THE EFFICACY OF FAECAL MICROBIOTA TRANSPLANTATION FOR THE TREATMENT OF IRRITABLE BOWEL SYNDROME: A SISTEMATIC REVIEW AND META-ANALYSIS

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A dissertation submitted in partial fulfillment of the requirements for the Degree of Masters in Metabolism and Human Nutrition

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ABREVIATIONS

AE	Adverse events
CDI	*Clostridiodes difficile* infection
CI	Confidence Interval
FMT	Faecal Microbiota Transplantation
GI	Gastrointestinal
GRADE	Grading of Recommendations Assessment, Development and Evaluations
IBS	Irritable Bowel Syndrome
IBS-C	Irritable Bowel Syndrome Constipation-predominant type
IBS-D	Irritable Bowel Syndrome Diarrhea-predominant type
IBS-M	Irritable Bowel Syndrome Mixed type
IBS-SSS	Irritable bowel Syndrome Severity Scoring System
IBS-U	Irritable Bowel Syndrome Unclassified type
MeSH	Medical Subject Headings
mo	month
OR	Odds Ratio
p	*P*-value
PICO	Population, Intervention, Comparator and Outcomes
PRISMA	Preferred Reporting Items for Systematic Review and Meta-Analyses
PROSPERO	International Prospective Register of Systematic Reviews
QOL	Quality Of Life
RCT	Randomised Controlled Trial
RR	Relative Risk
wk	week

ABSTRACT

Introduction: With a prevalence of approximately 4-10% worldwide, irritable bowel syndrome (IBS) is a common gastrointestinal disease characterised by abdominal pain and altered bowel habits. Despite the substantial effect on patient's quality of life and high costs to health care services, new treatment strategies for IBS are relatively poor.

Objective: To clarify the effectiveness of faecal microbiota transplantation (FMT) in the treatment of IBS and, importantly, to identify which is the most clinically efficient administration procedure.

Design: A literature search was carried out on Cochrane, MEDLINE, Scopus and Web of Science databases. Randomised controlled trials (RCTs) recruiting adult patients with IBS that compared FMT with placebo were eligible. Data were pooled to obtain a relative risk (RR) of symptom improvement, with a 95% confidence interval (CI). The quality of the evidence for each outcome was assessed using Grading of Recommendations Assessment, Development and Evaluation (GRADE) system.

Results: Seven RCTs (489 participants with age range between 25 and 63) met the eligibility requirements for our systematic review and meta-analysis. Although FMT seems not to be effective in global improvement of IBS symptoms, subgroup analysis shows that FMT through gastroscopy or nasojejunal tube are effective on IBS treatment (RR 3.03; 95% CI 1.94-4.73; I^2 = 10%, $p < 0.00001$). When considering non-oral FMT administration, patients with constipation symptoms are likely to benefit from FMT administration for IBS treatment ($p = 0.003$ for the difference between subgroups). Fresh faecal material and bowel preparation seem also to have an impact on FMT efficacy ($p = 0.03$ and $p = 0.01$, respectively).

Conclusion: Our meta-analysis revealed, for the first time, a set of critical steps with potential impact on the effectiveness of FMT as clinical procedure to treat IBS.

Keywords: faecal microbiota transplantation, irritable bowel syndrome, intestinal microbiota, meta-analysis.

RESUMO

Introdução: Com uma prevalência mundial de aproximadamente 4-10%, a síndrome do intestino irritável (SII) é uma doença gastrointestinal caracterizada por dor abdominal e hábitos intestinais alterados. Apesar do seu efeito substancial na qualidade de vida do paciente e dos elevados custos associados, as novas estratégias de tratamento para a SII são ainda escassas.

Objetivo: Clarificar a eficácia do transplante de microbiota fecal (TMF) no tratamento da SII e, sobretudo, identificar qual o procedimento de administração clinicamente mais eficiente.

Desenho: Foi realizada uma pesquisa bibliográfica nas bases de dados Cochrane, MEDLINE, Scopus e Web of Science. Ensaios clínicos controlados e aleatorizados recrutando pacientes adultos com SII que compararam o TMF com placebo, foram elegíveis. Os dados foram agrupados para obter um risco relativo (RR) de melhoria dos sintomas, com um intervalo de confiança (IC) de 95%. A qualidade da evidência para cada resultado foi avaliada usando o sistema Grading of Recomendations Assessment, Development and Evaluation (GRADE).

Resultados: Sete estudos (489 participantes com faixa etária entre os 25 e os 63 anos) cumpriram os requisitos de elegibilidade da revisão sistemática e foram incluídos na meta-análise. Embora os resultados da meta-análise apresentem uma aparente ineficácia do transplante fecal na melhoria dos sintomas de SII, a análise por subgrupos revela que o TMF administrado por gastroscopia ou tubo nasojejunal é eficaz no tratamento da SII (RR 3.03; IC 95% 1.94-4.73; I^2 = 10%, $p < 0.00001$). Além disso, excluindo a intervenção com administração por cápsulas, verificou-se que os pacientes com sintomas de obstipação provavelmente beneficiam do TMF no tratamento da SII ($p = 0.003$ para a diferença entre os subgrupos). A utilização de material fecal fresco e a preparação intestinal demonstraram também ter impacto na eficácia do TMF ($p = 0.03$ e $p = 0.01$, respetivamente).

Conclusão: Esta meta-análise revelou, pela primeira vez, um conjunto de fatores com potencial impacto na eficácia do TMF como procedimento clínico no tratamento da SII.

Palavras-chave: transplante de microbiota fecal, síndrome do intestino irritável, microbiota intestinal, meta-análise

CONTENTS

TABLE LIST

FIGURE LIST

1. INTRODUCTION

1.1 Irritable bowel syndrome

1.1.1 *Definition, prevalence, and its impact on patient quality of life*

Irritable bowel syndrome (IBS) can be defined as a symptom-based functional bowel disorder characterized by abdominal pain and altered bowel habits in the absence of detectable structural or biochemical abnormalities[1]. With a prevalence of approximately 4-10% worldwide[2,3], IBS is one of the most prevalent gastrointestinal (GI) disorders and a cause of substantial burden to healthcare services and society[4]. This syndrome is one of the primary causes for consultations in gastroenterology outpatient clinics, as well as in primary care, and is the most common reason for referral to gastroenterology clinics[5]. Due to its relapsing and chronic nature, this condition can impact patient's social interactions, reduce health-related quality of life and lower the work productivity[5,6].

1.1.2 Diagnostic criteria

Since patients with IBS do not have readily identifiable underlying structural abnormalities, the diagnosis is made based on a symptom-based diagnostic criteria, the Rome criteria[1], as recurrent abdominal pain, on average for at least 1 day per week, associated with 2 or more of the following: related to defecation; a change in frequency of stool, and a change in form (appearance) of stool (table 1). Criteria must be fulfilled for the last 3 months with symptom onset at least 6 months before diagnosis[1]. Based on the dominant stool form or consistency, as assessed by the Bristol Stool Form Scale[7] (table 2), IBS can be classified into 4 subtypes: diarrhea-predominant type (IBS-D), constipation-predominant type (IBS-C), mixed type (IBS-M) or unclassified type (IBS-U)[5].

Additionally, IBS patients can be further classified as sporadic (nonspecific), post-infectious or inflammatory bowel disease-associated IBS. In contrast to sporadic IBS, post-infectious IBS occurs after an episode of infectious gastroenteritis[8]; and inflammatory bowel disease-associated IBS indicates IBS-like symptoms in patients with clinically quiescent inflammatory bowel disease[9].

Table 1.The Rome IV criteria for IBS[1].

1A. Recurrent abdominal pain, on average, at least 1 day per week in the last 3 months and associated with two or more or the following:

a. Related to defecation;

b. Associated with a change in frequency of stool;

c. Associated with a change in form of stool.

1B. Criteria fulfilled for the last 3 months with symptom onset at least 6 months before diagnosis:

IBS-C	IBS-D	IBS-M	IBS-U
≥25% of bowel movements of Bristol stool form types 1 or 2, and <25% of Bristol stool form types 6 or 7.	≥25% of bowel movements of Bristol stool form types 6 or 7, and <25% of Bristol stool form types 1 or 2.	≥25% of bowel movements of Bristol stool form types 1 or 2, and ≥25% of bowel movements of Bristol stool form types 6 or 7.	Patients who meet criteria for IBS, but who do not fall into one of the other three subgroups according to Bristol stool form type.

Abbreviations: IBS-C: IBS Constipation predominant-type; IBS-D: IBS Diarrhea predominant-type; IBS-M: IBS mixed type; IBS-U: IBS unclassified type. Adapted from *Gut 2021;70:1214-1240*[10].

Table 2. Bristol stool form scale[7].

Type 1	Separate hard lumps, like nuts (hard to pass).
Type 2	Sausage-shaped but lumpy.
Type 3	Like a sausage but with cracks on its surface.
Type 4	Like a sausage or snake, smooth and soft.
Type 5	Soft blobs with clear-cut edges (passed easily).
Type 6	Fluffy pieces with ragged edges, a mushy stool.
Type 7	Watery, no solid pieces.

Adapted from *Aliment Pharmacol Ther 2016;44(7):693-703*[7].

1.1.2 *Etiology and pathophysiology*

IBS etiology is broad and not clearly understood[11]. This syndrome is currently described as a disorder of disturbed gut–brain interactions that involves a complex interaction between genetic, psychological and environmental factors that lead to GI motility dysfunction and altered visceral sensations[11,12]. Evidence suggests that several different underlying mechanisms are involved in IBS pathophysiology. Altered gut-brain interactions, visceral hypersensitivity[13] and psychosocial distress[5] seem to play an important role. Recent studies have also suggested intestinal immune activation and increased permeability[14], food hypersensitivity[15], and altered microbiome[16] as important factors that may contribute to IBS pathogenesis (figure 1).

***Figure 1**. An overview of important pathophysiological factors in IBS.*

In fact, not only intestinal dysbiosis has been recognized by the Rome Foundation Working Team as a plausible contributing aspect to this condition[17], but also several studies have shown an altered microbiota composition in patients with IBS, supporting an important role of the intestinal microbiota on IBS etiology[16,18–20]. Patients with severe IBS have been shown to have lower microbial richness and abundance of methane-producing *Methanobacteriales* and *Prevotella* enterotypes, and an increased abundance of Bacteroides enterotype[16,20]. Tap *et al.*[16] also found IBS symptom severity to be negatively associated with microbial richness, presence of methanogenic microorganisms, exhaled CH4, and abundance of *Prevotella* species. Furthermore, there is evidence suggesting that *Clostridiodes difficile* infection (CDI) can trigger IBS. According to a recent systematic review, after CDI diagnosis over 20% of patients develop post-infectious IBS (PI-IBS) symptoms such as diarrhea and abdominal discomfort[21]. However, despite symptoms similarities with IBS-D, PI-IBS is associated with distinct pathophysiologic alterations including gut microbiota alterations[22]. This heterogeneity created significant challenges in the development of effective therapeutic strategies[12]. Due to this, and to the fact the intestinal microbiota plays a key role in intestinal immunity and inflammation[23,24], manipulation of its composition has been proposed as a treatment strategy for IBS.

1.1.3 Current treatment recommendations for IBS

According to the latest British guidelines on the management of irritable bowel syndrome[10], the treatment of IBS should be directed towards the predominant symptom, or symptoms, experienced by the patient and should follow a sequence that begins with dietary and lifestyle style advice. If symptoms persist, first-line and second-line pharmacological treatments are recommended according to the corresponding IBS subtypes[10], as detailed in figure 2.

Dietary and lifestyle style advice: First, regular exercise is recommended, as there is some evidence from RCTs that it can be beneficial for IBS patients[25,26], particularly those with constipation[25]. Second, treatment should continue with general dietary recommendations, such as emphasizing the importance of eating regular meals, limiting alcohol and caffeine intake, maintaining an adequate hydration state, reducing processed food consumption and ingesting soluble fibre[10,27]. Probiotics[10] and low FODMAP (fermentable oligosaccharides, disaccharides, monosaccharides and polyols) diets[10,27] may also be considered if general dietary recommendations reveal unsuccessful.

First and second-line treatments: For IBS- M or IBS-U, antispasmodics drugs and peppermint oil can be used as first-line treatments of abdominal pain. If patients fail to respond, central neuromodulators can be used as second-line[10,27]. For IBS-C and IBS-D, laxatives and the anti-diarrheal loperamide can be used as first-line treatments for constipation and diarrhoea, respectively[10,27]. If patients with constipation fail to respond to laxatives, a trial of intestinal secretagogue linaclotide is recomended[10]. For patients with diarrhoea, the 5-hydroxytryptamine- 3 receptor agonists alosetron and ramosetron appear to be the most effective second-line drugs[10,27]. If medical treatment is unsuccessful, patients should be referred for psychological therapy particularly cognitive behavioural therapy and gut-directed hypnotherapy the options with the largest evidence base[10,27].

Despite the variety of available treatments for IBS, current options are often ineffective, and many patients remain unsatisfied with the medical treatment received[28,29]. Therefore, there is a large gap to be fulfilled regarding the development of novel therapeutic options against IBS, and FMT has already been proposed as a promising option for IBS in the future[10,27].

