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REVIEW ARTICLE

# Effectiveness of fecal microbiota transplant for the treatment of *Clostridioides difficile* diarrhea: a systematic review and meta-analysis

R.Á. Pomares Bascuñana[1], V. Veses[2] and C.C. Sheth[1]

1 Department of Medicine, Faculty of Health Sciences, Universidad Cardenal Herrera, CEU Universities, Valencia, Spain
2 Department of Biomedical Sciences, Faculty of Health Sciences, Universidad Cardenal Herrera, CEU Universities, Valencia, Spain

**Significance and impact of the study:** In the era of antimicrobial resistance, infections due to *Clostridioides difficile* are on the rise. In this review we examine the use of FMT as a possible avenue to fight gastrointestinal infections produced by *C. difficile*. Published studies in this field vary greatly in design and outcome variables, leading to ambiguity when considering their clinical application. We believe that there is a significant lack of objective systematic reviews to inform clinical decision-making. Our objective is to evaluate the overall effectiveness and safety of FMT, as well as possible differences in effectiveness on the bases of the administration route employed.

**Keywords**
adverse effects, *Clostridioides difficile* diarrhea, effectiveness, fecal microbiota transplant, safety.

**Correspondence**
Chirag Sheth, Faculty of Health Sciences, Calle Ramón y Cajal s/n, 46115 Alfara del Patriarca, Valencia, Spain.
E-mail: chirag.sheth@uchceu.es

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## Abstract

*Clostridioides difficile* is a major cause of health-care related infections and antibiotic-associated diarrhea. High recurrence rates following antibiotic treatment, along with the emergence of hypervirulent and multidrug resistant ribotypes makes essential the development of safe, effective, novel therapies for the treatment of *C. difficile* infections. The primary outcome evaluated in this meta-analysis was the effectiveness of fecal microbiota transplantation (FMT). Secondary outcomes were the proportion of patients suffering adverse effects along with the most effective administration route. The mean treatment effectiveness was 82% (95% CI: 75-89). Overall, patients receiving FMT via colonoscopy experienced more adverse effects than patients whom received enema, or oral capsules (71·6% vs 40·2%, and 35·3% respectively). Comparing administration of FMT by colonoscopy versus enema resulted in a Hedges' *g* of −0·74 (95% CI of −0·9 to −0·58), indicating a slight advantage in favor of colonoscopy. The comparison between colonoscopy and capsule returned a Hedges' *g* of 0·44 (95% CI of 0·20–0·69), indicating that delivery of the FMT by capsule was statistically significantly more effective. FMT provides an effective and safe treatment for *C. difficile* diarrhea. Further research into the efficacy of different preparation protocols is needed.

## Introduction

*Clostridioides difficile* is responsible for the majority of health-care related infections and antibiotic-associated diarrhea, with an increasing morbidity and mortality globally in the last few years (Banawas 2018; Cho *et al.* 2018). This anaerobic sporulated bacteria proliferates following antibiotic administration and hospitalization, and results in *C. difficile* infection (CDI). Symptoms of CDI can range from mild diarrhea to pseudomembranous colitis (including diarrhea, abdominal pain, fever, nausea, vomiting and leukocytosis) (Wilson 2019). CDI causes up to 30·7% of hospital infections and diarrhea cases, with 29 300 deaths annually in USA (Banawas 2018; Cho *et al.* 2018). Current treatment options for CDI include administration of vancomycin, fidaxomicin or metronizadol (Cho *et al.* 2018). Studies show that recurrence after antibiotic treatment ranges from 15 to 28% of cases (Nelson *et al.* 2017). Compounding the problem, authors have described the emergence of novel hypervirulent and

multidrug resistant (ribotypes 027 or 176) strains of *C. difficile*. These factors have elevated the threat of *C. difficile* in the current general antimicrobial crisis outlined by the World Health Organization (Herbert *et al.* 2019; Polivkova *et al.* 2016; WHO 2020).

In this context, it is essential to increase efforts in the search for innovative methods to combat CDI. The emergence of novel approaches for CDI such as fecal microbiota transplant (FMT) could prove beneficial if we could demonstrate that their efficacy and safety prove equal or superior than current antibiotic regimes. The effectiveness of alternative therapeutic strategies such as FMT would therefore act on multiple levels—one as direct therapeutic agents, and the other, in providing alternatives to traditional antibiotic therapy, thereby reducing the increase in antibiotic resistance in this highly plastic, microbial pathogen. FMT was first used in the fourth century in China. However, it is not until 1958 when Eisenman and coworkers use FMT for the treatment of pseudomembranous colitis (Smits *et al.* 2013). It is now known that prolonged antimicrobial therapy can give rise to dysbiosis in the gut microbiota, characterized by a reduction in Bacteroides and Firmicutes colonization, thereby favoring the overgrowth of *C. difficile* (Aroniadis and Brandt 2013).

In FMT, a fecal solution from a donor is administered into the intestinal tract of a recipient (Cho *et al.* 2018). The fecal material for transplantation can be delivered via a nasogastric tube, upper tract endoscopy, colonoscopy, enema or capsules, amongst other mechanisms. FMT aims to restore the gut microbial balance in patients with CDI (Aroniadis and Brandt 2013; Khoruts *et al.* 2010). Patients likely to benefit from a FMT are those with moderate to severe infections that do not respond to antibiotic treatment; or those with multiple recurrences (at least three mild-moderate episodes or two episodes that require hospital admission) (Societat Catalana de Digestologia 2020).

