (Table 3). [30-32, 39]

Efficacy of FMT in IBS

All five trials provided dichotomous data for response or non-response to FMT. [30-32, 38, 39] When data were pooled there were 79 (50.0%) of 158 patients assigned to FMT who failed to respond, compared with 56 (51.4%) of 109 assigned to placebo. The RR of IBS symptoms not improving after FMT versus placebo was 0.98 (95% CI 0.58 to 1.66), with significant heterogeneity detected between studies ($I^2 = 78\%$, P = 0.001) (Figure 2). There were too few studies to assess for funnel plot asymmetry.

We performed a subgroup analysis according to method of administration of the intervention. Placebo capsules were superior to capsules containing donor stool when data were pooled from two trials, [30, 38] containing 100 patients, with 32 (64.0%) of 50 patients assigned to capsules containing donor stool failing to respond to therapy, compared with 16 (32.0%) of 50 allocated to placebo capsules (RR = 1.96; 95% CI 1.19 to 3.20, $I^2 = 14\%$, P =

0.28). FMT from donor stool delivered via colonoscopy was superior to autologous stool when data were pooled from two RCTs, [31, 32] with 26 (39.4%) of 66 patients randomised to donor stool failing to respond to therapy, compared with 24 (64.9%) of 37 receiving autologous stool (RR = 0.63; 95% CI 0.43 to 0.93, $I^2 = 0\%$, P = 0.71). The NNT was 4 (95% CI 3 to 22). There were 21 (50.0%) of 42 patients assigned to FMT from donor stool delivered via nasojejunal tube who failed to respond to therapy, compared with 16 (72.7%) of 22 given autologous stool via the same route in one trial (RR = 0.69; 95% CI 0.46 to 1.02). [39]

Further subgroup analyses are provided in Table 4. Analysis according to the route of administration of the intervention demonstrated no benefit via the upper gastrointestinal tract in three pooled studies (RR = 1.35; 95% CI 0.58 to 3.14), [30, 38, 39] but a beneficial effect when the lower gastrointestinal tract was used when data were pooled from two studies (RR = 0.63; 95% CI 0.43 to 0.93, NNT = 4; 95% CI 3 to 22). [31, 32] When type of control intervention used was studied, donor stool demonstrated efficacy over autologous stool in three pooled trials (RR = 0.66; 95% CI 0.50 to 0.87, NNT = 3; 95% CI 3 to 11). [31, 32, 39] However, inert placebo was superior to oral capsules of donor stool when data were pooled from two RCTs (RR = 1.96; 95% 1.19 to 3.20). [30, 38] Three of the trials only recruited patients with IBS-D or IBS-M, [31, 38, 39] and we managed to obtain data for a fourth trial for only those individuals with IBS-D or IBS-M. [32] When data from patients with IBS-D or IBS-M in these four trials were pooled there was no clear benefit of FMT (RR = 0.79; 95% CI 0.54 to 1.15).

Safety of FMT in IBS

Complete adverse events data were provided by three of the trials. [30-32] Of the other two RCTs, one stated that adverse event rates did not differ between groups, [38] and there were no serious adverse events, and the other did not report any adverse events data at all. [39] When data were pooled from the three RCTs, there were 29 (30.9%) of 94 patients assigned to FMT

who reported at least one adverse event, compared with 25 (38.5%) of 65 allocated to placebo. The RR of adverse events with FMT versus placebo was 0.93 (95% CI 0.45 to 1.92), with significant heterogeneity detected between studies ($I^2 = 61\%$, P = 0.08) (Figure 3). Individual adverse events were incompletely reported by individual RCTs.