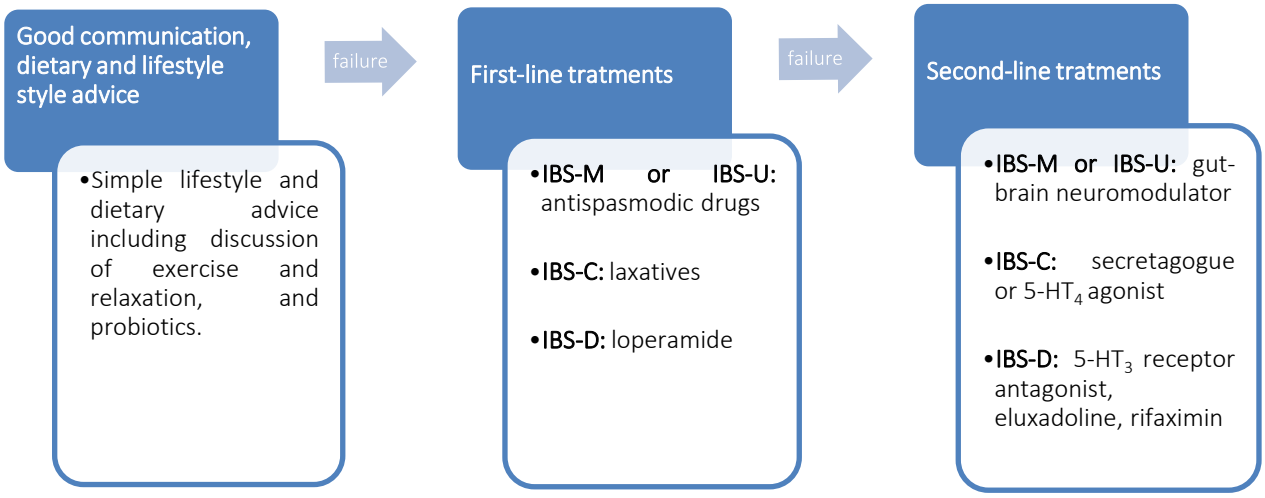

***Figure 2**. Treatment recommendations for IBS, according to the British Society of Gastroenterology[10].*

1.2 Faecal microbiota transplantation

1.2.1 Definition and considerations about regulation and procedure

Faecal microbial transplantation (FMT) is a technique in which faecal material containing a minimally manipulated community of microorganisms is transferred from a donor to a recipient (including autologous transfers) with the intention to restore the diversity of the gut microflora of the latter[30]. FMT can be administered either directly to the colon - via colonoscopy, or less frequently, via flexible sigmoidoscopy or an enema - or to the upper gastrointestinal tract via nasoenteric tubes, gastroscopy, or capsule ingestion[31].

At European and International level, there is no consensus regarding the regulatory status of FMT. Despite this, the European Committee on Organ Transplantation[30] recommends a similar approach to that applied to other tissues and cells in order to ensure quality and safety, with specific recommendations concerning: donor selection and preparation, faeces processing and storage, requirements for implementing a faecal microbiota transplantation centre, recipients preparation, and faecal matter delivery and patients monitoring. These recommendations are overall in line with the general guidelines provided by the European Consensus conference on faecal microbiota transplantation in clinical practice[32], that defines indications and methodologies for the use of FMT in the treatment of CDI (figure 3).

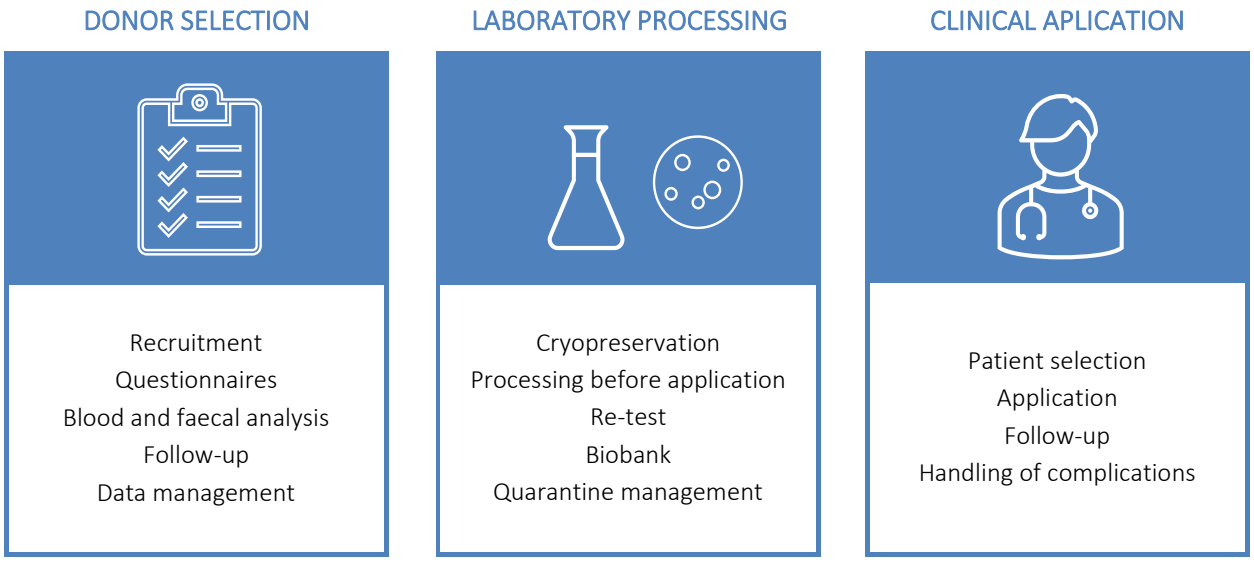

Figure 3. *Clinical application framework for faecal microbiota transplantation, including donor selection, laboratory processing and clinical application. Adapted from Aliment Pharmacol Ther 2016;44(7):693-7037.*

1.2.2 Mechanisms and current indications

The mechanisms behind the efficacy of FMT are not fully understood[33]. The key rationale for using FMT as a treatment is that it restores the gut microbial communities[32]. However, the relative importance of the gut microbiota in the overall pathogenesis is different from one disease to another[33]. For instance, it has been noted that in *Clostridiodes difficile* infection (CDI) changes in the composition of the gut microbiota represent the predominant factor of pathogenesis[34], and in IBD they play a very important role[35]. In fact, based on this concept of repopulating the intestine microbiota, FMT has been proven to be effective for the treatment of CDI[32,36,37], and so far, no major differences have been found between the different FMT administration procedures[37]. Beyond the treatment of CDI, FMT has also been investigated in other disorders associated with the alteration of gut microbiota such as inflammatory bowel disease, including ulcerative colitis[38,39] and Crohn's disease[40,41], metabolic syndrome[42,43], and functional gastrointestinal disorders, such as IBS[44–46].

1.2.3 Current knowledge about the efficacy of faecal microbiota transplantation in irritable bowel syndrome.

The efficacy of FMT as a treatment for IBS remains unclear. Results from the three first systematic reviews and meta-analysis about this subject were inconsistent and did not support a beneficial effect of FMT for IBS treatment[44,47,48], showing though opposite effects according with the type of FMT administration procedure.

Since the publication of the first systematic reviews focusing on the efficacy of FMT on IBS, novel RCTs have been published[49,50], followed by two updated systematic reviews and meta-analyses[46,51] in the last few months. These last two studies showed that FMT may improve the quality of life of IBS patients but is not effective in overall symptoms improvement. Wu *et al.*[46] also revealed that patients may benefit from FMT when administered via colonoscopy or gastroscopy but did not showed statistically significant results, neither analysed colonoscopy and gastroscopy separately.

Overall, none of the recent reviews have provided a detailed subgroup analysis and an in-depth discussion that addresses the various methodological dimensions that can affect the effectiveness of FMT in IBS.

2. AIMS

The aim of this study was to conduct a systematic review and meta-analyses of RCTs to clarify the effectiveness of FMT in the treatment of IBS and, importantly, which is the most clinically efficient administration procedure.

This study also aims to provide a detailed subgroup analysis to assess the impact of methodological factors on the effectiveness of faecal transplantation and narrative synthesis of the findings from the analysed studies.

3. METHODOLOGY

3.1 Design and registration

This systematic review and meta-analysis was developed in accordance with the preferred reporting items for systematic reviews and meta-analyses (PRISMA) statement[52] and the Cochrane Handbook for Systematic Reviews of Interventions[53] guidelines. The protocol was registered (CRD42021252141) in the International Prospective Register of Systematic Reviews (PROSPERO) (appendix 1).

3.2 Selection criteria

We defined inclusion and exclusion criteria in accordance to the PICO (Population, Intervention, Comparator and Outcomes)[54] strategy. Inclusion criteria were: (1) prospective, randomised, double-blind, placebo-controlled trials (parallel group or first arm of cross-over); (2) study populations formed by adult patients older than 16 years old and diagnosed with IBS defined by accepted symptom-based criteria including Manning, Kruis, Rome I, Rome II, Rome III, or Rome IV (Population); (3) studies comparing FMT (Intervention) with a placebo of FMT excipients or an autologous FMT (Comparator); (4) studies reporting improvement in global IBS symptoms (Outcome); and (5) studies with a minimum follow-up period of 1 week. Review articles, systematic reviews, meta-analysis, letters, conference abstracts, case reports, case series, position papers, and authors' replies were excluded. Only studies published in English were included.

3.3 Search strategy

To identify eligible reviews, we searched on MEDLINE, Scopus and Web of Science databases on the July 27th, 20211. Both medical subject headings (MeSH) terms and free text terms referring to fecal microbiota transplantation combined with terms referring to irritable bowel syndrome were used. Search terms for fecal microbiota transplantation were "faecal" or "fecal" or "feces" or "faeces" or "microbiota" or "microflora" or "fecal flora" or "faecal flora," and "transplant" or "transfusion" or "implant" or "donor" or "enema" or "transfer" or "FMT". Search terms for irritable bowel syndrome were "IBS" or "irritable bowel syndrome". The PubMed search strategy was converted to search in other databases (appendix 2).

3.4 Study selection

We used the online application Rayyan[55] to remove duplicates and to screen the remaining articles for eligibility, according to the screening criteria. Two independent reviewers screened both titles and abstracts of articles for relevance; and full-text articles were reviewed when title and abstract did not provide enough information. Once potentially relevant studies were identified, full-text articles were then assessed for eligibility according to previously established criteria (appendix 3). Excluded trials and the reasons for exclusion were recorded, and any disagreement between reviewers was resolved through discussion. The reference lists of the included and excluded articles were screened to ensure that no relevant studies were missed.

3.5 Data extraction

Data items were extracted by two authors using a standard data extraction form (appendix 3). For each study, first author, year of publication, country of origin, sample characteristics, methods, and outcomes were extracted. Data were extracted as intention-to-treat analyses, with dropouts assumed to be no responders to FMT. In cases where information was missing or incomplete, correspondence authors were contacted requesting for further information.

3.6 Risk of bias assessment

Risk of bias of individual studies were assessed by two independent reviewers using the updated Cochrane Risk of Bias (RoB 2.0) tool recommended by Cochrane Collaboration[56]. The following five domains were assessed: (1) bias due to the randomization process, (2) bias due to deviations from intended interventions, (3) bias due to missing outcome data, (4) bias in the measurement of the outcome, and (5) bias in the selection of the reported result. Regarding the evaluation of the third domain (missing bias), 10% of missing and missing above 5% with imbalances between arms were classified with “some concerns”. The overall risk of bias was classified as “high risk”, having “some concerns”, and “low risk”. Reviewers were blinded to each other’s assessment, and disagreements were solved by reaching consensus.

3.7 Quantitative synthesis

Relative risk (RR) was used as an effect measure for the dichotomous variable 'treatment responders'. Effect measures were reported along with the 95% confidence interval (CI).

The Cochran's Q (significance level of 0.1) and I^2 tests were used to assess heterogeneity. According to the Cochrane guidelines[53], the I^2 values were interpreted as follows: 0% to 40% might not be important, 30% to 60% may represent moderate heterogeneity, 50% to 90% may represent substantial heterogeneity, 75% to 100% represent considerable heterogeneity.

Pooled estimates were computed and weighted using generic inverse-variance with random-effect. A *P*-value < 0.05 was considered as statistically significant. Statistical analysis was performed using Review Manager (RevMan), version 5.4, The Cochrane Collaboration, 2020.

3.8 Grading the evidence

Funnel plots were used to assess evidence of publication bias. Quality assessment of the evidence for each outcome was performed by two independent authors using the Grading of Recommendations Assessment, Development and Evaluation (GRADE)[57]. The meta-analysis was scored with a maximum of 10 points, according to (1) risk of bias, (2) precision, (3) heterogeneity, (4) directness, (5) publication bias, (6) funding bias, (7) effect-size, and (8) dose–response. Based on the final score, we classified the quality of the evidence as "high", "moderate", "low", or "very low".

4. RESULTS

4.1 Study selection

The literature search identified 5866 citations, narrowed to 4136 after duplicates were removed. Of these, 3869 abstracts were excluded from the initial screening by not involving FMT and IBS, resulting in 267 abstracts for review. Then, reviewers examined the abstracts and manuscripts based on previously determined eligibility criteria, further excluding 243 references. Of the 24 remaining citations, careful full-text review excluded 17, as detailed in figure 4. Therefore, 7 RCTs [49,50,58–62] (full manuscripts) were eligible and included in our analysis.