FMT is a promising novel approach for CDI, that has shown encouraging results in clinical trials (Kassam *et al.* 2013). Additionally, FMT is economically favorable due to low costs of patient and recipient preparation, sample obtention and administration procedures, which make it ideal for large scale application in hospitals. This meta-analysis aims to evaluate the global effectiveness of FMT. Secondary objectives include a description of the proportion of patients suffering from adverse effects and an evaluation of the most efficacious administration route.

## Results and discussion

### Search results

A total of 5266 articles were retrieved in PubMed, Cochrane and Science Direct databases initially, using the specificied keywords (see Eligibility). Following screening, 233 articles were evaluated according to inclusion and exclusion criteria. 15 articles were finally included in the review and meta-analysis (Fig. 1).

### Study characteristics

Data was evaluated from an aggregate of 1168 patients. The main characteristics of the articles used in this review can be found in Table 1. All the included studies are primary articles, specifically clinical trials ($n = 12$) and cohort ($n = 3$). Excluded articles were removed from this review due to the following exclusion criteria: (i) Studies not published in the last 5 years. (ii) Subjects not suffering from *C. difficile* diarrhea. (iii) Studies not conducted in humans. (iv) Language other than English. (v) Studies in infants, children and adolescents (aged under 19). (vi) Article type incorrect: Case reports, case series, journal articles, editorial, systematic reviews.

Results of the quality assessment of the primary research articles included in this review, using the CASP tool are presented in Fig. S1. None of the included articles were found to have a high risk of bias across all evaluated domains. All studies had "low concern" with regards to applicability. All 15 studies adressed a clearly focused question and rated positively on the article selection process.

Figure S1a shows the results of the CASP analysis of the included clinical trials whilst Fig. S1b shows the analysis of the included cohort studies.

### Effectiveness of FMT

FMT is a highly effective therapy for the treatment of *C. difficile* diarhoea, as all articles in this review reported an effectiveness (defined as the absence of diarrhea between 8 and 13 weeks following treatment) of between 78 and 100%. A Forest plot was constructed to calculate and demonstrate the summary statistic for treatment effectiveness, which was shown to be 82% (95% CI: 75-89) (Fig. 2a). The $I^2$ statistic of heterogeneity was calculated and found to be 20%, suggesting that the selected studies presented a high degree of homogeneity in the results, and hence there was no need to further explore the data via a subgroup or moderator analysis.

The dispersion and heterogeneity of included studies was presented as a funnel plot (Fig. 2b). Our calculations ($I^2$ value) demonstrate that the included studies were highly homogenous in their distribution irrespective of differences in standard error between the studies.

The Egger regression test returned an estimated intercept of 0·43 ($P = 0·511$). This suggests that the degree of data asymmetry was low and not statistically significant,