DISCUSSION

This systematic review and meta-analysis has evaluated the efficacy of FMT in the treatment of IBS, synthesizing evidence from the available RCTs conducted to date. Five trials fulfilling inclusion criteria were identified and eligible. When data from all studies were pooled, there was no significant improvement in IBS symptoms with FMT versus placebo. When data were pooled from studies reporting adverse events, no severe adverse events were reported; total adverse events were more frequent among placebo patients than among those assigned to FMT, although this difference was not statistically significant. We were able to examine the impact of the method and route of administration of FMT, and the type of intervention used in the control arm. The pooled result may have been influenced by the method and route of administration of FMT. Donor stool delivered via colonoscopy or nasojejunal tube seemed to be more effective than autologous stool delivered via the same route, whereas no beneficial effect was found when a stool capsule was compared with a placebo capsule. Subgroup analyses also revealed a potential benefit on IBS symptoms when FMT was administered via the lower gastrointestinal tract, but no benefit was observed when the upper gastrointestinal tract was used.

We used a contemporaneous and exhaustive search strategy, in order to identify all potentially relevant RCTs, two of which were published only in abstract form. [38, 39] The judging of eligible studies and the data extraction were carried out by two investigators independently, with discrepancies resolved by consensus. We also contacted authors of included studies to obtain additional data, where required. Finally, a random effects model was used to pool data, in order to provide a more conservative estimate of the range of efficacy of FMT in IBS.

Our systematic review has some limitations, mainly due to the characteristics of the published literature identified. Our analyses are limited by the low number of available

studies, and the quality of the reported data, as none of the trials we identified was at low risk of bias. Most studies were undertaken in Europe, except for one trial performed in the US, limiting generalisability. Interestingly, despite some of the pioneers of FMT being based in Australia, [40] there were no RCTs from this geographic region. Another limitation was the variability between trials in terms of working protocols including route of delivery, population of patients with IBS recruited, comparator intervention, and criteria used to define symptomatic response to FMT. This may have contributed to the significant heterogeneity observed when pooling data from all studies. This heterogeneity appeared to resolve in some of our subgroups analyses, although with only two or three studies included in most of these analyses, an alternative explanation would be a reduction in power to detect any heterogeneity.

The fact that there was no significant improvement in IBS symptoms with FMT when all studies were pooled could be explained by several factors. First, the role of gut microbiota in contributing to IBS symptoms is still uncertain. Although a distinct microbial profile of subjects with IBS has been identified by one group of investigators, these findings should be considered as preliminary only. [21] This uncertainty may limit enthusiasm towards manipulating the microbiota as a treatment for IBS, as the therapeutic target is still not clear. Second, as mentioned above, the different working protocols of the included studies, including route of delivery of FMT, choice of control intervention, and the IBS subtypes studied may have contributed to the lack of efficacy overall. Finally, the use of bowel lavage may also have affected the results of individual trials. Only the three fully published studies, two of which used the lower gastrointestinal route of administration, reported that they used bowel lavage as part of their FMT protocol. [30-32] There is evidence to suggest that bowel lavage itself can alter the faecal microbiota in healthy individuals, [41, 42] although whether this in itself has any effect on symptoms in people with IBS is uncertain.

In our subgroup analyses, there appeared to be a beneficial effect of FMT on IBS symptoms when administration occurred via the lower gastrointestinal tract, but no benefit was observed when delivery was via the upper gastrointestinal tract. The lack of efficacy of the upper gastrointestinal route could be explained by the potential for the development of small intestinal bacterial overgrowth after delivery of faeces into the jejunum or ileum although, as hydrogen breath testing was not performed in these studies, this is speculative. There was also no benefit according to IBS subtype, although we grouped patients with IBS-D and IBS-M together, and were not able to provide a meaningful estimate of efficacy in patients with IBS-C due to the small numbers of patients enrolled with the latter.

Another finding from our subgroup analyses was the apparently higher efficacy of donor stool than autologous stool, but not than an inert placebo capsule. Perhaps the results in individual studies would have been different if either an inert placebo was used, rather than autologous stool, or if a capsule containing autologous stool had been used, rather than a placebo capsule. The use of autologous stool as a placebo has been debated, as some studies have suggested it is as effective as donor FMT in inducing remission in ulcerative colitis, [28] and achieves cure rates in excess of 60% for recurrent *C. difficile* infection in RCTs. [43] In patients with IBS, however, a delivery of concentrated autologous microbiota, which is dysbiotic by definition, may have exacerbated symptoms in a subset of patients. Moreover, efficacy data were not grouped for different IBS subtypes in any study. This could represent a limitation, as using the same microbiota to treat different groups of symptoms is potentially unsound.