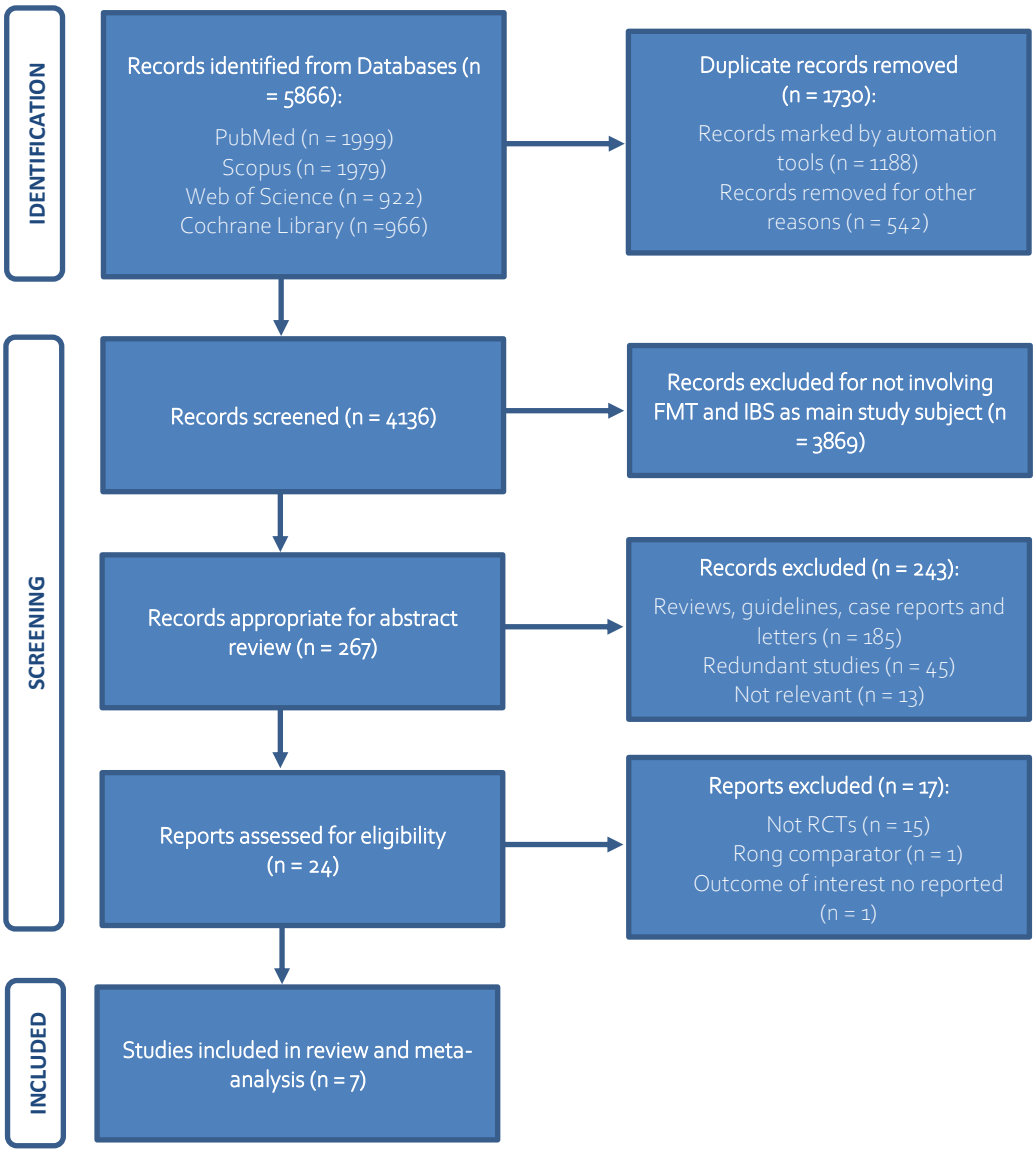

***Figure 4**. PRISMA study flow diagram describing the process of study selection.*

4.2 Study characteristics

Detailed characteristics of the included RCTs are summarized in Table 3. Among 7 included RCTs, that were published between 2017 and 2021, 7 were carried out in Europe[49,50,59–62] and one was conducted in the USA[58]. The sample size of each study ranged from 17 to 165 participants with a total of 489 adults included in these RCTs. However only 465 were analyzed since none of the studies reported a true intention-to-treat analysis. A total of 298 patients were allocated to the FMT group, and 140 to the control group.

The age of the participants ranged between 25 and 63 year old and all studies included patients with IBS from both genders with a predominance of females in 6 studies[49,50,59–62]. The criteria used for the diagnosis of IBS were Rome III or Rome IV. One study included IBS-D only[58], two studies included IBS without predominant constipation[61,62] and four studies included all 4 subtypes of IBS[49,50,59,60]. FMT was administered using colonoscopy in 3 study[50,60,62], gastroscopy in 1 study[49], nasojejunal tube in 1 study[61], and oral capsules in 2 studies[58,59]. The 5 nonoral route studies performed single-dose administration of donor or autologous fecal microbiota preparation and the 2 oral capsule FMT studies used multiple doses (3 and 12) of donor fecal microbiota or placebo (FMT excipients only). The follow-up time varied between 4[49], 6[58–60] and 12 months[50,61,62]. As first outcome, all studies aimed to evaluate the improvement in gastrointestinal symptoms after transplantation, identified by a decrease in the irritable bowel syndrome severity scoring system (IBS-SSS)[63] ≥ 75 points at 12 weeks in one study[62], a decrease in IBS-SSS≥ 50 points at 12 weeks in 4 studies[49,50,58,59] and by other parameters in two studies[60,61].

Table 3. Characteristics of studies included in the systematic review.

Study	Country/ Setting	Sample size (n analysed)	Median or Mean age[1] /years[2]	% Females[2]	Diagnostic criteria used for IBS and subtypes[2] of IBS recruited	FMT route	Intervention/dose	Control
Aroniadis et al. 2019[58]	USA/ Primary, secondary, and tertiary care (three centres)	48 (45)	I: 33 (27-48) C: 48 (28-48)	I: 36 C: 39	ROME III; 100% IBS-D	Oral capsules	75 FMT capsules containing 50 g faeces from 1 of 4 donors	Placebo capsules not containing faecal microbiota
Halkjaer et al. 2018[59]	Denmark/ Tertiary care (two centres)	52 (46)	I: 37.3 (12,5) C: 35.5 (10.6)	I: 68 C: 69	ROME III; 33.3% IBS-C, 29.4% IBS-D, 37.3% IBS-M	Oral capsules	300 FMT capsules containing 144 g faecal matter derived from 600 g pooled donor faeces (4 donors)	Placebo capsules not containing faecal microbiota
Holster et al. 2019[60]	Sweden/ Tertiary care (single centre)	17 (16)	I: 34 (27-49) C: 39 (33-43)	I: 52 C: 65	ROME III; 25.0% IBS-C, 56.2% IBS-D, 18.8% IBS-M	Colonoscopy	30g donor faeces from 1 of 2 donors mixed with isotonic saline and 10% glycerol to a final volume of 150mL	Autologous
Johnsen et al. 2017[62]	Norway/ Primary care (single centre)	90 (83)	I: 44 (33-54) C: 45 (34-57)	I: 65 C: 68	ROME III; 53.0% IBS-D, 47.0% IBS-M	Colonoscopy	50–80 g pooled donor faeces (2 donors) mixed with 200 mL isotonic saline and 50 mL 85% glycerol	Autologous
Lahtinen et al. 2020[50]	Finland/ Tertiary care (three centres)	55 (49)	I: 47.3 (16.8) C: 46.3 (14.3)	I: 52 C: 65	ROME IV; 51% IBS-D, 4,3% IBS-M, 34.7% IBS-O or IBS-U	Colonoscopy	30g donor faeces (1 donor) homogenized in 100–200 mL of water	Autologous
El-Salhy et al. 2020[49]	Norway/ Tertiary care (single centre)	165 (164)	I_{60}: 39.3(13.2) I_{30}: 39.2 (12.4) C: 41.2 (13.7)	I: 79 C: 85	ROME IV; 37.8% IBS-C, 38.4% IBS-D, 23.8% IBS-M	Gastroscopy	30 and 60 g donor faeces (1 single "super donor") mixed with 40 mL isotonic saline	Autologous
Holvoet et al. 2021[61]	Belgium/ Tertiary care (single centre)	64 (62)	I: 40 [25-59] C: 36 [18-63]	I: 69 C: 41	ROME III; 100% IBS-D or IBS-M	Nasojejunal tube	50–80 g pooled donor faeces (2 donors) mixed with isotonic saline and glycerol	Autologous

Abbreviations: C: control group; FMT: faecal microbiota transplantation; IBS: irritable bowel syndrome; IBS-C: constipation predominant IBS; IBS-D: diarrhea predominant IBS; IBS-M: IBS with mixed stool pattern; IBS-O: other, IBS in remission (not meeting the Rome III criteria at the baseline); IBS-U: unsubtyped IBS; I: intervention group; I_{30}: intervention group with 30g doses; I_{60}: intervention group with 60g doses; USA: United States of America; y: years. [1]Age are median (IQR), median [range], or mean (SD); [2]at baseline

Table 3. *cont.*

Study	Frequency/ Duration	Follow-up	Primary outcome	Secondary outcomes	Main findings	Risk of Bias
Aroniadis et al. 2019[58]	25 capsules daily × 3 days[1]	6 mo.	Decrease in IBS-SSS≥ 50 points at 12 wk.	IBS-QOL, HADS, Bristol stool scale scores and microbiota profiles.	No significant differences in IBS symptoms improvement, QOL, depression, anxiety, stool consistency and microbiome profiles between intervention and control groups. Significant similarity[2] between the patient and donor microbiota 1wk after FMT.	Unclear
Halkjaer et al. 2018[59]	25 capsules daily × 12 days	6 mo.	Decrease in IBS-SSS≥ 50 points at 12 wk.	IBS-QOL and microbiota diversity.	Significant improvement in IBS symptoms and QOL in the placebo group compared to the intervention group. Significant similarity[4] between the patient and donor microbiota after FMT.	Unclear
Holster et al. 2019[60]	Once	6 mo.	Decrease in gastrointestinal symptom rating scale-IBS of ≥30%	IBS-SSS, IBS-QOL, HADS, visceral sensitivity and microbiota composition.	No significant differences in IBS symptoms improvement, QOL, anxiety and visceral sensitivity between intervention and control groups. No significant similarity[2] between the patient and donor microbiota after FMT.	Low
Johnsen et al. 2017[62]	Once	12 mo.	Decrease in IBS-SSS> 75 points at 12 wk.	Decrease in IBS-SSS > 75 points at 12 mo.	Significant improvement in IBS symptoms in the intervention group compared to the control group.	Low
Lahtinen et al. 2020[50]	Once	12 mo.	Decrease in IBS-SSS≥ 50 points at 12 wk.	IBS-QOL, BDI, BAI, microbiota composition and faecal water content.	No significant differences in IBS symptoms improvement, QOL, depression, anxiety and stool consistency between intervention and control groups. Significant similarity[4] between patient and donor microbiota after FMT at all points after intervention, significantly higher in the intervention group compared to the control group.	Unclear
El-Salhy et al. 2019[49]	Once	4 mo.	Decrease in IBS-SSS≥ 50 points at 12 wk.	IBS-QOL, FAS, SF-NDI, dysbiosis index and microbiota profiles.	Significant improvement in IBS symptoms, QOL, fatigue and dyspepsia in the intervention group compared to the control group. Significant changes in microbiota abundance[3] in the intervention group but not in the placebo group.	Low
Holvoet et al. 2021[61]	Once[1]	12 mo.	Improvement in overall symptoms and abdominal bloating at 12 wk.	IBS symptom scores by using daily diary, IBS-QOL and microbiota composition.	Significant improvement in IBS symptoms and QOL in the intervention group compared to the control group. No significant similarity between the patient and donor microbiota after FMT.	High

Abbreviations: BDI: Beck Depression Inventory; BAI: Beck Anxiety Inventory; FAS: Fatigue Assessment Scale; FMT: faecal microbiota transplant; HADS: Hospital Anxiety and Depression Scale; IBS-QOL: irritable bowel syndrome – Quality of Life Questionnaire; IBS-SSS: irritable bowel syndrome - Severity Symptom Scale; mo.: months; QOL: quality of life; SF-NDI: Short-Form Nepean Dyspepsia Index; VAS: Visual Analogue Score; wk.: weeks; [1]First intervention of a crossover study; [2]Jensen-Shannon Distance; [3]GA-map Dysbiosis; [4]Mann-Whitney U test.

4.3 Risk of Bias Assessment

According to the Cochrane Collaboration tool[56] 3 RCTs presented "some concerns" and 1 was classified as having "high risk" of bias. Three studies presented "some concerns" in the third domain – missing outcome data,[50,58,59] and 1 presented "high risk" in the fourth and fifth domains[61] (figure 5). According to funnel plot analysis, there is no evidence of publication bias (figure 6). However, it should be highlighted that we found 3 studies registered on Clinicaltrials.gov completed more than 18 months ago, whose results were still not published.