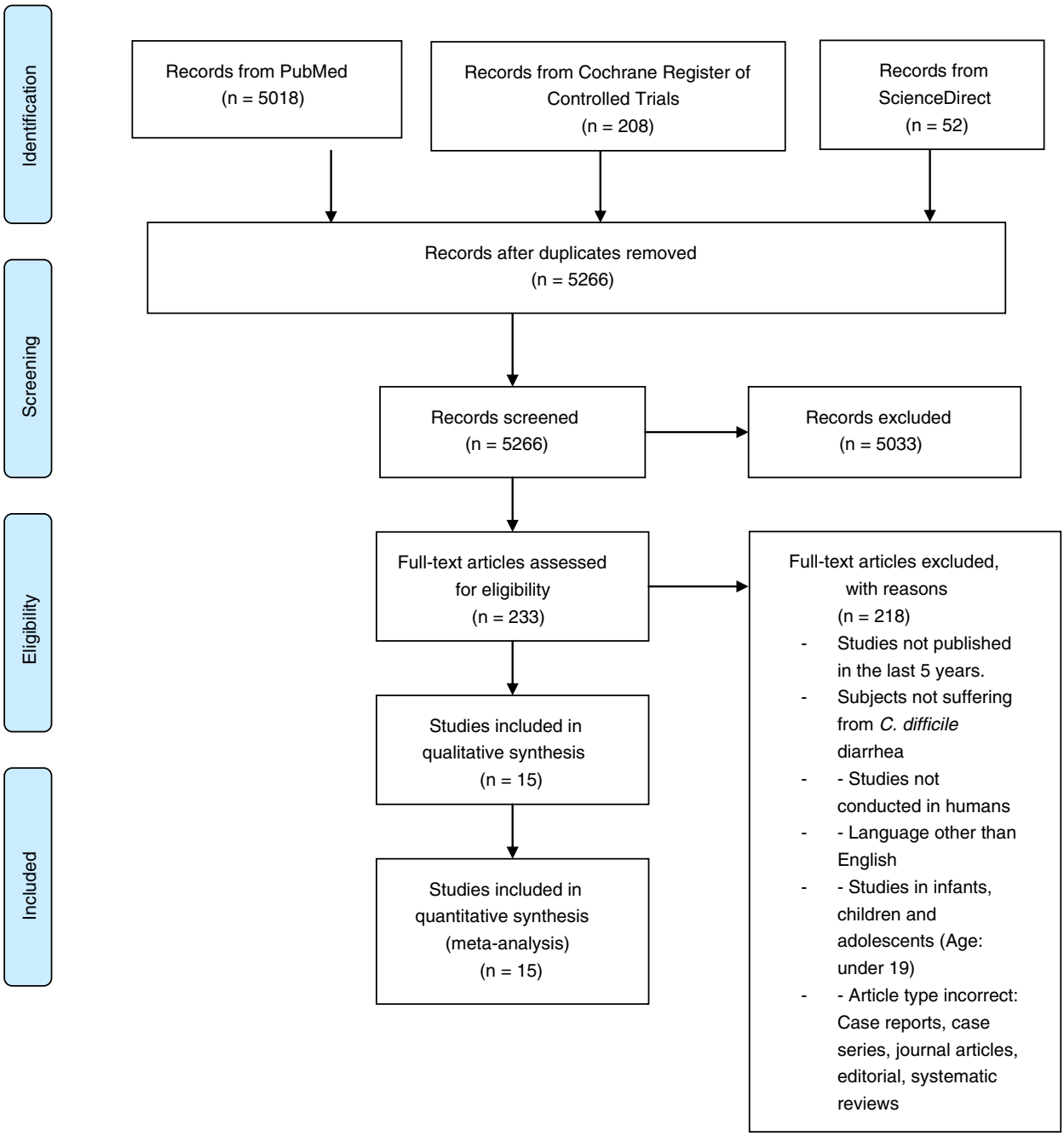



**Figure 1** The PRISMA flow diagram outlines the search and selection process applied for the preparation of this review

confirming low publication bias. These analyses demonstrate the remarkable homogeneity and suggests a low overall bias in our analysis.

Furthermore, FMT´s efficacy increases with the number of doses (Cammarota *et al.* 2015; Agrawal *et al.* 2016; Ianiro *et al.* 2018; Juul *et al.* 2018; Hagel *et al.* 2016), as it has been shown, in general, that patients who had received two treatment doses have lower rates of disease recurrence. As an exception, in the clinical trial by Dubberke, two doses of FMT were superior to the administration of one dose, however the results were not statistically significant (Dubberke *et al.* 2018).

Recent clinical trials show vancomycin is superior to metronidazole for non-severe CDI, with a percentage

**Table 1** Summary of the descriptive characteristics of the included studies

| Author | N° of participants | Mean age ± SD | Recovery | Regression | Administration route | N° AE/patients | Adverse effects | Type of article |
|---|---|---|---|---|---|---|---|---|
| Dubberke *et al.* (2018) | 127<br>2 FMT: 41<br>2 placebo: 44<br>1 FMT: 42 | 18+ years | Overalll efficacy 89%<br>95/107<br>2 FMT: 25/41 (61%)<br>2 placebo: 20/44 (45%)<br>1 FTM: 28/42 (67%) | 12 (11%)<br>16 (29%)<br>24 (55%)<br>14 (33%) | Enema | 379 AE 82 p (blinded phase) | Mild GI disorders, infections and nervous disorders | Clinical trial |
| Kelly *et al.* (2016) | 46 | 75 | Donor FMT 20/22 (90·9%)<br>Autologous 15/24 (62·5%) | 2/22 (9·1%)<br>9/24 (37·5%) | Colonoscopy | N/S | Abdominal pain, bloating, vomiting, flatulence, constipation, diarrhea, fever, anorexia, constipation | Clinical trial |
| Lee *et al.* (2016) | 232<br>Fresh: 118<br>Frozen: 114 | 72·65 ± 15·97 | Protocol<br>Fresh 76/91 (83·5%)<br>Frozen 74/87 (85·1%)<br>mITT<br>Fresh 81/108 (75%)<br>Frozen 78/111 (70·3%) | Protocol<br>Fresh 15<br>Frozen 13<br>mITT<br>Fresh 27<br>Frozen 33 | Enema | 29 | Diarrhea (70%), abdominal cramps, nausea, constipation, flatulence | Clinical trial |
| Orenstein *et al.* (2016) | 31 | 18+ years | 27/31 (87·1%) | 4 (12·9%) | Enema | 188 in 28 patients | Diarrhea, flatulence, abdominal pain, cramping, constipation, infections | Clinical trial |
| Cammarota *et al.* (2015) | 39<br>FMT: 20<br>Vancomycin: 19 | 71 (29-89) | 18/20 (90%) | 2 (10%) | Colonoscopy | 31 patients | Diarrhea (94%), bloating, abdominal cramping (60%). | Clinical trial |
| Jiang *et al.* (2017) | 72<br>Fresh 25<br>Lyophilised 23<br>Frozen 24 | Fresh: 75 (19-97)<br>Frozen: 62·5 (33-88)<br>Lyophilised: 63 (20-87) | Overall efficacy 87%<br>63/72<br>Fresh 25/25<br>Lyophilised 16/23<br>Frozen 20/24 | Overall: 9<br>Fresh: 0<br>Lyophilised: 7<br>Frozen: 4 | Colonoscopy | 76 patients | Nausea, mild diarrhoea, transient abdominal discomfort (86%), fatigue (8%), headache (6%) and weight gain (3%) | Clinical trial |
| Jiang *et al.* (2018) | 65<br>Lyophilized capsules: 31<br>Frozen enema: 34 | 18+ years<br>67 (20-95)<br>63 (28-97) | 56/65 (86%)<br>Lyophilized capsules: 26/31 (84%)<br>Frozen enema: 30/34 (88%) | Lyophilized capsules: 5/31 (16%)<br>Frozen enema: 4/31 (12%) | Enema | 152 in first 7 d<br>227 in 3 first 3 months | Diarrhea, nausea, vomiting, abdominal cramps/pain, fecal urgency, flatulence, constipation, others | Clinical trial |