An alternative explanation for our findings could be the lack of standardisation of the selected donors. In most studies, it appeared that donors were recruited without checking their microbial profile. Only one study selected the potential donor with the highest

abundance of butyryl-CoA transferase gene in their faeces, [32] due to the fact that some investigators have reported that abundance of butyrate-producing bacteria is reduced in patients with IBS, compared with health individuals. [44] As two of the studies were only published in abstract form they may have checked microbial profiles of donors, but not reported this. [38, 39] A non-standardised approach works well in *C. difficile* infection, where the gut microbiota is already profoundly disturbed, and a healthy microbial biomass is therefore effective in curing the disease. However, it may not be as effective in IBS, where specific microbiota signatures that characterise different clusters of patients with IBS, [45] or that have been associated with disease severity, [21] have been identified, and specific microbial changes have been associated with symptoms relief after FMT. [46] Finally, in all studies, treatment with FMT lasted at most 12 days. Although a single FMT treatment usually works in recurrent *C. difficile* infection, [47] which is an acute infectious disease, it may not be enough to be effective for the treatment of a chronic disorder, such as IBS. Repeated administration of FMT led to successful induction of remission in ulcerative colitis in two RCTs, [27, 48] although all advantages given by donor FMT over autologous FMT were lost after less than 5 months in patients with metabolic syndrome. [49]

Another group have just published a meta-analysis examining this issue. [50] However, the authors of this meta-analysis only identified four RCTs, recruiting 254 patients. They did not identify the study by Holster *et al.*, [32] although it would have been available in abstract form at the time they conducted their searches. This meant that they only included data from one RCT using colonoscopy to administer the FMT. In addition, they included data from four patients with microscopic colitis detected after histological analysis of random colonic biopsies in one of the eligible trials, [31] whose data we excluded from our analysis.

In conclusion, this systematic review and meta-analysis showed no advantage of FMT

over placebo in relieving symptoms in patients with IBS when data from all RCTs were considered, although the lower gastrointestinal route of delivery may be effective. The disparity in individual trial results is likely to be explained by differences in working protocols, and by the lack of a personalised approach to modulating the microbiota in these RCTs. However, there have been only a few eligible trials conducted to date, so it is difficult to draw any firm conclusions. Future studies should test FMT in more specific groups of patients with IBS, such as those with a predominant stool type, and report efficacy according to recommended composite symptom-based endpoints. They should also include in their design a study of the microbial profiles of donors and patients, in order to find a match between them and tailor the treatment accordingly. Finally, an approach that uses long-term administration will be required to understand more clearly whether there is a role for FMT in treating IBS.

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Guarantor of the article: ACF is guarantor.

Specific author contributions: GI, LHE, CJB, and ACF conceived and drafted the study. GI, LHE, CJB, and ACF collected all data. ACF, CJB, and LHE analysed and interpreted the data. LHE, GI, CJB, AG, GC and 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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FIGURES

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

Figure 2. Forest Plot of Randomised Controlled Trials of Faecal Microbiota Transplantation Versus Placebo in Irritable Bowel Syndrome.

Figure 3. Forest Plot of Adverse Events in Randomised Controlled Trials of Faecal Microbiota Transplantation Versus Placebo in Irritable Bowel Syndrome.

Table 1. Eligibility Criteria.

Randomised controlled trials.
Adults (participants aged ≥18 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 faecal microbiota transplantation with placebo.
Minimum duration of follow-up 7 days.
Dichotomous assessment of response to therapy in terms of effect on global IBS symptoms following therapy.†

*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. Characteristics of Randomised Controlled Trials of Faecal Microbiota Transplantation Versus Placebo in Irritable Bowel Syndrome.