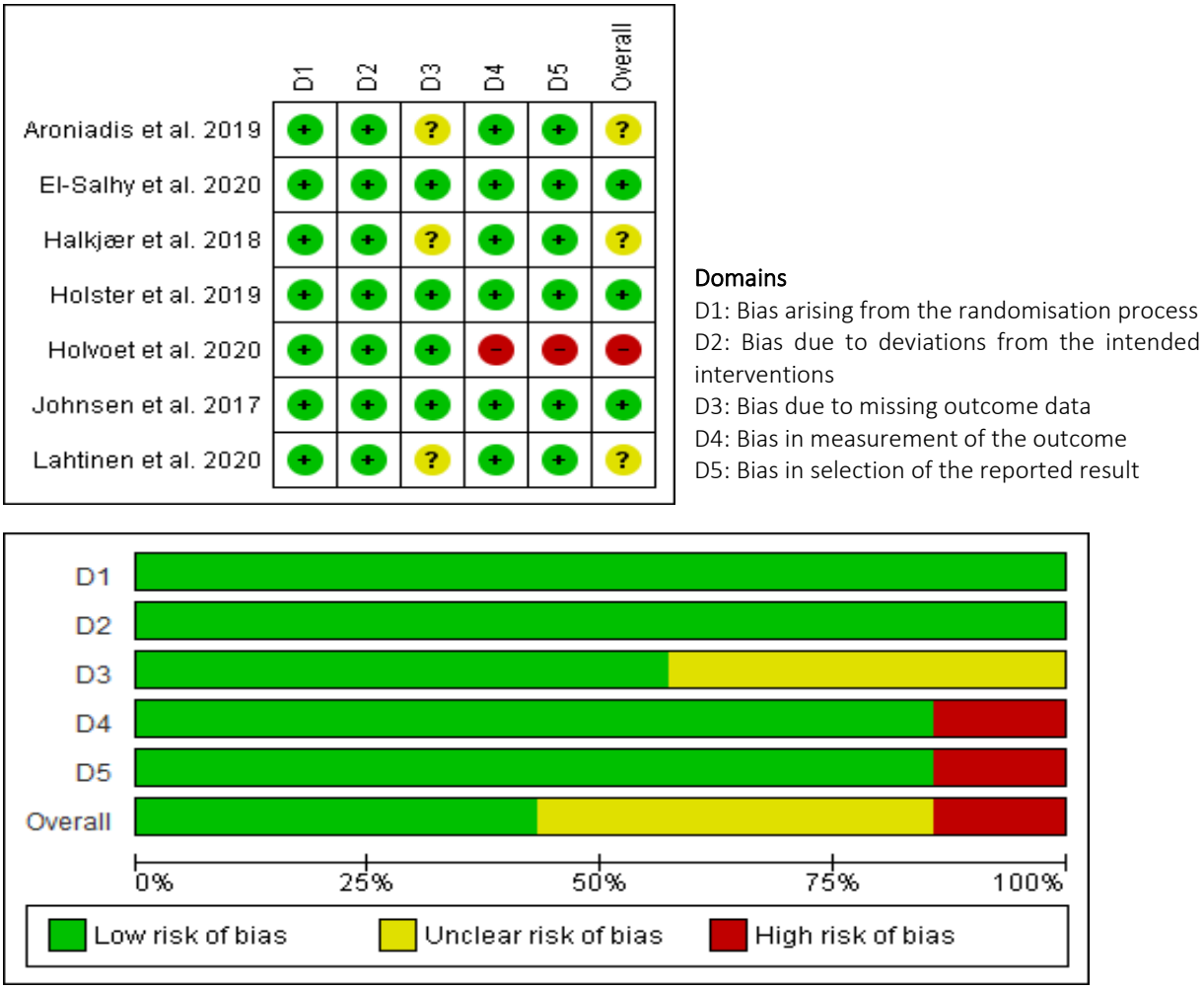

***Figure 5**. Risk of bias: judgements about each risk of bias domain presented as percentages across all included studies.*

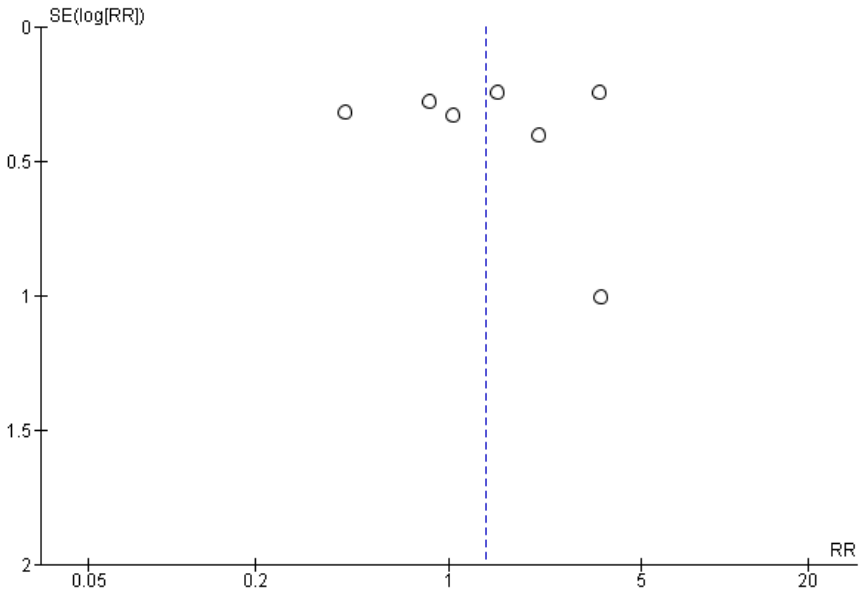

Figure 6. Publication bias plot analysis. The RR of FMT responders is plotted on the x axis and the SE of the RR is plotted on the y axis. The vertical dotted line denotes the mean value of the RRs reported by the 7 included studies. Abbreviations: FMT: fecal microbiota transplantation; RR: risk ratio; SE: standard error.

4.4 GRADE Assessment

Based on the GRADE assessment (table 4), the quality of the current body of evidence was "very low" mainly due to the serious risk of bias, the imprecision of effect estimates. The heterogeneity in the methodology of FMT and placebo interventions between studies also affected the quality, especially in studies with capsules administration.

Table 4. GRADE summary of evidence on the efficacy of FMT in IBS by administration method.

Components	Nº of participants	FMT	Placebo	Relative effect (95% CI)	Absolute effect (95% CI)	Certainty of the evidence	Importance
Overall symptoms improvement	489 (7 RCTs)	185/298 (62.1%)	75/191 (39.3%)	RR 1.35 (0.75 to 2.43)	**137 more per 1.000** (from 98 fewer to 562 more)	⊕◯◯◯ Very low[a,b,c]	CRITICAL
Symptoms improvement via oral capsules	100 (2 RCTs)	19/49 (38.8%)	33/51 (64.7%)	RR 0.61 (0.30 to 1.22)	**252 fewer per 1.000** (from 453 fewer to 142 more)	⊕◯◯◯ Very low[a,b,c]	CRITICAL
Symptoms improvement via colonoscopy	162 (3 RCTs)	51/96 (53.1%)	24/66 (36.4%)	RR 1.36 (0.93 to 1.99)	**131 more per 1.000** (from 25 fewer to 360 more)	⊕⊕⊕◯ Moderate[a]	CRITICAL
Symptoms improvement via gastroscopy or nasojejunal tube	227 (2 RCTs)	115/153 (75.2%)	18/74 (24.3%)	RR 3.03 (1.94 to 4.73)	**494 more per 1.000** (from 229 more to 907 more)	⊕⊕◯◯ Low[a,b]	CRITICAL

Abbreviations: CI: confidence interval; FMT: faecal microbiota transplantation, IBS: irritable bowel syndrome, RCT: randomised controlled trial; RR: risk ratio

GRADE Working Group grades of evidence

High certainty: very confident that the true effect lies close to that of the estimate of effect.

Low certainty: confidence in the effect estimate is limited: the true effect may be substantially different from the estimate of the effect.

Very low certainty: very little confidence in the effect estimate: the true effect is likely to be substantially different from the estimate of the effect.

[a]Downgraded one level due to imprecision.

[b]Downgraded one level due to risk of bias.

[c]Downgraded one level due to inconsistency.

4.5 IBS symptoms improvement

From the 489 participants allocated, 465 were included in the analysis of the primary outcome with an overall symptoms response rate of 66% (185/282) in patients assigned to FMT, and 41% in patients assigned to placebo (75/183), at 12 weeks of follow-up (figure 7). Considering an intention-to-treat approach, the clinical response rate at 12 weeks was 62% (185/298) in the FMT group, and 39% in the placebo group (75/191) (figure 8). No significant difference in global improvement of IBS symptoms was observed between groups (RR 1.35; 95% confidence interval (CI) 0.75-2.43, p = 0.31 from random effects). Moreover, a significant heterogeneity was identified across all studies (I^2 = 82%) (figure 8). Given these results, a subgroup analyses to further explore possible heterogeneity sources was performed.

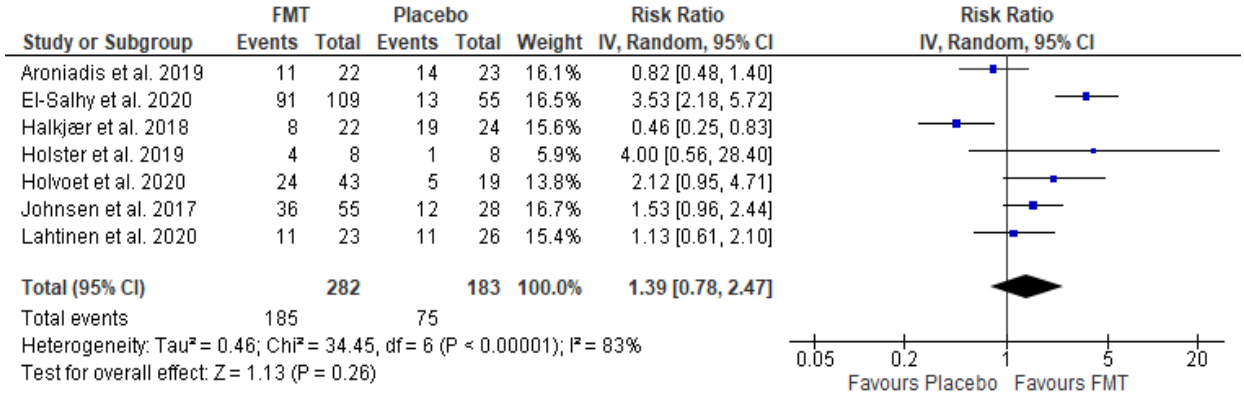

Figure 7. Forest plot of all studies for efficacy of FMT vs placebo on global improvement of IBS symptoms (without intention-to-treat analysis). Abbreviations: CI: confidence interval; FMT: fecal microbiota transplantation; IBS: irritable bowel syndrome; RR: risk ratio.

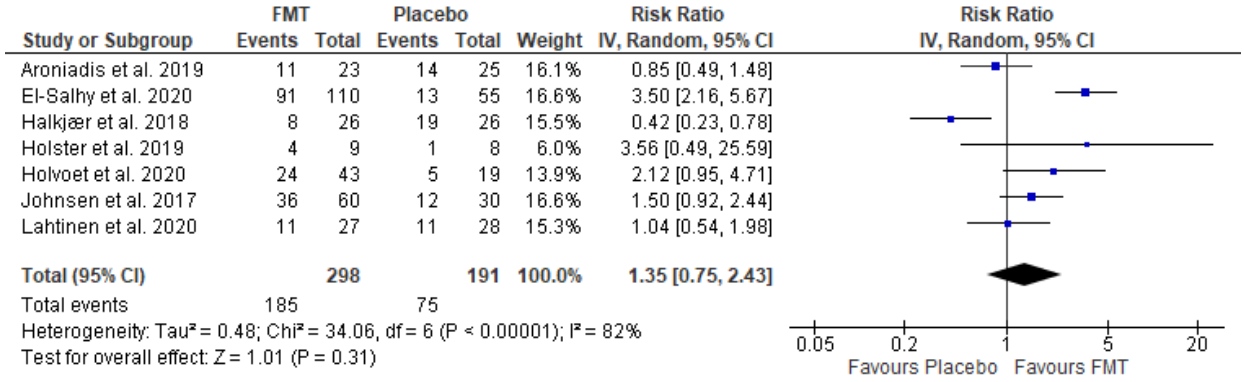

Figure 8. Forest plot of all studies for efficacy of FMT vs placebo on global improvement of IBS symptoms (with intention-to-treat analysis). Abbreviations: CI: confidence interval; FMT: fecal microbiota transplantation; IBS: irritable bowel syndrome; RR: risk ratio.

4.5.1 Delivery method of FMT

Subgroup analyses found that delivery method significantly influences the efficacy of FMT in the treatment of IBS (p = 0.0003, for subgroup differences) (table 5 and 6). Accordingly, FMT was associated with symptoms improvement compared with placebo (RR 3.03; 95% CI 1.94-4.73, I^2 = 10%) in gastroscopy and nasojejunal tube.[49,61] By contrast, no significant improvement was found in colonoscopy[50,60,62] and oral capsules[58,59] (RR 1.36; 95% CI 0.93-1.9; I^2 = 0% and RR 0.61; 95% CI 0.30–1.22, I^2 = 64%, respectively) (figure 9).