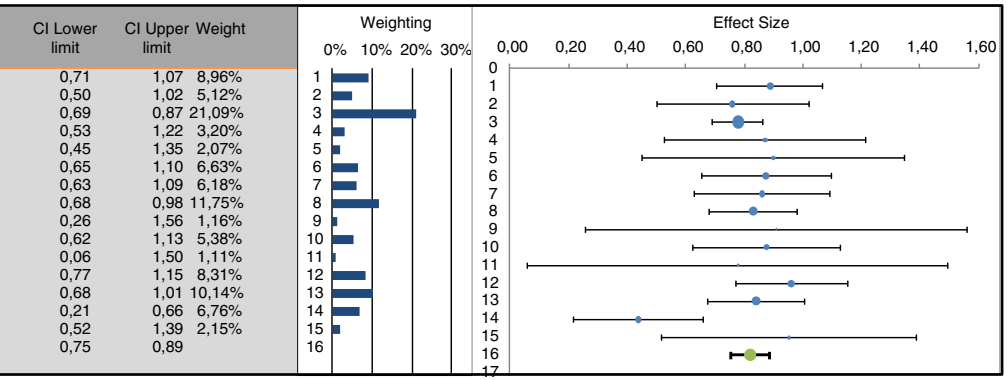
(continued)

**Table 1** (continued)

| Author | N° of participants | Mean age ± SD | Recovery | Regression | Administration route | N° AE/patients | Adverse effects | Type of article |
|---|---|---|---|---|---|---|---|---|
| Agrawal *et al.* (2016) | 146<br>RCDI: 89<br>SCDI: 45<br>CCDI: 12 | 78·6 (65-97) | Overall primary cure rate: 82·9% (121/146)<br>Overall secondary cure rate: 95·9% (140/146)<br>RCDI: 82%<br>SCDI: 91%<br>CCDI: 66% | Early recurrence<br>25 patients<br>Late recurrence<br>6 patients<br>RCDI: 18%<br>SCDI: 9%<br>CCDI: 34% | Colonoscopy<br>Enema, EGD<br>Sigmoidoscopy, enteroscopy | 11 patients | Diarrhea (7) and constipation (4) | Retrospective cohort |
| Friedman-Korn *et al.* (2018) | 34<br>FMT: 11<br>Control: 23 | 82<br>FMT: 78 ± 14<br>Control: 83 ± 8 | FMT: 10/11. 90%.<br>Control: 9/23. 39%. | FMT: 1/11. 10%.<br>Control: 14/23. 61% | Colonoscopy, EGD | **0** | Aspiration of transplant material and deterioration during sedation | Prospective cohort |
| Ianiro *et al.* (2018) | 56<br>FMT-S: 28<br>FMT-M: 28 | 75 | FMT-S: 21/28 (75%)<br>FMT-M: 28/28 (100%) | FMT-S: 7 (25%)<br>FMT-M: 0 (0%) | Colonoscopy | 78 patients | Mild diarrhea (38) and constipation (40) | Clinical trial |
| Juul *et al.* (2018) | 20<br>FMT: 9<br>Metronidazole: 11 | 18+ years | Overall efficacy: 78%<br>7/9<br>FMT: 5/9. 56%<br>Metronidazole: 5/11. 45% | Overall: 22%<br>FMT: 4/9<br>Metronidazole: 6/11 | Enema | 1 patient | Foul stool smell | Clinical trial |
| Kao *et al.* (2017) | 116. 105 complete trial<br>Capsule: 57<br>Colonoscopy: 59 | 58 | Overall efficacy: 96·2%<br>101/105<br>Capsule: 51/53<br>Colonoscopy: 50/52 | 3·8%. 4/105<br>Capsule: 2/53<br>Colonoscopy: 2/52 | Colonocopy<br>Capsule | 14 in 10 patients | Capsule group vs colonoscopy group<br>Nausea (3 vs 1)<br>Vomiting (2 vs 1)<br>Fever (0 vs 1)<br>Abdominal pain (1 vs 5) | Clinical trial |
| Hagel *et al.* (2016) | 133 | 75 (59·5–81·5) | Primary cure rate: 84·2% −101/120-(d 30) 78·3% −72/92-(d 90). Secondary cure rate: 87·5% −105/120-(d 30) 85·9% −79/92-(d 90) | 1 regression<br>19/120 (d 30)<br>29/92 (d 90)<br>2 regression<br>15/120 (d 30)<br>13/92 (d 90) | Duodenal (59)<br>Colonoscopy (55)<br>Capsule (13)<br>Gastric (4) | 16 patients (12%) | Nausea, fever, emesis, abdominal pain, belching, throat/retroesternal discomfort | Retrospective cohor |
| Hota *et al.* (2017) | 30<br>FMT<br>Vancomyicin | FMT: 75·7<br>Vancomycin: 69·6 | FMT: 7/16. 43·75%<br>Vancomycin: 7/12. 58·33% | FMT: 9/16.<br>Vancomycin: 5/12 | Enema | Early (0-7 d): 16<br>Late: 11 | Fever, vomiting, fatigue, abdominal pain, bloating, incontinence, stool smell | Clinical trial |
| Garza-Gonzalez *et al.* (2019) | 21. FMT (13) vs FMT-Lactobacillus (8) | 61 years (17-91) | Overall efficacy: 95·2%<br>20/21 | Overall: 4·8%<br>1/21 | Capsules | 9 patients | Burping, constipation, vomiting | Clinical trial |

