PROFESSOR MICHAEL A KAMM (Orcid ID : 0000-0003-3319-7199)

DR GILAAD GERSHON KAPLAN (Orcid ID : 0000-0003-2719-0556)

DR SIEW C NG (Orcid ID : 0000-0002-7706-3988)

Article type : Systematic Review with Meta-analysis

Systematic Review with Meta-analysis: Review of Donor Features, Procedures and Outcomes in 168 Clinical Studies of Faecal Microbiota Transplantation

Cheuk Yin Lai[1#], Joanne Sung[1#], Felix Cheng[1#], Whitney Tang[1], Sunny H Wong[2], Paul KS Chan[3,5], Michael A Kamm[4], Joseph JY Sung[2], Gilaad Kaplan[5], Francis KL Chan[2,5], Siew C Ng[2,5]

1. Department of Medicine and Therapeutics, The Chinese University of Hong Kong, Hong Kong, China
2. Department of Medicine and Therapeutics, Institute of Digestive Disease, State Key Laboratory of Digestive Disease, LKS Institute of Health Sciences, The Chinese University of Hong Kong, Hong Kong, China
3. Department of Microbiology, The Chinese University of Hong Kong, Hong Kong, China.
4. St Vincent's Hospital and University of Melbourne, Melbourne, VIC, Australia
5. Departments of Medicine and Community Health Sciences, The University of Calgary, Calgary, Canada
6. Center for Gut Microbiota Research, The Chinese University of Hong Kong, Hong Kong, China

# equal contribution

Correspondence:

Siew C Ng, MBBS, MRCP, PhD

Department of Medicine and Therapeutics

Chinese University of Hong Kong, Hong Kong

Tel: 852 2632 1420

Fax: 852 2637 3852

E-mail: siewchienng@cuhk.edu.hk

Short running title: FMT and donor

Abbreviations: BMI, body mass index; FMT, faecal microbiota transplantation

Authorship statement: All authors have contributed to the study design, study identification, data collection, and manuscript revision. All authors have seen and approved the manuscript. Siew C Ng, as the guarantor of the article, had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Acknowledgements: The authors have no additional acknowledgements.

Manuscript word count: 3695

Structured summary

Background: Faecal microbiota transplantation (FMT) is effective for *Clostridium difficile* infections (CDI) refractory to standard treatment, and is being studied in other diseases. **Aims:** We evaluated donor characteristics, procedures and clinical outcomes of FMT. **Methods:** We systematically reviewed FMT studies published up to 29 August 2018 using MEDLINE (R) and EMBASE and identified clinical studies with FMT donor information. We reported data on donor characteristics, screening criteria, administration, clinical outcomes and adverse events. **Results:** Amongst 5,267 reports, 239 full-text

articles were screened and 168 articles were included. FMT was performed commonly for CDI (n=108) and inflammatory bowel disease (IBD) (n=31). We reported characteristics of 1,513 donors [58% male; mean age, 34.3 years; mean body mass index, 21.6]. Donor in Asia were younger than the West (mean age 30.7 versus 32.9, p=0.00075). Less than 50% of studies screened donor for transmittable pathogens. Final cure rate for CDI was 95.6% [95% confidence interval (CI), 93.9%-97.1%] and final remission rates for ulcerative colitis (UC) and Crohn's disease (CD) were 39.6% (95% CI, 25.4%-54.6%) and 47.5% (95% CI, 29.4%-65.8%), respectively. Cure rates in CDI and final remission rates for CD and UC were comparable across all routes of FMT administration. Overall adverse event incidence was <1%, mostly GI-related. Adverse events rates did not differ between routes of FMT administration or indication. **Conclusions:** In a systematic review assessing donor characteristics and FMT efficacy, we observed heterogeneity in donor selection, application and outcomes of FMT. These data can facilitate standardization of FMT protocols for various diseases.

Keywords: donor; recipient; clostridium difficile; inflammatory bowel disease

Introduction

Faecal Microbiota Transplantation (FMT) involves the engraftment of the gut microbiota of healthy individuals into diseased recipients to reconstitute a normal intestinal microbial composition. It is currently been used to treat recurrent and refractory Clostridium difficile infections worldwide (CDI)[1,2]. There is also increasing investigation into other conditions such as inflammatory bowel disease (IBD), irritable bowel syndrome, obesity, acute graft-versus-host diseases and autism[3-7]. The United States Food and Drug Administration has approved that investigational new drug (IND) application is not required for the use of FMT for treatment of recurrent CDI but except for stool banks distributing stool to physicians[8]. Recent frameworks have promoted the need of easy access for patients to FMT as well as efficacy and safety data for regulators and providers. However, research in FMT beyond the indication of CDI remains limited[9].