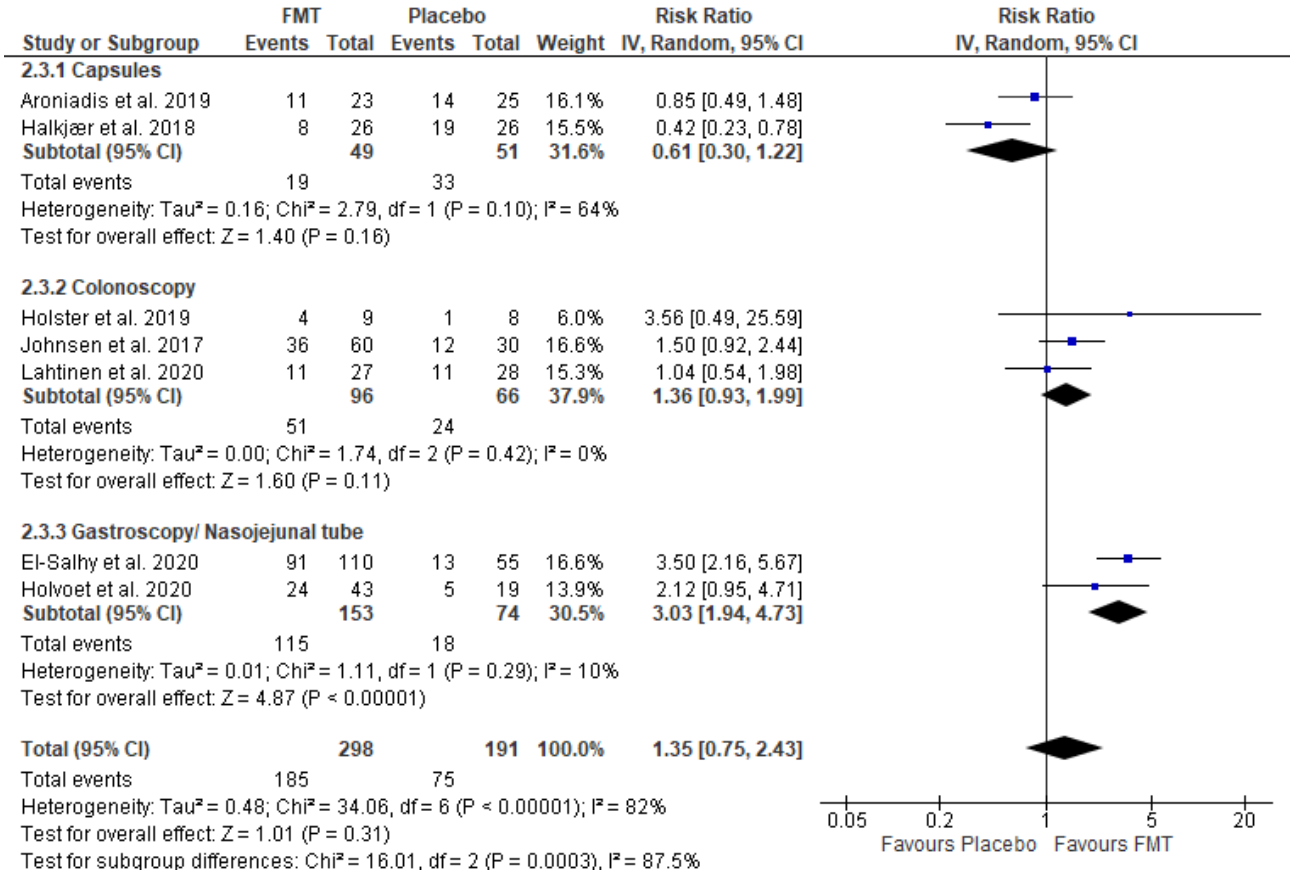

***Figure 9**. Forest plot of all studies for efficacy of FMT vs placebo on global improvement of IBS symptoms by delivery method. Abbreviations: CI: confidence interval; FMT: fecal microbiota transplantation; IBS: irritable bowel syndrome; RR: risk ratio.*

4.5.2 Dose of FMT

To assess a dose-response in FMT efficacy we separated RCTs that used in total ≥ 50g of faecal material from the remaining studies. Subgroup analyses showed that dose does not significantly influence the efficacy of FMT in the treatment of IBS (p = 0.76, for subgroup differences), with no

significant improvement with a dose ≥ 50g [49,59,62] and < 50g[49,50,58,60,61] of faecal material (RR 1.35; 95% CI 0.42-4.37; I^2 = 93% and RR 1.67; 95% CI 0.89-3.15; I^2 = 74%, respectively) (figure 10 and table 5). When studies with capsules were excluded from the subgroup analysis, the dose influence remained not statistically significant (p = 0.8 for subgroup differences) with a high heterogeneity in both groups, with ≥ 50g and <50g of faecal material (RR 2.38; 95% CI 0.96-5.87; I^2 = 86% and RR 2.06; 95% CI 1.10-3.88; I^2 = 61%, respectively) (table 6).

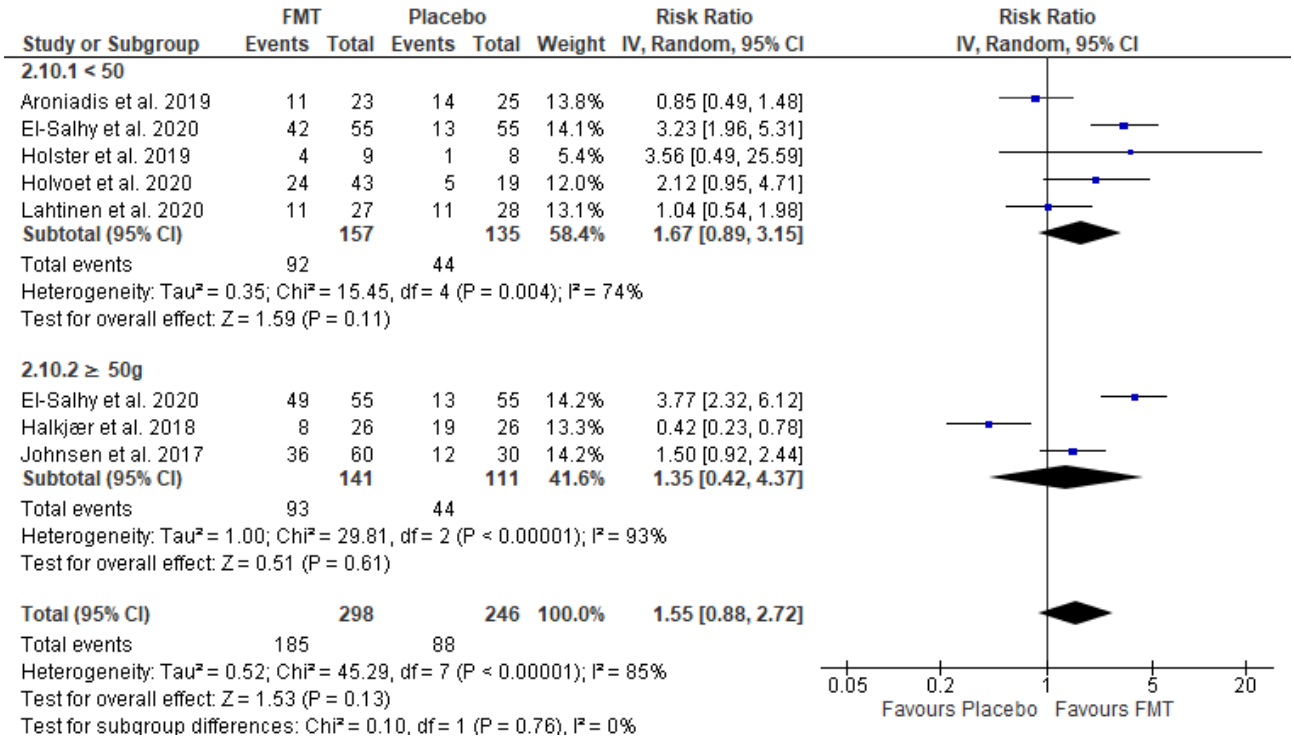

***Figure 10**. Forest plot of all studies for efficacy of FMT vs placebo on global improvement of IBS symptoms by faecal material dose. Abbreviations: CI: confidence interval; FMT: fecal microbiota transplantation; IBS: irritable bowel syndrome; RR: risk ratio.*

4.5.3 Fresh vs frozen faecal material

Subgroup analyses found that freezing faecal samples does not significantly influence the efficacy of FMT in the treatment of IBS (p = 0.44, for subgroup differences). However, when fresh faecal samples were used[60,61], FMT was associated with improvement of global IBS symptoms compared with placebo (RR 2.28; 95% CI 1.09-4.78; I^2 = 0%), while when frozen[49,50,58,59] and both frozen and fresh[62] faeces were used no significant improvements were found (RR 1.08; 95% CI 0.43-2.72; I^2 = 90% and RR 1.50; 95% CI 0.92-2.44, respectively) (figure 11 and table 5).

Subgroup analysis with exclusion of studies with capsules also found that freezing faecal samples does not significantly influence the efficacy of FMT in the treatment of IBS (p = 0.64, for subgroup

differences). While when fresh faecal samples were used[60,61] FMT was associated with improvement of global IBS symptoms compared with placebo (RR 2.28; 95% CI 1.09-4.78; I^2 = 0%), when frozen[49,50,58,59] and both frozen and fresh[62] faeces were used no significant improvements were found (RR 1.94; 95% CI 0.59-6.40; I^2 = 89% and RR 1.50; 95% CI 0.92-2.44, respectively) (table 6).

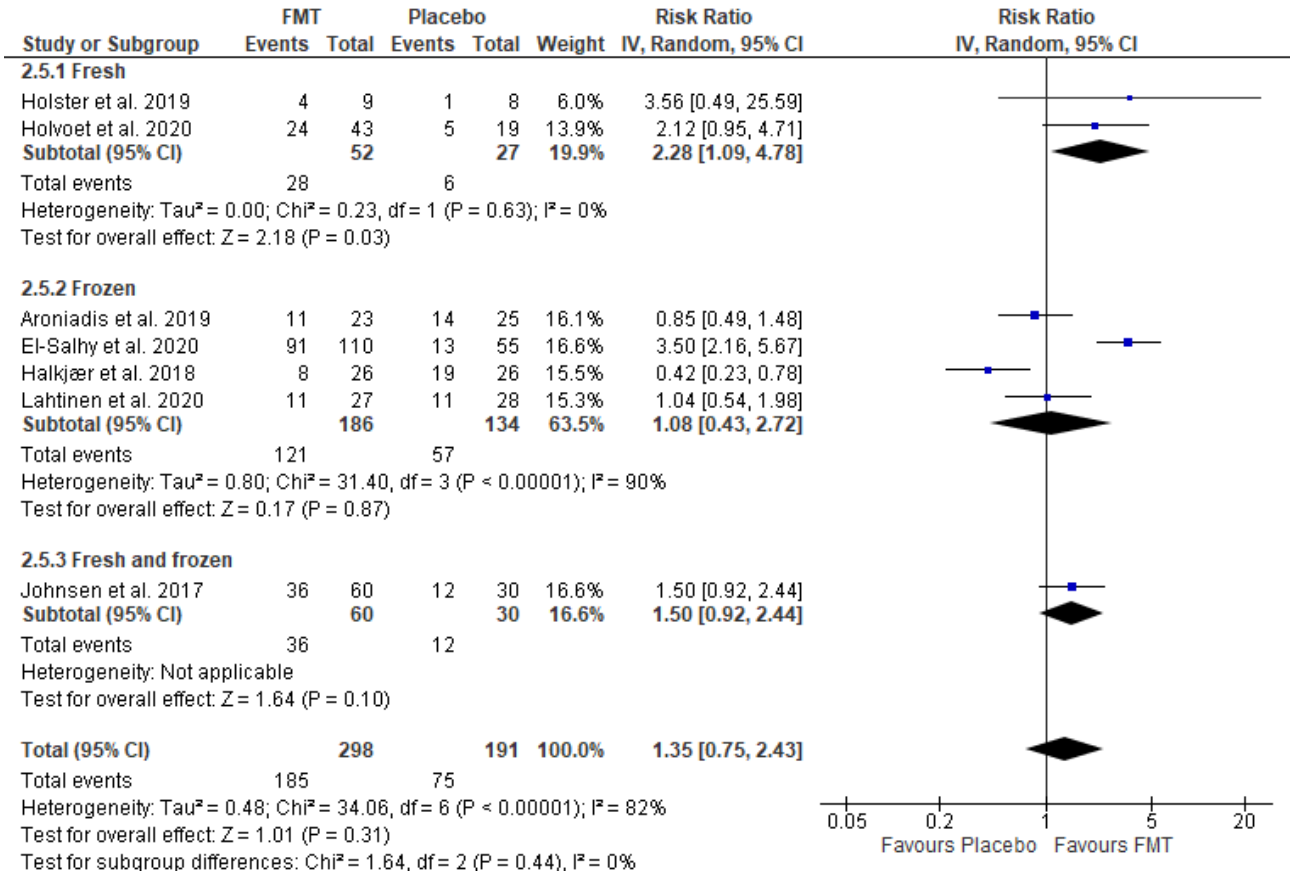

***Figure 11**. Forest plot of all studies for efficacy of FMT vs placebo on global improvement of IBS symptoms between studies that used fresh FMT, frozen FMT and both. Abbreviations: CI: confidence interval; FMT: fecal microbiota transplantation; IBS: irritable bowel syndrome; RR: risk ratio.*

4.5.4 Bowel lavage

Subgroup analyses showed that bowel lavage does not significantly influence the efficacy of FMT in the treatment of IBS (p = 0.8, for subgroup differences). In both groups, with bowel lavage[59–62] and without bowel lavage[49,50,58], FMT was not associated with improvement of global IBS symptoms compared with placebo (RR 1.26; 95% CI 0.53-2.97; I^2 = 80%, and RR 1.48; 95% CI 0.58-3.75; I^2 = 88%, respectively) (figure 12 and table 5).

When studies with capsules were excluded from the subgroup analysis, the influence of bowel lavage remained not statistically significant (p = 0.84 for subgroup differences). However, when bowel lavage

was made[60–62] FMT was associated with a significant improvement of global IBS symptoms compared with placebo (RR 1.70; 95% CI 1.13-2.55; I^2 = 0%; p = 0.01), while without bowel lavage[49,50] no significant improvement was found (RR 1.94; 95% CI 0.59-6.40; I^2 = 89%) (table 6).