**(a)**

| # | Study name | Effect size | CI Lower limit | CI Upper limit | Weight |
|---|---|---|---|---|---|
| 1 | Dubberke, 2018 | 0,89 | 0,71 | 1,07 | 8,96% |
| 2 | Kelly, 2016 | 0,76 | 0,50 | 1,02 | 5,12% |
| 3 | Lee, 2016 | 0,78 | 0,69 | 0,87 | 21,09% |
| 4 | Orenstein, 2016 | 0,87 | 0,53 | 1,22 | 3,20% |
| 5 | Cammarota, 2015 | 0,90 | 0,45 | 1,35 | 2,07% |
| 6 | Jiang, 2017 | 0,88 | 0,65 | 1,10 | 6,63% |
| 7 | Jiang, 2018 | 0,86 | 0,63 | 1,09 | 6,18% |
| 8 | Agrawal, 2016 | 0,83 | 0,68 | 0,98 | 11,75% |
| 9 | Friedman-Korn, 2018 | 0,91 | 0,26 | 1,56 | 1,16% |
| 10 | Ianiro, 2018 | 0,88 | 0,62 | 1,13 | 5,38% |
| 11 | Juul, 2018 | 0,78 | 0,06 | 1,50 | 1,11% |
| 12 | Kao, 2017 | 0,96 | 0,77 | 1,15 | 8,31% |
| 13 | Hagel, 2016 | 0,84 | 0,68 | 1,01 | 10,14% |
| 14 | Hota, 2017 | 0,44 | 0,21 | 0,66 | 6,76% |
| 15 | Garza-González, 2019 | 0,95 | 0,52 | 1,39 | 2,15% |
| 16 | SummaryStatistic | 0,82 | 0,75 | 0,89 | |




**(b)**

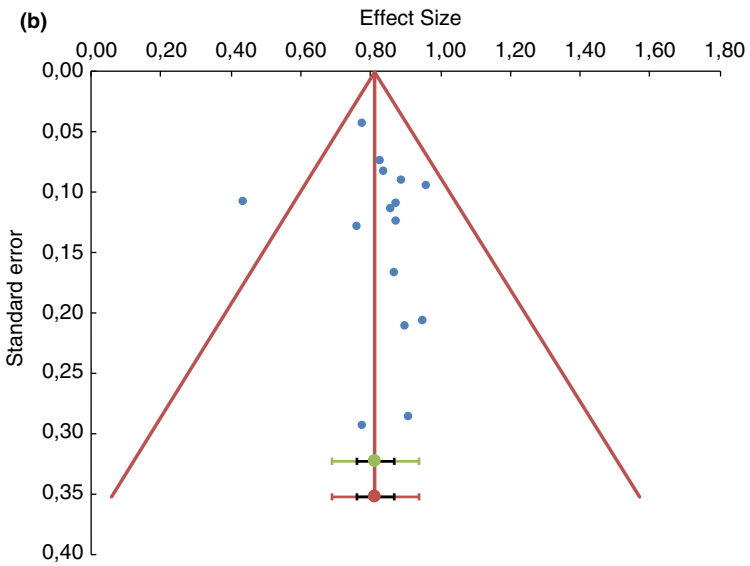



**Figure 2** (a) Forest Plot showing the proportion of treated patients recovering following FMT treatment. Combined effect size (grey circle) is 82% (95% CI: 75-89). (b) Funnel Plot showing the dispersion and heterogeneity of included studies (blue circles). The trim-and-fill adjustment identified no possible missing studies (open circles). (●) Combined effect size; (●) Adjusted CES; (●) Inputed Data Points.

resolution of 87% compared to 78% for metronidazole (Dieterle *et al.* 2019). Other studies showed that fidaxomicin was more effective than vancomycin for achieving symptomatic cure, with a 71% recovery rate (Nelson *et al.* 2017). Overall, our study shows equivalent or superior effectiveness of FMT when compared to the gold standard antibiotic regimes (vancomycin and fidaxomicin).

### Safety of FMT

Adverse effects after FMT were reported in 14 of the 15 articles included in the review. Most affected the gastrointestinal tract, the most frequent symptom being diarrhea not caused by *C. difficile* (Cammarota 2015; Agrawal *et al.* 2016; Orenstein *et al.* 2016; Jiang *et al.* 2017; Kelly *et al.* 2016; Lee *et al.* 2016; Ianiro *et al.* 2018; Jiang *et al.* 2018). However, all adverse effects were mild and self-resolved within several days. Hota *et al.* (2017) reported fewer incidences of adverse effects in the FMT treatment group when compared to the control group receiving standard antibiotic therapy. The nature of the adverse effects however, were comparable between the two groups (abdominal pain, tenderness and bloating. Some authors such as Friedman-Korn *et al.* (2018) and Kao *et al.* (2017) report differences in the percentage of adverse effects between groups of participants based on the administation route of the treatment. In the Friedman-Korn study, the authors showed that FMT via colonoscopy was associated with less risk and a lower rate of adverse affects as compared to

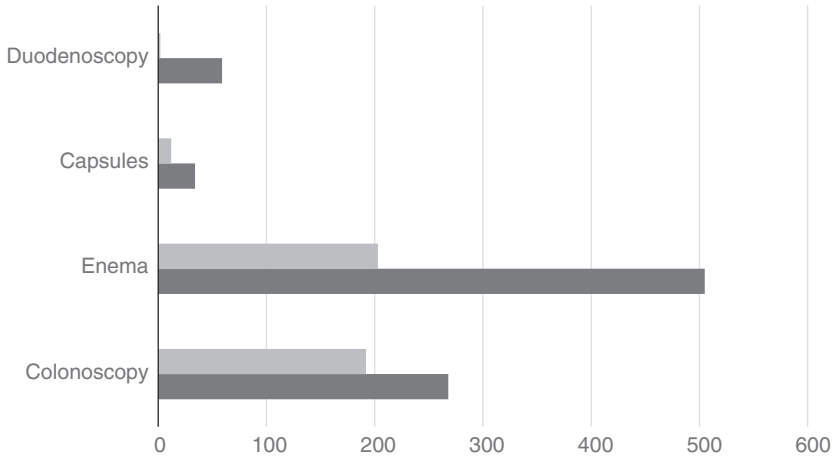



**Figure 3** Total of patients treated with FMT and number of patients who experienced adverse effects divided by administration route. (○) Patients reporting adverse effects; (▒) Patients treated.