Importantly, standardized FMT procedures are lacking, particularly on donor characteristics and selection. Although International recommendations exist for FMT, they are rarely implemented rigorously in practice[10]. Several meta-

analyses have reported data on the efficacy and safety of FMT for CDI but few have analysed donor characteristics or microbial profile[2,11-16]. Even widely used protocols have highly variable donor selection methods which are mostly limited to screening for infections and co-morbidities[17-19]. We performed a systematic review of all FMT clinical studies without language restriction up to 29th August 2018. This review comprehensively examined donor characteristics and screening protocols as well as FMT procedures and outcomes.

Methods

Data Sources and Searches

This systematic review followed the PRISMA 2009 guidelines. Search terms related or synonymous to FMT were utilized on OvidSP: MEDINE (R) (1946 to Present) and EMBASE (1910 to Present) up to 29th August, 2018. All identified papers were catalogued using EndNote X7. Detailed search strategies are outlined in **Appendix 1**.

Study selection

We included original studies containing donor information and fulfilling pre-defined inclusion and exclusion criteria. Systematic reviews, meta-analyses, conference presentations, letters or correspondences were excluded. Non-English reports were evaluated using Google Translate or members proficient in that language. In the first selection round, three reviewers (F.H.C, C.Y.L, C.Y.S.) independently catalogued all papers using predefined criteria, with inter-reviewer cross-checking. In the second phase, full texts of all identified reports were reviewed for suitability of inclusion. Disagreements were resolved by consensus with reviewers W.T and S.C.N.

Data Extraction and Quality Assessment

Extracted data was catalogued into variables under publication information (main author's name, publication year, study period/type), donor clinical characteristics (population, age, gender, donor-patient relationship), donor exclusion criteria and screening tests, FMT delivery methods (pre-medications, bowel lavage, FMT material mass/route/preparation), outcomes and adverse events (AE). Furthermore, we emailed all 168 corresponding authors to clarify

any uncertainties or missing data. 46 authors responded (27%) and 24 provided additional information.

Paper qualities were rated using the US National Heart, Lung and Blood Institute quality assessment tool for controlled intervention studies, observational cohort/cross-sectional studies, before-after (pre-post) studies with no control group, or case series, whichever was appropriate for the study type concerned[20]. For assessment of follow-up duration, 3 months or 90 days was selected as the cut-off for adequate follow-up period. **Appendix 2** shows quality assessments of individual papers.

Data synthesis and analysis

Data were stratified by continent of study or main disease indicating FMT, with descriptive statistics including percentages and central tendencies. P-values for FMT remission rates were calculated with the meta-analysis process described below; otherwise, p-values were obtained via Student's t-test, based on pooled individual patient data. For each variable, only studies with available data were analysed; the number involved is indicated in the tables. Studies containing multiple patient groups with distinct conditions or geographic origin were split into two components.

Data on donor clinical characteristics, exclusion criteria, screening tests and FMT delivery were pooled by study location; those involving populations from different continents were designated "multi-continent". Where unspecified, patient/donor populations were assumed to originate from the locality of the main author's institution. Studies with ambiguous or absent screening criteria were not analysed; for those listing selective criteria, unmentioned tests were considered as not performed. Studies referencing protocols from previous papers were assumed to have identical screening criteria.

Data on outcomes and AEs were pooled by main disease under study and route of FMT administration. FMT route was designated as upper GI for administrations by nasogastric/nasojejunal tube, esophagogastroduodenoscopy or oral capsule; those using colonoscopy or enema were lower GI, with the

remainder using mixed or unknown approaches.

Primary cure rate was defined as cure after a single FMT. Final cure rate was defined as the rate of cure after multiple FMTs. For studies with multiple FMT infusions, if it was intended as a single, multi-dose regimen, it was counted as 1 FMT; otherwise it would be counted as separate FMTs. In these cases, primary outcomes may be assessed on multiple occasions. For the purpose of our analysis, primary cure/remission rate (i.e. cure after 1 FMT) was calculated based on the first assessment which is the initial assessment after 1st FMT for all studies while final cure rate (i.e. cure after multiple FMT) was calculated based on the last assessment. A meta-analysis with pooled-proportions was performed for cure/remission rates in CDI, UC and CD studies. The Freeman-Tukey (double arcsine) transformation method and random effect model were used. The cure/remission rates were calculated using population remaining at primary outcome assessment; where participants were excluded from per-protocol analysis but still reported data from follow-up (e.g. by telephone interview), initial patient populations were used instead. Cure/remission rate was compared between different route of FMT in CDI, UC and CD using Wald-type test. All meta-analyses were performed in R version 3.4.1 using the "metafor" package.