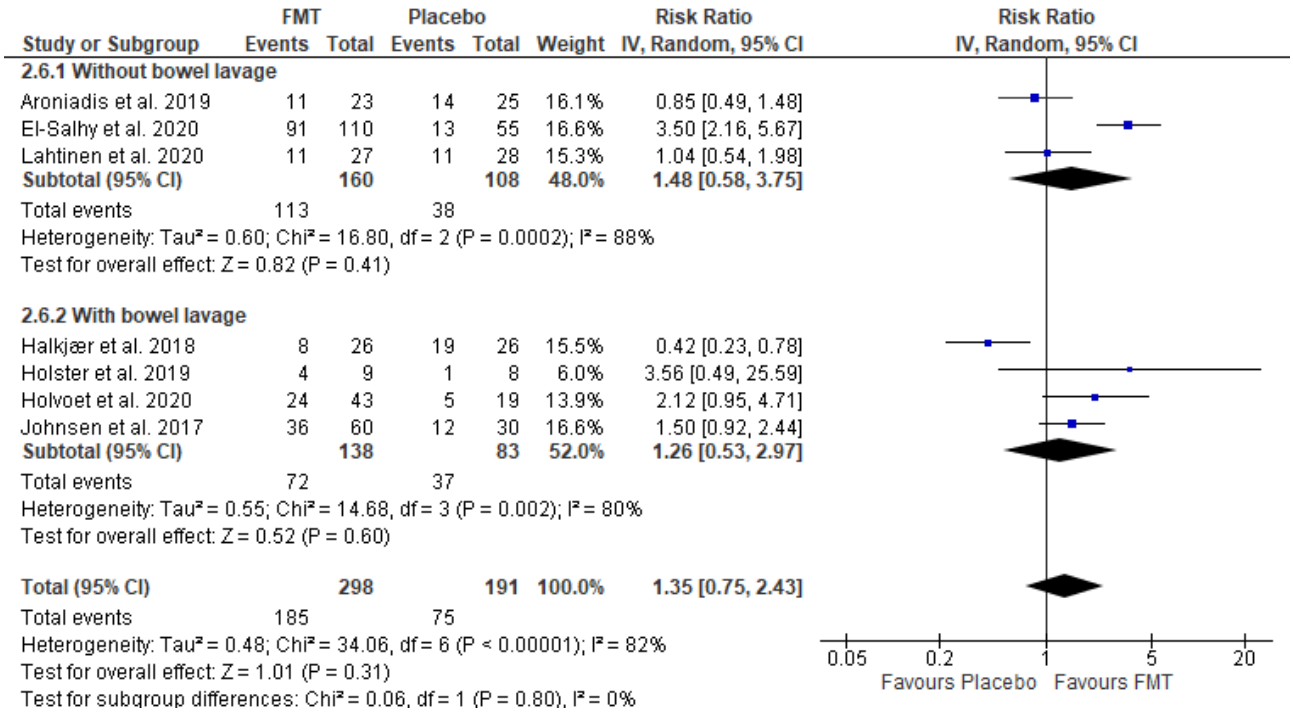

Figure 12. Forest plot of all studies for efficacy of FMT vs placebo on global improvement of IBS symptoms by bowel preparation. Abbreviations: CI: confidence interval; FMT: faecal microbiota transplantation; IBS: irritable bowel syndrome; RR: risk ratio.

4.5.5 IBS subtype

Two RCTs[49,59] performed subgroup analysis based on IBS subtype and found no differences in the response rate at 12 weeks associated with IBS subtypes. Since only these two studies grouped efficacy data for different IBS subtypes, and that some did not include participants with IBS-C, we divided RCTs based on the presence[49,59,60] or absence[50,58,61,62] of IBS-C.

Subgroup analyses showed that IBS subtype does not significantly influence the efficacy of FMT in the treatment of IBS (p = 0.77, for subgroup differences). In both groups, with and without constipation type, FMT was not associated with improvement of global IBS symptoms compared with placebo (RR 1.61; 95% CI 0.30-8.69; I^2 = 93%, and RR 1.25; 95% CI 0.87-1.79; I^2 = 31%, respectively) (figure 13 and table 5).

However, when studies with capsules were excluded, subgroup analyses found that IBS subtype significantly influence the efficacy of FMT in the treatment of IBS (p = 0.003, for subgroup

differences). In both groups, with and without constipation type, FMT was associated with improvement of global IBS symptoms (RR 3.50; 95% CI 2.19-5.60; I^2 = 0%; p < 0.00001, and RR 1.44; 95% CI 1.02-2.04; I^2 = 0%; p = 0.04, respectively) (table 6).

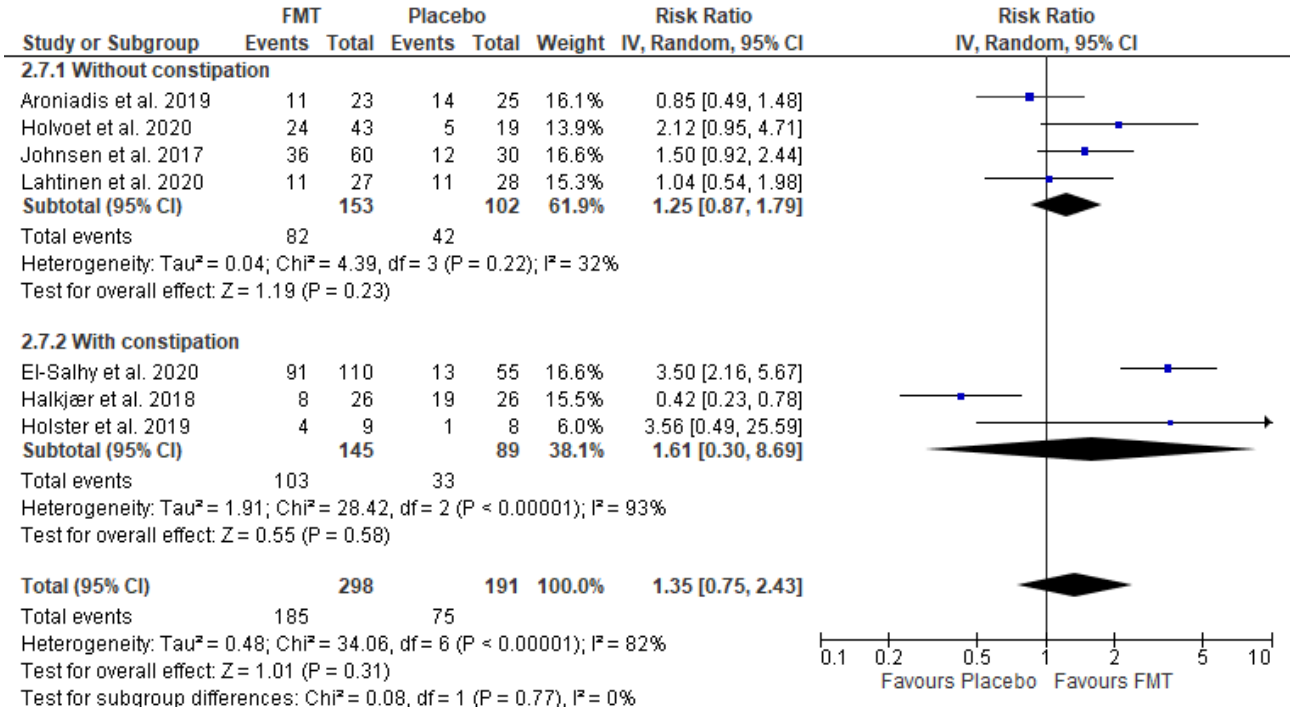

Figure 13. Forest plot of all studies for efficacy of FMT vs placebo on global improvement of IBS symptoms by subtype of IBS. Abbreviations: CI: confidence interval; FMT: fecal microbiota transplantation; IBS: irritable bowel syndrome; RR: risk ratio.

Table 5. Subgroup analyses of comparisons of FMT vs placebo in IBS.

	No. of RCTs	No. of patients	RR (95% CI)	I^2	p^1
All studies	7	489	1.35 (0.75-2.43)	82%	
Method of administration					0.0003
Capsules[58,59]	2	100	0.61 (0.30-1.22)	64%	
Colonoscopy[50,60,62]	3	162	1.36 (0.93-1.99)	0%	
Gastroscopy/Nasojejunal tube[49,61]	2	227	3.03 (1.94-4.73)*	10%	
Total dose					0.76
≥ 50g[49,59,62]	3	252	1.35 (0.42-4.37)	93%	
< 50g[49,50,58,60,61]	5	292	1.67 (0.89-3.15)	74%	
FMT sample preparation					0.44
Fresh[60,61]	2	79	2.28 (1.09-4.78)*	0%	
Frozen[49,50,58,59]	4	320	1.08 (0.43-2.72)	90%	
Both[62]	1	90	1.50 (0.92-2.44)	-	
Bowel preparation					0.80
With bowel preparation[59–62]	4	221	1.26 (0.53-2.97)	80%	
Without bowel preparation[49,50,58]	3	268	1.48 (0.58-3.75)	88%	
IBS subtypes					0.77
With constipation type[49,59,60]	3	234	1.61 (0.30-8.69)	93%	
Without constipation type[50,58,61,62]	4	255	1.25 (0.87-1.79)	32%	

Abbreviations: CI: confidence interval; FMT: faecal microbiota transplantation; GI: gastrointestinal; IBS: irritable bowel syndrome; No: number; RCTs: randomised controlled trials; RR: risk ratio. [1]Test for subgroup differences. *$P < 0.05$.

Table 6. Subgroup analyses of comparisons of FMT vs placebo in IBS between studies that delivered FMT trough colonoscopy, gastroscopy and nasojejunal tube.

	No. of RCTs	No. of patients	RR (95% CI)	I^2	p^1
All studies	5	389	1.94 (1.17-3.22)	63%	
Total dose					0.8
≥ 50g[49,62]	2	200	2.38 (0.96-5.87)	86%	
< 50g[49,50,60,61]	4	244	2.06 (1.10-3.88)*	61%	
FMT sample preparation					0.64
Fresh[60,61]	2	79	2.28 (1.09-4.78)*	0%	
Frozen[49,50,58,59]	2	220	1.94 (0.59-6.40)	89%	
Both[62]	1	90	1.50 (0.92-2.44)	-	
Bowel preparation					0.84
With bowel preparation[60–62]	3	169	1.70 (1.13-2.55)*	0%	
Without bowel preparation[49,50]	2	220	1.94 (0.59-6.40)	89%	
IBS subtypes					0.003
With constipation type [49,60]	2	182	3.50 (2.19 - 5.60)**	0%	
Without constipation type [50,61,62]	3	207	1.44 (1.02 - 2.04)*	0%	

Abbreviations: CI: confidence interval; FMT: faecal microbiota transplantation; GI: gastrointestinal; IBS: irritable bowel syndrome; No: number; RCTs: randomised controlled trials; RR: risk ratio. [1]Test for subgroup differences. *$P < 0.05$; **$P < 0.001$.

4.6 Safety of FMT in IBS

Complete adverse events (AEs) data were available for five studies.[49,50,59,60,62]. In total, four serious AEs were reported. Two patients developed diverticulitis 2 months after FMT and experienced several diverticulitis attacks before FMT)[62]. 1 developed transient vertigo and nausea after the FMT procedure, requiring a few hours of observation in the hospital[62] and 1 committed suicide[61].

After pooling data from the five studies, 53 (23%) of 232 patients assigned to FMT reported at least one adverse event, compared with 44 (30%) of the 147 allocated to placebo. No significant difference in the total number of AEs was observed in patients receiving FMT compared control patients (RR 0.88; 95% CI 0.55-1.41), with moderate heterogeneity between studies (I^2 = 52%, p = 0.08) (figure 14).

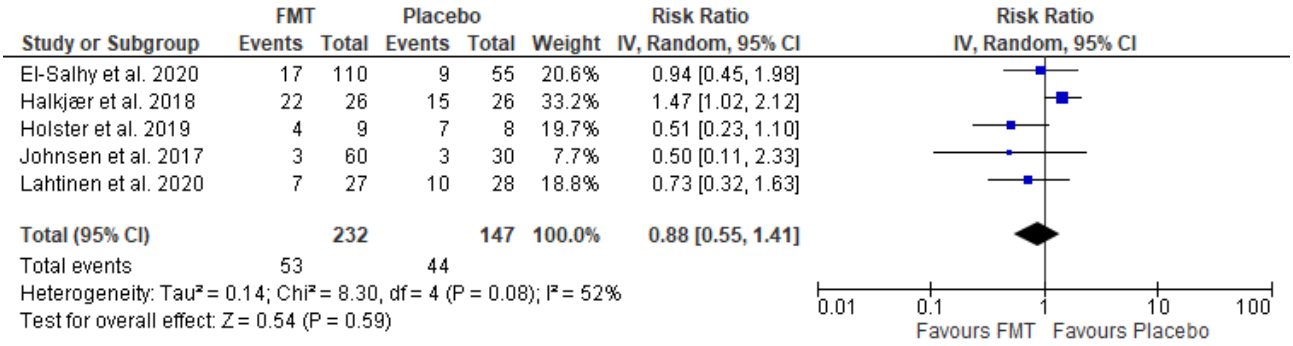

Figure 14. Forest plot of adverse events. Abbreviations: CI: confidence interval; FMT: fecal microbiota transplantation; IBS: irritable bowel syndrome; RR: risk ratio.

5. DISCUSSION

5.1 Summary of Evidence

We performed an up-to-date systematic review and meta-analysis to evaluate the short-term efficacy of FMT in IBS. For the first time, we identified critical clinical procedure that should be controlled to make FMT more effective in the treatment of IBS.

Using the endpoint of global improvement in IBS symptoms at 12 weeks after FMT, 7 RCTs involving 489 participants have yielded statistically inconclusive results, with no significant difference in global improvement between FMT and placebo, and significant heterogeneity of results. To explore the methodological factors that may have contributed to this high heterogeneity, we carried out a subgroup analysis targeting the following variables: delivery method, dosage, fresh or frozen stools, bowel lavage and IBS subtypes.

Regarding the FMT delivery method, multiple-dose oral liquid capsules[58,59] or colonoscopy[50,60,62] showed no benefit, while gastroscopy and nasojejunal tubes[49,61] demonstrated a clinical in IBS symptoms improvement compared to placebo.