esophagogastroduodenoscopy, in which stool aspiration was reported in two people (Friedman-Korn *et al.* 2018). The authors of the Kao study revealed that 12·5% of patients receiving FMT via colonoscopy reported adverse effects, whilst 5·4% of the group receiving oral treatment via capsules reported undesirable side effects (Kao *et al.* 2017). Kelly *et al.* (2016) and Lee *et al.* (2016) did not report the number of patients experiencing adverse effects. Figure 3 summarizes the proportion of patients experiencing adverse effects. Overall, patients receiving FMT via colonoscopy experienced more adverse effects than patients whom received enema, or oral capsules (71·6% *vs* 37·1%, and 23·1% respectively). With regards to the administration of FMT via esophagogastroduodenoscopy, authors described a low overall proportion of patients suffering from adverse effects (3·4%), however, clinically, the adverse effects appeared to be more serious (stool aspiration).

### Impact of administration route in efficacy

Colonoscopy is regarded as the FMT treatment route of choice by practitioners as it allows observation of the colon and collection of biopsy samples if necessary (Agrawal *et al.* 2016). The Hedges' *g* value of the standardized mean difference in treatment effectiveness between patients receiving FMT by colonoscopy versus another administration route were calculated. Negative values favor colonoscopy over the alternative and positive values favor the alternative. Comparing administration of FMT by colonoscopy versus enema resulted in a Hedges' *g* of −0·74 (95% CI of −0·90 to −0·58), indicating an advantage in effectiveness in favor of colonoscopy. A comparison of treatment effectiveness between colonoscopy and capsule returned a Hedges' *g* of 0·44 (95% CI of 0·20–0·69), indicating that delivery of the FMT by capsule was statistically significantly more effective than colonoscopy. Finally, an analysis of administration by colonoscopy as compared to esophagogastroduodenoscopy showed that a Hedges' *g* of −2·28 (95% CI of −2·63 to −1·93) indicating that colonoscopy was significantly more effective as a delivery method when used for the treatment of CDI by FMT (Fig. 4). This last conclusion should be examined carefully, however, as only one article gave data about cures and recurrences of the disease after FMT by esophagogastroduodenoscopy. Furthermore, Ianiro *et al.* (2018) presented data showing that there was no statistically significant difference in the treatment effectiveness between the two routes of administration. Further investigation is required to confirm these results.

| # | Study name | Hedges'g | CI Lower limit | CI Upper limit |
|---|---|---|---|---|
| 1 | Colonoscopy vs Enema | -0,74 | -0,90 | -0,58 |
| 2 | Colonoscopy vs Capsule | 0,44 | 0,20 | 0,69 |
| 3 | Colonoscopy vs Duodenoscopy | -2,28 | -2,63 | -1,93 |

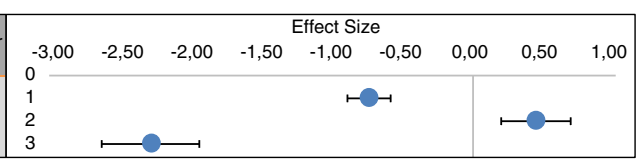



**Figure 4** A Forest plot showing the Hedges' *g* value of the standardized mean difference in treatment effectiveness (combined studies) between patients receiving FMT by colonoscopy versus another administration route. Negative values favor colonoscopy while and positive values favor the alternative

### Limitations

Preparation protocols for the FMT were not an intended analysis variable of this review and meta-analysis, hence no conclusions are presented in regards with differences in effectiveness when different preparation protocols are used. One study reported no statistically significant differences between using fresh and frozen stools (efficacies of 83·5 and 85·1% respectively; Lee *et al.* 2016). Kelly *et al.* (2016) reported significant differences in effectiveness between allogenic and autologous FMT (90·9% vs 62·5%). Nonetheless, thorough analysis of the effectiveness and safety of the current available protocols requires attention. Current protocols recommend application of FMT following the second recurrence of CDI, however Garza-Gonzalez *et al.* (2019) suggest that the response rate may be further improved by applying FMT as a first-round therapy, taking advantage of a lower dysbiosis at an earlier stage.

FMT has been proven to be an effective (82% recovery) and safe (mild side effects) treatment for *C. difficile* diarrhea as compared to gold standard antibiotic therapy. Additional benefits of FMT include avoidance of antibiotic use and therefore the potential of reducing antimicrobial resistance. Patients receiving FMT via colonoscopy experienced more adverse effects than patients whom received enema, or oral capsules. Furthermore, oral administration of capsules showed superior effectiveness when compared to colonoscopy and enema. Further research into the most effective clinical protocols to prepare the FMT as well as more comparative studies on administration routes are needed.

## Materials and methods

### Protocols and registration

This systematic review was created in accordance with the Preferred Reporting System for Systematic Reviews and Meta-Analyses (PRISMA) (Moher *et al.* 2009). The protocol has been registered at the International Prospective Register of Systematic Reviews (PROSPERO).

### Eligibility

We searched for original articles reporting outcomes in individuals with diagnoses of *C. difficile* diarrhea. In order to locate potentially suitable studies, we conducted several searches using three electronic databases (PubMed, Cochrane, Science Direct). The keywords used were "F*cal microbiota transplant*" AND "Clostridium difficile diarrh*a" and "F*cal microbiota transplant*" AND "Clostridioides difficile diarrh*a". A manual search for articles was also carried out and, when necessary authors were contacted directly for unpublished data and additional information.