Mean follow-up period and number of individual FMTs performed per patient were calculated as weighted mean by dividing the total follow-up period and total number of FMTs by the total number of patients across studies, using initial patient populations to account for participant attrition during follow-up. AEs were grouped by systems, and means were calculated by dividing the total number of patient having the specific AE by the total number of patients across studies. Those obviously or stated to be irrelevant – e.g. fall and hip fracture – were not analysed. Finally, we excluded from analysis studies with indeterminate frequencies for each AE; e.g. those giving total AE number only.

Results

5,267 records in total were identified from both databases, and 3803 remained after removal of duplicates by OvidSP. Of 239 papers remaining after screening,

40 were duplicates, and 31 were excluded after full-text review (**Figure 1**). 168 papers were ultimately included: 82 were from North America, 43 from Europe, 32 Asia, 4 Australia, 1 South America, 1 Africa and 5 were multi-continental. Countries that contributed most papers were the United States (U.S.) (n=68), China (n=22), Canada (n=10), and the Netherlands (n=8). The total patient population across all studies was 5,958, with patient populations of individual studies ranging from 2 to 462. 20 studies were randomized controlled trials, whilst the rest were uncontrolled studies. Publications relating to FMT have increased yearly: 17 papers were published in 2014, 24 in 2015, 36 in 2016 and 37 in 2017.

Indications for FMT

FMT was performed most frequently for CDI (n=108; 64.3%): 75 studies involved recurrent CDI, 26 involved refractory and recurrent CDI, and seven included subjects with concurrent IBD, transplant recipients or immunocompromised. The second commonest indication for FMT was IBD (n=31; 18.5%), of which 71.0% (n=22) involved ulcerative colitis. Other miscellaneous studies investigated slow transit constipation (n=6), IBS (n=3), and carriage of antibiotic-resistant bacteria (n=2). **Appendix 3** shows the study characteristics based on FMT indication.

Donor characteristics

117 studies including one study divided into two components provided specified characteristics of 1,513 FMT donors and 3,097 patients, with a donor-to-patient ratio of 1:2.0. 55 studies (33%) reported the gender of 485 donors, of which 58% were males. 38 papers (22.8%) reported the age of 325 donors; mean donor age was 34.3 years (range, 5-94). Asian studies had younger donors than Western studies (mean age, 30.7 versus 32.9, p=0.00075).

Only nine studies (5%) reported donor BMI; most accepted BMIs between 18.5 and 30, and the mean was 21.5. 100 studies reported donor-patient relationships for 1318 donors, of which 80% used unrelated donors. Overall, 22% of donors were first-degree relatives, 2% second-degree relatives, and 72% universal (i.e. unrelated) donors – of this last group, 13% were spouses or

partners of recipients, while 17% were non-specified family or household members. **Table 1** summarizes donor characteristics across 167 studies, with data for each continent in **Appendix 4**.

Donor selection

148 studies (88%) including 2 studies split into 2 components each reported 91 different exclusion criteria for donor selection based on medical history, categorized into personal (6), blood-borne disease risk (10), medication (18), GI diseases (15), immune diseases (4), metabolic diseases (3), cancers (2), infection (14), and others (19). Criteria varied substantially. Recent antibiotic use was the most frequent exclusion criterion (94%), but only utilized in 83% of European studies.

The most frequent exclusion criteria besides antibiotics involved GI diseases: irritable bowel syndrome (71%), IBD (70%), constipation (65%), diarrhoea (63%), communicable GI diseases (57%), GI surgery (57%), recent travel to high-risk/diarrhoea-endemic areas (53%), malignancy (62%), or systemic autoimmunity (51%). Other common exclusion criteria concerned exposure-risk to blood or body fluid-borne pathogens: high-risk sex/behaviour (51%), tattoos or piercings (42%), HIV/viral hepatitis exposure (39%) etc. However, less than 50% studies excluded other significant diseases like metabolic syndrome (41%), polyposis (32%), variant Creutzfeldt-Jakob disease risk (30%), psychiatric/neurological illness (29%), or diabetes mellitus (20%).