In terms of delivery efficacy, the difference observed between capsule and upper gastrointestinal routes could be due to microbial viability disparities in the FMT content after delivery. Indeed, a higher bacterial viability is expected when donor microbiota is directly released in the gastrointestinal tract of the receiver. Nevertheless, other methodological shortcomings may have contributed to the low efficacy of studies with colonoscopy and capsules administration. Namely, and considering the FMT administrated by colonoscopy, two of the three studies[60,62] used different cut-offs for treatment response, which may have led to an underestimation of FMT efficacy in these RCTs. Responses were defined by a decrease of more than 75 points assessed by IBS-SSS[63] in Johnsen *et al*.[62], and at least 30% in the total GSRS[64] symptom score in Holster *et al*.[60]. Considering capsules administration, in Aroniadis *et al.*[58], less than 30 g of faecal transplant was administered, despite of 30g being the dose recommended by the European Committee on Organ Transplantation[30] and the European Consensus[32]. Furthermore, in Halkjaer *et al*.[59] faecal transplants were stored at -20ºC before transfer to recipients, when the aforementioned guidelines recommend a storage temperature of -80°C to avoid enzymes activation, and consequently, the degradation of sensitive microbial populations (*e.g., Bacteroidetes*)[65]. Due to that, FMT should be prepared and processed in stool banks in accordance with the European and International consensus guidelines for Good Manufacturing Practices, that

describe the best practices for collecting, preparing and clinical application of human donor stool[30]. Taking this into account, and considering that FMT is equally effective through capsules or colonoscopy for patients with recurrent CDI[66], more well-designed studies with high-quality faecal transplants are needed to confirm whether capsules are comparable or not to other FMT methods of administration in IBS treatment. Indeed, faecal material preparation and capsule material are relevant factors for FMT viability that should be considered. Lyophilised FMT has already shown promising results and could simplify FMT treatment[67,68].

Considering the FMT dosage, of the seven RCTs analysed in this study, only three used a dose of 50g or more[49,59,62]. El-Sahly *et al.*[49] showed that the group that received a 60g dose had a higher response rate compared to the group that only received 30g. Yet, our meta-analysis shows that a dose higher than 50g per transplant did not result in greater improvement in IBS global symptoms compared with lower than 50g. However, due to the low number of RCTs included in this meta-analysis and the higher heterogeneity yielded (93%), it is difficult to draw solid conclusions regarding the adequate FMT dose. Indeed, a systematic review and meta-analysis about FMT use in recurrent CDI showed that most studies used at least 50 g of faeces, and those using a higher dose than 50g resulted in a higher response rate,[47] even though the minimum recommended dose is 30g[30,32]. Furthermore, Quraishi et al.[69] showed that repeated transfusions were associated with a higher clinical success on the treatment of recurrent and refractory CDI.

When comparing fresh *versus* frozen FMT, our meta-analysis found a significant improvement in IBS symptoms when patients received fresh donor stools. However, as already mentioned by Wu et al.[46], interpretation of this result should be done carefully since the fresh FMT was exclusively delivered through colonoscopy and nasojejunal tube, and because of the high heterogeneity (I^2 = 90%) among studies using frozen FMT. In fact, one of the most recent studies, showed promising results on the effectiveness of frozen FMT[49]. Thus, the efficacy of frozen FMT for IBS treatment needs to be clarified due to its advantage in terms of implementation in the routine clinical practice, benefits of frozen faecal material, and the evidence of its efficacy for recurrent CDI[70].

Subgroup analyses revealed that bowel lavage may improve the effectiveness of non-capsule FMT. These results are in line with previous findings that suggest that bowel preparation can alter the faecal microbiota of healthy individuals[71,72] and with the last European consensus on FMT in clinical practice[32] that states that recipients should be submitted to bowel preparation using polyethylene glycol before FMT via upper GI route or by colonoscopy.

Finally, we found that IBS subtype may influence the FMT efficiency delivered through colonoscopy, gastroscopy and nasojejunal tube. This may be related to the different characteristics between IBS subtypes, both in terms of clinical manifestations and in terms of microbiota alteration. In fact, a longitudinal multi-omic analysis of the gut microbiome, metabolome, host epigenome and transcriptome, identified subtype-specific and symptom-related variations in microbial composition and function, by revealing a role of the gut microbiota in modulating purine metabolism and host gastrointestinal function[73]. Thus, some consideration should be given to stratifying randomised controlled trials by IBS subtype, to clarify whether the existence of constipation symptoms influences the effectiveness of the FMT.

5.2 Global considerations about RCTs evaluating the FMT efficacy on IBS treatment

In a clinical trial design, one methodological consideration that may affect the efficacy of FMT in IBS treatment, and cause heterogeneity, is the lack of standardisation of the recruited patients. Only two of the RCTs included in our review[49,58] considered the diagnostic of small intestinal bacterial overgrowth (SIBO) in the exclusion criteria. SIBO causes GI symptoms such as abdominal pain, bloating, gas, distension, flatulence, and diarrhea, that can lead to an incorrectly diagnosed IBS.[74] Likewise, PI-IBS, that frequently occurs after an episode of infectious gastroenteritis,[8] and may have a different microbiota signature,[22] was only considered as an exclusion criteria in two trials.[49,60] Aroniadis *et al.*[58] revealed a trend toward greater improvement in PI-IBS patients who received FMT, according to a post-hoc analysis.

Also of importance, is the fact that only four studies[49,58–60] excluded participants supplemented with probiotics prior to FMT and none gave specific instructions regarding the diet during the follow-up time, other than to keep it stable. Indeed, only one study[62] reported changes in dietary habits during the follow-up. Diet can affect many aspects of gut physiology such as motility, permeability, microbiome, visceral sensation, brain-gut interactions, immune regulation and neuro-endocrine function[75], being a relevant confounding variable. Thus, participants' background diet and changes in diet during intervention should be reported in order to exclude any effect on IBS symptoms.

Regarding medication, only one study[49] excluded patients that were under concomitant IBS medication, such as antimotility, antispasmodic and antidepressants drugs[10]. As medication may mask IBS symptoms and affect gut microbiota composition[76], its intake should be monitored before FMT and during follow-up.

Additionally, as discussed in a previous review[46], placebo response rate and faecal donor selection may also play an important role in the efficacy of FMT. According to recommendations of the European FMT Working Group[32], potential donors should undergo four steps to be selected: 1) a written questionnaire, 2) a general clinical examination, 3) a blood and stool testing, and 4) a further questionnaire in the donation day. The aim of this selection is to prevent AEs potentially related with the infusion of a deleterious faecal microbiota in the recipient[30]. The use of FMT that is produced under the best manufacturing practices, in a stool bank from accredited healthcare facilities, ensures the quality of faecal donation.

Finally, regarding the microbiota analysis of recipients and donors, we verified a great heterogeneity in the type of analysis performed between studies, making it difficult to carry out comparisons between them. Only a few RCTs[50,58,60,61] assessed the similarity between recipient and donor microbiomes after FMT and between FMT responders and non-responders, showing inconsistent results. This discrepancy could be related to the different parameters of the microbiota studied and the different techniques used, highlighting the importance of standardising procedures and techniques to understand whether the FMT was successfully engrafted and whether microbiota alterations are related or not to symptoms improvement. Future studies are also required to better understand mechanisms through which changes in gut microbiota and engraftment affect outcomes in IBS patients.

5.3 Strengths and Limitations

Several limitations in this systematic review should be acknowledged. First, our analyses are limited by the low number of available studies and the quality of reported data. Second, most studies were performed in Europe, limiting generalisability. Third, in order to perform subgroup analysis, we simply divided FMT dosage and IBS subtypes in two groups since we did not have access to raw data from all studies. Fourth, we did not assess the microbiota changes and the impact on quality of life. This aspect was already addressed in a previous meta-analysis[46] that found a significant improvement in quality of life of IBS patients 12 weeks after FMT. Fifth, the diversity of study populations may have contributed to the heterogeneity of the results. For instance, some studies included patients with different disease severities and patients had a wide age range.

Nevertheless, our systematic review brings a comprehensive overview of the methodological limitations of clinical trials design, as well as of differences in FMT interventions performed so far.

6. CONCLUSION AND PERSPECTIVES

Before this study, FMT was known as a safe technique, associated with an improvement in quality of life, but not effective for IBS treatment. The present systematic review with meta-analysis found that FMT may not only be safe, but also effective in IBS treatment, in terms of global symptoms improvement, when administered via gastroscopy or nasojejunal tube. Results also showed that bowel preparation may affect FMT efficacy in IBS treatment and that patient's IBS subtypes may also have impact in FMT effectiveness.

Future RCTs may benefit from a stratification by IBS subtype and disease severity, but also from stricter participants recruitment criteria. Thus, the RCTs should exclude participants with the diagnosis of confounding diseases, as well as they should consider background diet and change in diet during intervention and the use of medication before and during the follow-up period.

More well-designed RCTs are needed to firstly assess whether the efficacy of capsules made of materials that allow the delivery of FMT to a specific intestinal location, is comparable with other delivery method. Secondly, the impact of stool formulation (fresh, liquid frozen or even lyophilized), the FMT dose-response and lastly, the bowel lavage preparation on FMT efficacy must be investigated. This knowledge will contribute to optimize and standardize FMT procedures for IBS and to assess its true clinical impact.

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8. APPENDIX

Appendix 1 – PROSPERO registration protocol

NIHR | National Institute for Health Research

PROSPERO
International prospective register of systematic reviews

Citation

Tânia Rodrigues, Sofia Rodrigues Fialho, João Araújo, Rita Rocha, André Moreira-Rosário. The efficacy of fecal microbiota transplantation for the treatment of irritable bowel syndrome: a systematic review and meta-analysis. PROSPERO 2021 CRD42021252141 Available from: https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42021252141

Review question

Is fecal microbiome transplantation an effective treatment for irritable bowel syndrome?

Searches

A search of the literature will be conducted on the following bibliographic databases: PubMed, The Cochrane Library, Scopus and Web of Science. In addition, the references from selected articles will be hand searched for eligible studies.

The medical literature will be searched using both as Medical Subject Headings (MeSH) terms and as free text referring to fecal microbiota transplantation combined with terms referring to irritable bowel syndrome. Search terms for fecal microbiota transplantation will be "faecal" or "fecal" or "feces" or "faeces" or "microbiota" or "microflora" or "fecal flora" or "faecal flora," and "transplant" or "transfusion" or "implant" or "donor" or "enema" or "transfer" or "FMT". Search terms for irritable bowel syndrome will be "IBS" or "irritable bowel syndrome" or IBS related symptoms.

The timeframe for the retrieval will range from the respective dates of database inception to the date of the beginning of the search.

Types of study to be included

RCT

Condition or domain being studied

Irritable bowel syndrome (IBS) is a common symptom-based functional bowel disorder characterized by abdominal pain and altered bowel habits in the absence of detectable structural or biochemical abnormalities.

Recent studies have shown an altered microbiota composition in patients with IBS, supporting an important role for the intestinal microbiota in IBS etiology and therefore, manipulation of the gut microbiota has been proposed as a treatment strategy for IBS.

Fecal microbial transplantation (FMT) is a technique in which gut bacteria are transferred from a healthy donor to a patient, with the intention of correcting imbalances in microbial community in the gut. This technique has been proven to be effective for the treatment of recurrent Clostridium difficile infection, by inhibiting its colonization. However, the efficacy of FMT as a treatment for IBS remains unclear.

Participants/population

Adult patients older than 16 years with IBS defined by accepted symptom-based criteria including Manning, Kruis, Rome I, Rome II, Rome III, or Rome IV.

Intervention(s), exposure(s)

Fecal microbiota transplantation.

Comparator(s)/control

Placebo consisting of only the FMT excipients or an autologous FMT.

Main outcome(s)

Changes in the symptom scores.

Additional outcome(s)
Changes in quality-of-life scores.

Changes in microbiota profiles.

Data extraction (selection and coding)
Two reviewers will independently search and evaluate every study according to the Cochrane Handbook for Systematic Reviews of Interventions, and the following four procedures will be undertaken: (1) Irrelevant literature will be eliminated by screening the titles and abstracts of the references retrieved in the searches; (2) The results will be organized and all duplicates will be removed; (3) Literature not meeting the inclusion criteria will be eliminated based on a full text assessment; (4) All literature reporting identical clinical studies will be amalgamated. Independently, the two reviewers will use a data extraction form to extract data about participants, randomization, interventions, outcomes, duration, follow-up, reasons for discontinuation, numbers of treatment-related adverse events, author information, and conflicts of interest. A third author will arbitrate any disagreement between authors.

Risk of bias (quality) assessment
Two reviewers will independently evaluate the version 2 of the Cochrane risk-of-bias tool for randomized trials (RoB 2) (Higgins 2019). Any disagreement between the two review authors will be resolved by consensus or consulting a third reviewer.

Strategy for data synthesis
A narrative synthesis of the findings from the included studies will be performed. Meta-analysis will be performed when appropriated using Review Manager 5.4 (The Nordic Cochrane Centre, The Cochrane Collaboration). Pooled meta-analytical results will be estimated using random-effects models. Clinical and methodological heterogeneity will be assessed by carefully evaluating the design, participant characteristics and other relevant study characteristics of the included studies. We will assess the degree of statistical heterogeneity between studies visually from inspection of the forest plot. Moreover, the degree of statistical heterogeneity between studies will be formally assessed using Cochran Q test (significance level of 0.1), supplemented by the I^2 statistic. The I^2 values will be interpreted as follows: 0% to 40% might not be important; 30% to 60% may represent moderate heterogeneity; 50% to 90% may represent substantial heterogeneity; 75% to 100% considerable heterogeneity (Higgins 2019). Subgroup and sensitivity analyses will be performed in order to explore the source of heterogeneity and results consistency.

Analysis of subgroups or subsets
A subgroup analysis will be performed based on FMT delivery methods, IBS subtypes and outcome measures.

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NIHR | National Institute for Health Research

PROSPERO
International prospective register of systematic reviews

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Type and method of review
Meta-analysis, Systematic review

Anticipated or actual start date
01 April 2021

Anticipated completion date
28 March 2022

Funding sources/sponsors
None.