### Inclusion and exclusion criteria

Full-text articles published in English during the last 5 years involving adult human participants aged over 19 years old were included for analysis. Clinical trials and analytic observational studies (cohorts and case-control) were included. The following study types were excluded from this review: biographies, directories, editorial, lectures, commentaries, abstracts, reviews, meta-analysis. Studies that did not focus on the subject of study or used pediatric patients were also excluded.

### Data extraction

The titles, abstracts, results and conclusions of the articles identified from the search results were screened. The included articles were then evaluated with respect to the exclusion criteria. The following information was collected from the full-text articles comprising the final selection: author(s), publication year, country, number of participants, subject ages, treatment given, the proportion of recoveries and recurrences, administration route of the FMT therapy and adverse effects. Articles were reviewed by authors RPB and VV. RPB collected the necessary data from the chosen articles for subsequent evaluation and VV and CCS cross-checked data for suitability.

### Quality assessment measures

The CASP quality assessment tool was used to appraise primary research articles (Clinical Appraisal Skills Programme 2020).

### Outcome measures

The primary evaluated outcome was FMT effectiveness. Secondary outcomes include the proportion of patients suffering from adverse effects and the efficacy of the administration routes used for the delivery of FMT. Recovery was defined as the absence of diarrhea between 8 and 13 weeks following treatment.

### Statistical analysis

Statistical analysis was carried out on all collected data prior to presentation of the results. CASP data analysis was carried out by calculating the proportion "Yes", "No" and "Don't know/Can't tell" responses as a function of the total number of questions. The proportion of "Yes" responses correlates with the study quality.

The Meta-Essentials tool was used to carry out all the meta-analysis calculations (Suurmond *et al.* 2017). Briefly, the tool requires the use of a set of Microsoft Excel workbooks that, following data input, automatically carry out the required statistics and generate the necessary tables and figures.

The summary statistic for the effectiveness of FMT treatment and that of the comparison of administration routes for the delivery of FMT was calculated and presented via a Forest plot, using a fixed-effects model and a 95% confidence level. The data used to construct the Forest plot was also used to estimate the extent of heterogeneity via the calculation of the $I^2$ value.

An analysis of possible publication bias was carried out and displayed in the form of a funnel graph. The Meta-Essentials tool allows the calculation and adjustment for the estimate of the combined effect size in order to correct for potential publication bias. A detailed explanation of the calculation can be found in the Meta-Essentials user manual (van Rhee *et al.* 2015). The funnel graph was also used to carry out significance testing for publication bias. The Egger test was used as the Begg and Mazumdar test is unreliable for meta-analyses with a small number of included studies such as this one.

## Funding

No grants supported the current study.

## Ethical statement

This systematic review and meta-analysis was carried out in accordance with the Helsinki guidelines, and approved by the Ethics Committee of CEU Cardenal Herrera University (authorization number CEI20/067).

## Conflict of Interest

No conflict of interest declared.

## References

Agrawal, M., Aroniadis, O.C., Brandt, L.J., Kelly, C., Freeman, S., Surawicz, C., Broussard, E. & Stollman, N. *et al.* (2016) The long-term efficacy and safety of fecal microbiota transplant for recurrent, severe, and complicated *Clostridium difficile* infection in 146 elderly individuals. *J Clin Gastroenterol* **50**, 403–407. https://doi.org/10.1097/MCG.0000000000000410.

Aroniadis, O.C. and Brandt, L.J. (2013) Fecal microbiota transplantation: past, present and future. *Curr Opin Gastroenterol* **29**, 79–84.

Banawas, S.S. (2018) *Clostridium difficile* infections: a global overview of drug sensitivity and resistance mechanisms. *Bio Med Res Int.* **2018**(8414257), 1–9. https://doi.org/10.1155/2018/8414257.

Cammarota, G., Masucci, L., Ianiro, G. *et al.* (2015) Randomised clinical trial: faecal microbiota transplantation by colonoscopy vs. vancomycin for the treatment of recurrent *Clostridium difficile* infection. *Aliment Pharmacol Ther* **41**, 835–843.

Cho, J.M., Pardi, D.S. and Khanna, S. (2018) Update on treatment of *Clostridioides difficile* infection. *Mayo Clin Proced* **95**, 758–769.

Clinical Appraisal Skills Programme(2020). https://casp-uk.net

Dieterle, M.G., Rao, K. and Young, V.B. (2019) Novel therapies and preventative strategies for primary and recurrent *Clostridium difficile* infections. *Ann N Y Acad Sci* **1435**, 110–138.

Dubberke, E.R., Lee, C.H., Orenstein, R. *et al.* (2018) Results from a randomized, placebo-controlled clinical trial of a RBX2660—a microbiota-based drug for the prevention of recurrent *Clostridium difficile* infection. *Clin Infect Dis* **67**, 1198–1204.

Friedman-Korn, T., Livovsky, D.M., Maharshak, N., Aviv Cohen, N., Paz, K., Bar-Gil Shitrit, A., Goldin, E. and Koslowsky, B. (2018) Fecal transplantation for treatment of *Clostridium difficile* infection in elderly and debilitated patients. *Dig Dis Sci* **63**, 198–203.

Garza-González, E., Mendoza-Olazaran, S., Morfin-Otero, R. *et al.* (2019) Intestinal microbiome changes in Fecal Microbiota Transplant (FMT) *vs.* FMT enriched with *Lactobacillus* in the treatment of recurrent *Clostridioides difficile* infection. *Can J Gastroenterol Hepatol* **2019**, 4549298.

Hagel, S., Stallmach, A. and Vehreschild, M. (2016) Fecal microbiota transplant in patients with recurrent *Clostridium Difficile* infection: a retrospective multicenter observational study from the microtrans registry. *Dtsch Arztebl Int* **113**, 583.