Study	Country and Number of Centres	Diagnostic Criteria Used for IBS and Subtypes of IBS Recruited	Primary Endpoint Used to Define Symptom Improvement Following Therapy and Time of Assessment	Number of Patients (% Female)	Active Intervention (Number of Patients)	Control Intervention (Number of Patients)
Aroniadis 2018 [38]	USA, 3 sites	Rome III criteria, 100% IBS-D	Decrease in IBS severity scoring system of ≥50 points at 12 weeks	48 (37.5)	25 FMT capsules containing 50g of donor stool per day for 3 days (24)	25 placebo capsules per day for 3 days (24)
Halkjaer 2018 [30]	Denmark, 2 sites	Rome III criteria, 33.3% IBS-C, 29.4% IBS-D, 37.3% IBS-M	Decrease in IBS severity scoring system of ≥50 points at 12 weeks	52 (68.6)	25 FMT capsules containing 50g of donor stool per day for 12 days (26)	25 placebo capsules per day for 12 days (26)
Holvoet 2018 [39]	Belgium, 1 site	Rome III criteria, 100% IBS-D or IBS-M	Self-reported adequate relief of symptoms at 12 weeks	64 (not reported)	Single FMT consisting of donor stool via nasojejunal tube (42)	Autologous stool via nasojejunal tube (22)

Johnsen 2018 [31]	Norway, 1 site	Rome III criteria, 53.0% IBS-D, 47.0% IBS-M	Decrease in IBS severity scoring system of ≥75 points at 12 weeks	86 (66.3)	Single FMT consisting of 50 to 80g of donor stool via colonoscopy (58)	50 to 80g of autologous stool via colonoscopy (28)
Holster 2019 [32]	Sweden, 1 site	Rome III criteria, 25.0% IBS-C, 56.2% IBS-D, 18.8% IBS-M	Decrease in gastrointestinal symptom rating scale-IBS of ≥30%	17 (50.0%)	Single 150mL FMT, which contained 30g of donor stool mixed with 0.9% sterile saline and 10% glycerol, via colonoscopy (8)	Single 150mL FMT, which contained 30g of autologous stool mixed with 0.9% sterile saline and 10% glycerol, via colonoscopy (9)

Table 3. Risk of Bias of Randomised Controlled Trials of Faecal Microbiota Transplantation Versus Placebo in Irritable Bowel Syndrome.

Study	Method of Generation of Randomisation Schedule	Method of Concealment of Treatment Allocation	Blinding	Evidence of Incomplete Outcomes Data	Evidence of Selective Reporting of Outcomes
Aroniadis 2018 [38]	Low	Unclear	Low	Low	Low
Halkjaer 2018 [30]	Low	Low	Low	High	Low
Holvoet 2018 [39]	Unclear	Unclear	Low	High	Low
Johnsen 2018 [31]	Low	Low	Low	High	Low
Holster 2019 [32]	Low	Low	Low	High	Low

Table 4. Subgroup Analyses of Randomised Controlled Trials of Faecal Microbiota Transplantation Versus Placebo in Irritable Bowel Syndrome.

	Number of trials	Number of patients	Relative risk of IBS symptoms not improving (95% confidence interval)	I^2 (P value)	NNT (95% CI)
Method of administration of the intervention					
Oral capsule [30, 38]	2	100	1.96 (1.19 to 3.20)	14% (0.28)	3 (1 to 16)
Colonoscopy [31, 32]	2	103	0.63 (0.43 to 0.93)	0% (0.71)	4 (3 to 22)
Nasojejunal tube [39]	1	64	0.69 (0.46 to 1.02)	N/A	N/A
Route of administration of the intervention					
Upper gastrointestinal tract [30, 38, 39]	3	164	1.35 (0.58 to 3.14)	85% (0.001)	N/A
Lower gastrointestinal tract [31, 32]	2	103	0.63 (0.43 to 0.93)	0% (0.71)	4 (3 to 22)
Type of placebo used					
Inactive placebo [30, 38]	2	100	1.96 (1.19 to 3.20)	14% (0.28)	3 (1 to 16)
Autologous stool [31, 32, 39]	3	167	0.66 (0.50 to 0.87)	0% (0.89)	4 (3 to 11)

N/A; not applicable