Conflicts of interest

Language
English

Country
Portugal

Stage of review [1 change]

Review Completed not published

Subject index terms status
Subject indexing assigned by CRD

Subject index terms
Fecal Microbiota Transplantation; Feces; Gastrointestinal Microbiome; Humans; Irritable Bowel Syndrome

Date of registration in PROSPERO
24 May 2021

Date of first submission
29 April 2021

Stage of review at time of this submission [2 changes]

Stage	Started	Completed
Preliminary searches	Yes	Yes
Piloting of the study selection process	Yes	Yes
Formal screening of search results against eligibility criteria	Yes	Yes
Data extraction	Yes	Yes
Risk of bias (quality) assessment	Yes	Yes
Data analysis	Yes	Yes

Revision note
To update the authors' current affiliation and the review status.

The record owner confirms that the information they have supplied for this submission is accurate and complete and they understand that deliberate provision of inaccurate information or omission of data may be construed as scientific misconduct.
The record owner confirms that they will update the status of the review when it is completed and will add publication details in due course.

Versions
24 May 2021
07 February 2022
31 July 2022

Appendix 2 – Query definition table

QUERY DEFINITION TABLE

#	PUBMED	SCOPUS	WEB OF SCIENCE	COCHRANE
1	"fecal"[Title/Abstract] OR "faecal"[Title/Abstract] OR "feces"[MeSH Terms] OR "feces"[Title/Abstract] OR "faeces"[Title/Abstract] OR "stool"[Title/Abstract] OR "gut"[Title/Abstract] OR "microbiota"[MeSH Terms] OR "microb*"[Title/Abstract] OR "microflora"[Title/Abstract]	TITLE-ABS-KEY(fecal) OR TITLE-ABS-KEY(faecal) OR TITLE-ABS-KEY(feces) OR TITLE-ABS-KEY(faeces) OR TITLE-ABS-KEY(stool) OR TITLE-ABS-KEY(gut) OR TITLE-ABS-KEY(microb*) OR TITLE-ABS-KEY(microflora)	TI=(fecal OR faecal OR feces OR faeces OR stool OR gut OR microb* OR microflora) OR AB=(fecal OR faecal OR feces OR faeces OR stool OR gut OR microb* OR microflora)	(fecal):ti,ab,kw OR (faecal):ti,ab,kw OR (feces):ti,ab,kw OR MeSH descriptor:[feces] explode all trees OR (stool):ti,ab,kw OR (gut):ti,ab,kw OR (microb*):ti,ab,kw OR MeSH descriptor: [microbiota] explode all trees OR (microflora):ti,ab,kw
2	"transplantation"[MeSH Terms] OR "transplant*"[Title/Abstract] OR "transfusion"[Title/Abstract] OR "implant*"[Title/Abstract] OR "donor*"[Title/Abstract] OR "enema"[Title/Abstract] OR "infusion"[Title/Abstract] OR "reconstitution"[Title/Abstract] OR "transfer"[Title/Abstract] OR]	TITLE-ABS-KEY(transplant*) OR TITLE-ABS-KEY(transfusion) OR TITLE-ABS-KEY(implant) OR TITLE-ABS-KEY(donor*) OR TITLE-ABS-KEY(enema) OR TITLE-ABS-KEY(infusion) OR TITLE-ABS-KEY(reconstitution) OR TITLE-ABS-KEY(transfer)	TI=(transplant* OR transfusion* OR implant OR donor* OR enema OR infusion OR reconstitution OR transfer OR bacteriotherapy) OR AB=(transplant* OR transfusion* OR implant OR donor* OR enema OR infusion OR reconstitution OR transfer)	MeSH descriptor: [transplantation] in all MeSH products OR (transplant*):ti,ab,kw OR (transfusion):ti,ab,kw OR (implant*):ti,ab,kw OR (donor*):ti,ab,kw OR (enema):ti,ab,kw OR (infusion):ti,ab,kw OR (reconstitution):ti,ab,kw OR (transfer):ti,ab,kw
3	"irritable bowel syndrome"[MeSH Terms] OR "irritable bowel syndrome"[Title/Abstract] OR "irritable colon"[Title/Abstract] OR "IBS"[Title/Abstract] OR "dysbiosis"[Title/Abstract] OR "quality of life"[Title/Abstract] OR "functional gastrointestinal disorders"[Title/Abstract] OR "functional gut disorders"[Title/Abstract] OR "functional bowel disorders"[Title/Abstract]	TITLE-ABS-KEY("irritable bowel syndrome") OR TITLE-ABS-KEY(irritable colon) OR TITLE-ABS-KEY("quality of life") OR TITLE-ABS-KEY("functional gastrointestinal disorders") OR TITLE-ABS-KEY("functional gut disorders") OR TITLE-ABS-KEY("functional bowel disorders")	TI=("irritable bowel syndrome" OR "irritable colon" OR dysbiosis OR "quality of life" OR "ibs severity scoring system" OR "functional gastrointestinal disorders" OR "functional gut disorders" OR "functional bowel disorders") OR AB=("irritable bowel syndrome" OR "irritable colon" OR "quality of life" OR "functional gastrointestinal disorders" OR "functional gut disorders" OR "functional bowel disorders")	MeSH descriptor: [irritable bowel syndrome] explode all trees OR (irritable bowel syndrome):ti,ab,kw OR (irritable colon):ti,ab,kw OR (IBS):ti,ab,kw OR MeSH descriptor: [dysbiosis] explode all trees OR (dysbiosis):ti,ab,kw OR (quality of life):ti,ab,kw OR (functional gastrointestinal disorders):ti,ab,kw OR (functional gut disorders):ti,ab,kw OR (functional bowel disorders):ti,ab,kw

Appendix 3 – Search and study selection protocol

SEARCH AND STUDY SELECTION PROTOCOL

1) SEARCH STRATEGY

1.1) Perform a systematic search in the selected databases by inserting the query corresponding to each one of them.

The medical literature will be searched using terms referring to fecal microbiota (#1) transplantation (#2) combined with terms referring to irritable bowel syndrome (#3). Run the search combining #1 AND #2 AND #3 (see query table).

1.2) Save the results obtained in each of the bases in a folder. Follow the instructions below.

PUBMED: Save > All results > Pubmed > Create File
SCOPUS: Text export > CSV Excel > Select Citation Information and Abstract & keywords > Export
WEB OF SCIENCE: Export to Endnote Desktop (CIW) > Select Author, Title, Source, Abstract > Export
COCHRANE: Select all > Export selected citations > CSV > Include abstract > Download

2) STUDY SELECTION

2.1 Sign up in https://www.rayyan.ai/. Accept the invitation to collaborate in the review.
2.2 Upload files with de search results from de folder.
Upload references > Select files
2.3 Identification - Examinate titles and eliminate duplicates.

- Eliminate duplicates;

(Detect duplicates → Unresolved → Resolve duplicate → Delete

2.4 Screening - Examinate titles and abstracts to identify studies. To help the identification add as keywords for inclusion: fecal microbiota transplantation, FMT, irritable bowel syndrome and IBS; and select "Highlights ON".

2.4.1 Examinate titles and, if necessary, abstracts and exclude articles that do not involve FMT and IBS (as the main issue). Use the following reasons:

- Not related to FMT
- Not related to IBS or bowel functional diseases

2.4.2 Examinate abstracts and exclude articles according to the following reasons:

- irrelevant studies,
- redundant studies (articles related to the same clinical trials)
- reviews, guidelines, and commentaries, other types of articles.

2.5 Assessing- Evaluate each full text article from the screening stage to assess the remaining eligibility criteria.

Studies will be considered for inclusion if they met the following criteria:

(i) prospective, randomized, double-blind, placebo-controlled trials (parallel group or first arm of cross-over);

(ii) adult patients older than 16 years with IBS defined by accepted symptom-based criteria including Manning, Kruis, Rome I, Rome II, Rome III, or Rome IV;

(iii) compared FMT with placebo consisting of only the FMT excipients or an autologous FMT;

(iv) outcome of improvement in global IBS symptoms;

(v) minimum duration of 8-week follow-up;

(vi) english language

2.6 Complete the flow diagram according to the PRISMA statement.

Appendix 4 – Prisma 2020 Checklist

PRISMA 2020 Checklist

Section and Topic	Item #	Checklist item	Location where item is reported
TITLE			
Title	1	Identify the report as a systematic review.	1
ABSTRACT			
Abstract	2	See the PRISMA 2020 for Abstracts checklist.	2
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of existing knowledge.	4
Objectives	4	Provide an explicit statement of the objective(s) or question(s) the review addresses.	5
METHODS			
Eligibility criteria	5	Specify the inclusion and exclusion criteria for the review and how studies were grouped for the syntheses.	6
Information sources	6	Specify all databases, registers, websites, organisations, reference lists and other sources searched or consulted to identify studies. Specify the date when each source was last searched or consulted.	6
Search strategy	7	Present the full search strategies for all databases, registers and websites, including any filters and limits used.	6
Selection process	8	Specify the methods used to decide whether a study met the inclusion criteria of the review, including how many reviewers screened each record and each report retrieved, whether they worked independently, and if applicable, details of automation tools used in the process.	6
Data collection process	9	Specify the methods used to collect data from reports, including how many reviewers collected data from each report, whether they worked independently, any processes for obtaining or confirming data from study investigators, and if applicable, details of automation tools used in the process.	7, 23
Data items	10a	List and define all outcomes for which data were sought. Specify whether all results that were compatible with each outcome domain in each study were sought (e.g. for all measures, time points, analyses), and if not, the methods used to decide which results to collect.	6, 7
	10b	List and define all other variables for which data were sought (e.g. participant and intervention characteristics, funding sources). Describe any assumptions made about any missing or unclear information.	7
Study risk of bias assessment	11	Specify the methods used to assess risk of bias in the included studies, including details of the tool(s) used, how many reviewers assessed each study and whether they worked independently, and if applicable, details of automation tools used in the process.	7
Effect measures	12	Specify for each outcome the effect measure(s) (e.g. risk ratio, mean difference) used in the synthesis or presentation of results.	7
Synthesis methods	13a	Describe the processes used to decide which studies were eligible for each synthesis (e.g. tabulating the study intervention characteristics and comparing against the planned groups for each synthesis (item #5)).	not applicable
	13b	Describe any methods required to prepare the data for presentation or synthesis, such as handling of missing summary statistics, or data conversions.	not applicable
	13c	Describe any methods used to tabulate or visually display results of individual studies and syntheses.	7
	13d	Describe any methods used to synthesize results and provide a rationale for the choice(s). If meta-analysis was performed, describe the model(s), method(s) to identify the presence and extent of statistical heterogeneity, and software package(s) used.	7
	13e	Describe any methods used to explore possible causes of heterogeneity among study results (e.g. subgroup analysis, meta-regression).	7
	13f	Describe any sensitivity analyses conducted to assess robustness of the synthesized results.	not applicable
Reporting bias assessment	14	Describe any methods used to assess risk of bias due to missing results in a synthesis (arising from reporting biases).	7

PRISMA 2020 Checklist

Section and Topic	Item #	Checklist item	Location where item is reported
Certainty assessment	15	Describe any methods used to assess certainty (or confidence) in the body of evidence for an outcome.	8
RESULTS			
Study selection	16a	Describe the results of the search and selection process, from the number of records identified in the search to the number of studies included in the review, ideally using a flow diagram.	9, (figure 1)
	16b	Cite studies that might appear to meet the inclusion criteria, but which were excluded, and explain why they were excluded.	9 (figure 1)
Study characteristics	17	Cite each included study and present its characteristics.	9, 10, 11 (table 1)
Risk of bias in studies	18	Present assessments of risk of bias for each included study.	12
Results of individual studies	19	For all outcomes, present, for each study: (a) summary statistics for each group (where appropriate) and (b) an effect estimate and its precision (e.g. confidence/credible interval), ideally using structured tables or plots.	(figure 2)
Results of syntheses	20a	For each synthesis, briefly summarise the characteristics and risk of bias among contributing studies.	12
	20b	Present results of all statistical syntheses conducted. If meta-analysis was done, present for each the summary estimate and its precision (e.g. confidence/credible interval) and measures of statistical heterogeneity. If comparing groups, describe the direction of the effect.	17 (table 3 and 4)
	20c	Present results of all investigations of possible causes of heterogeneity among study results.	14, 15, 17 (table 3 and 4)
	20d	Present results of all sensitivity analyses conducted to assess the robustness of the synthesized results.	not applicable
Reporting biases	21	Present assessments of risk of bias due to missing results (arising from reporting biases) for each synthesis assessed.	12
Certainty of evidence	22	Present assessments of certainty (or confidence) in the body of evidence for each outcome assessed.	12
DISCUSSION			
Discussion	23a	Provide a general interpretation of the results in the context of other evidence.	18
	23b	Discuss any limitations of the evidence included in the review.	18, 19, 20, 21
	23c	Discuss any limitations of the review processes used.	21
	23d	Discuss implications of the results for practice, policy, and future research.	21
OTHER INFORMATION			
Registration and protocol	24a	Provide registration information for the review, including register name and registration number, or state that the review was not registered.	6
	24b	Indicate where the review protocol can be accessed, or state that a protocol was not prepared.	6
	24c	Describe and explain any amendments to information provided at registration or in the protocol.	not applicable
Support	25	Describe sources of financial or non-financial support for the review, and the role of the funders or sponsors in the review.	23
Competing interests	26	Declare any competing interests of review authors.	23

PRISMA 2020 Checklist

Section and Topic	Item #	Checklist item	Location where item is reported
Availability of data, code and other materials	27	Report which of the following are publicly available and where they can be found: template data collection forms; data extracted from included studies; data used for all analyses; analytic code; any other materials used in the review.	not applicable

From: Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ 2021;372:n71. doi: 10.1136/bmj.n71
For more information, visit: http://www.prisma-statement.org/