Herbert, R., Hatcher, J., Jauneikaite, E. *et al.* (2019) Two-year analysis of *Clostridium difficile* ribotypes associated with increased severity. *J Hosp Infect* **103**, 388–394.

Hota, S.S., Sales, V., Tomlinson, G., Salpeter, M.J., McGeer, A., Coburn, B., Guttman, D.S., Low, D.E. *et al.* (2017) Oral vancomycin followed by fecal transplantation versus tapering oral vancomycin treatment for recurrent *Clostridium difficile* infection: an open-label, randomized controlled trial. *Clin Infect Dis* **64**, 265–271.

Ianiro, G., Masucci, L., Quaranta, G. *et al.* (2018) Randomised clinical trial: faecal microbiota transplantation by colonoscopy plus vancomycin for the treatment of severe refractory *Clostridium difficile* infection—single versus multiple infusions. *Aliment Pharmacol Ther* **48**, 152–159.

Jiang, Z.D., Ajami, N.J., Petrosino, J.F. *et al.* (2017) Randomised clinical trial: faecal microbiota transplantation for recurrent *Clostridium difficile* infection–fresh, or frozen, or lyophilised microbiota from a small pool of healthy donors delivered by colonoscopy. *Aliment Pharmacol Ther* **45**, 899–908.

Jiang, Z.-D., Jenq, R.R., Ajami, N.J., Petrosino, J.F., Alexander, A.A., Ke, S., Iqbal, T., DuPont, A.W. *et al.* (2018) Safety and preliminary efficacy of orally administered lyophilized fecal microbiota product compared with frozen product given by enema for recurrent *Clostridium difficile* infection: a randomized clinical trial. *PLoS One* **13**, e0205064.

Juul, F.E., Garborg, K., Bretthauer, M. *et al.* (2018) Fecal microbiota transplantation for primary *Clostridium difficile* infection. *N Eng J Med* **378**, 2535–2536.

Kao, D., Roach, B., Silva, M. *et al.* (2017) Effect of oral capsule–vs colonoscopy-delivered fecal microbiota transplantation on recurrent *Clostridium difficile* infection: a randomized clinical trial. *JAMA* **318**, 1985–1993.

Kassam, Z., Lee, C.H., Yuan, Y. and Hunt, R.H. (2013) Fecal microbiota transplantation for *Clostridium difficile* infection: systematic review and meta-analysis. *Am J Gastroenterol* **108**, 500–508.

Kelly, C.R., Khoruts, A., Staley, C. *et al.* (2016) Effect of fecal microbiota transplantation on recurrence in multiply recurrent *Clostridium difficile* infection: a randomized trial. *Ann Intern Med* **165**, 609–616.

Khoruts, A., Dicksved, J., Jansson, J.K. and Sadowsky, M.J. (2010) Changes in the composition of the human fecal microbiome after bacteriotherapy for recurrent *Clostridium difficile*-associated diarrhea. *J Clin Gastroenterol* **44**, 354–360.

Lee, C.H., Steiner, T., Petrof, E.O. *et al.* (2016) Frozen vs fresh fecal microbiota transplantation and clinical resolution of diarrhea in patients with recurrent *Clostridium difficile* infection: a randomized clinical trial. *JAMA* **315**, 142–149.

Moher, D., Liberati, A., Tetzlaff, J. *et al.* (2009) The PRISMA group. Preferred reporting ítems for systematic reviews and meta-analyses: The PRISMA Statement. *PLoS Med* **6**, e1000097.

Nelson, R.L., Suda, K.J. and Evans, C.T. (2017) Antibiotic treatment for *Clostridium difficile*-associated diarrhoea in adults. *Cochrane Database Syst Rev* **3**, CD004610.

Orenstein, R., Dubberke, E., Hardi, R. *et al.* (2016) Safety and durability of RBX2660 (microbiota suspension) for recurrent *Clostridium difficile* infection: results of the PUNCH CD study. *Clin Infect Dis* **62**, 596–602.

Polivkova, S., Krutova, M., Petrlova, K., Benes, J. and Nyc, O. (2016) *Clostridium difficile* ribotype 176 – a predictor for high mortality and risk of nosocomial spread? *Anaerobe* **40**, 35–40.

van Rhee, H.J., Suurmond, R. and Hak, T. (2015) *User manual for meta-essentials: Workbooks for meta-analysis (Version 1.4)*. Rotterdam, The Netherlands: Erasmus Research Institute of Management. www.erim.eur.nl/research-support/meta-essentials

Smits, L.P., Bouter, K.E.C., de Vos, W.M., Borody, T.J. and Nieuwdorp, M. (2013) Therapeutic potential of fecal microbiota transplantation. *Gastroenterology* **145**, 946–953.

Suurmond, R., van Rhee, H. and Hak, T. (2017) Introduction, comparison and validation of meta-essentials: a free and simple tool for meta-analysis. *Res Synth Methods* **8**, 537–553.

Societat Catalana de Digestologia. Trasplante microbiota fecal. http://www.scdigestologia.org/docs/patologies/es/Trasplantament_microbiota_fecal_es.pdf

Wilson, M. (2019) The indigenous microbiota of the gastrointestinal tract. In, *The Human Microbiota in Health and Disease. An Ecological and Community-Based Approach*. pp. 353–425. Boca Raton, FL: CRC Press.

World Health Organization (WHO). Antibiotic resistance. http://www.who.int/news-room/fact-sheets/detail/antibiotic-resistance

## Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Figure S1.** Summary table of Critical Appraisal Skills Programme (CASP). Tool for the evaluation of literature reviews.