Some studies excluded donors if they met exclusion criteria within a timeframe before screening. Three months was the commonest duration for antibiotics use (47% of all studies), hospitalization (7%), immunosuppressive drugs (5%) and probiotics (3%). Six months was most frequent for travel history to high-risk areas (16%) or tattoos/piercings (14%), while 12 months was commonest for HIV/viral hepatitis exposure (9%), high-risk sex or behaviour (8%), blood product reception (7%), incarceration (6%), and needle-stick injury (5%). Finally, 4% of studies excluded donors who, within the last five days, ingested substances the FMT recipients were allergic to. Most studies omitted timeframes for exclusion criteria other than tattoos/piercings and antibiotics use.

Several studies excluded donors with family histories of malignancy (11%), IBD (9%), systemic autoimmunity (1%) or family members with current non-specified communicable GI disease (1%). The most common BMI and age requirements were <30 (20%) and >18 or <60 years (3%) respectively. **Table 2** presents the more popular donor exclusion criteria among 148 studies, with all exclusion criteria sorted by continents in **Appendix 5 and 6**.

Donor screening tests

159 papers including 3 papers split into 2 components each described 48 different stool tests and 53 different blood tests. Most popular stool tests included C. difficile toxins (95%), parasites/ova (94%), Salmonella (53%), Shigella (53%), Campylobacter (53%) and Cryptosporidium (52%). Other pathogens were screened in less than 50% of studies, including Giardia (49%), general enteric pathogen screen (48%), Yersinia (42%), E. Coli O157 (38%) and H. pylori (32%).

The most frequent blood tests were human immunodeficiency virus (HIV) (98%), hepatitis C virus (98%), hepatitis B virus (98%), hepatitis A virus (86%) and syphilis (84%). Other pathogens were screened in less than one-third of studies, e.g. cytomegalovirus (CMV) (31%), human T-lymphotropic virus (28%), Epstein-Barr virus (EBV) (28%). Full blood count (27%), liver enzymes (27%), C-reactive protein (18%), and renal function tests (11%) were also commonly tested. Fewer Asian studies screened vancomycin-resistant enterococci (VRE) (6% vs 28%) and Giardia (28% vs 58%) than North American studies, whereas fewer North American studies screened CMV (14% vs 53%) and EBV (11% vs 55%) than European studies. **Table 3** shows the more popular screening tests among 159 studies, with all tests sorted by continents in **Appendix 7**.

Donor material and delivery methods

168 studies were analysed. 85% studies described stool preparations; 63% of these used fresh stool, 27% frozen, 10% used a mix of fresh and frozen stool. The infused mass ranged from 25g to 300g. Only 17% and 42% of studies used

proton-pump inhibitors (PPI) and antibiotics respectively as pre-medication. Of 10 different administration routes reported, colonoscopy was the commonest (30.4%), followed by mixed routes (25.6%) and nasogastric/nasoduodenal tube (6.6% each). The commonest single infusion site was the terminal ileum/cecum (17.9%), while 15.5% used multiple infusion sites including jejunum/colon, stomach/small intestine, or colon/terminal ileum. Asian studies preferred upper GI routes of administration (58.6% vs 20.7% for lower GI), whilst North American studies favoured the lower GI route (41.0% vs 23.9% for upper GI); European studies showed less preference, as 45.2% used upper GI while 40.5% used lower GI. **Appendix 8** summarizes FMT procedures for each continent.

Clinical Outcomes

For CDI, 132 studies involving 4,609 patients were analysed, yielding a primary cure rate of 87.7% (95% CI, 84.4%-90.7%), and the final cure rate was 95.6% (95% CI, 93.9%-97.1%). Patients underwent a mean of 1.20 FMTs (range,1-6), with a mean follow-up of 7.7 months (range, 0-364). Some studies listed follow-ups of 0 months with data on outcomes but without further explanation. Cure rates were both comparable between all routes of FMT administration for both primary and final cure rates. We also analysed 29 UC studies and 10 CD studies, involving 545 and 115 patients respectively. For UC, the primary remission rate was 34.0% (95% CI: 21.3%-47.8%) and the final remission rate was 39.6% (95% CI, 25.4%-54.6%); while that for CD was 52.3% (95% CI, 33.5%-70.8%) and 47.5% (95% CI, 29.4%-65.8%) respectively. UC patients received a mean of 7.02 of FMTs (range, 1-41), with a mean follow-up of 7.8 months (range, 1-66); the high number of FMTs was due to several studies with multi-FMT design. CD patients received a mean of 1.01 FMTs (range, 1-2), with a mean follow-up of 6.1 months (range, 1-15). FMT by upper GI route had higher primary remission rate than that by mixed routes of administration for both CD (66.3% vs 27.1%, p = 0.012) and UC (43.3% vs 12.8%, p = 0.047). No study reported using lower GI route alone in CD. Final remission rates were comparable across all FMT administration routes in both CD and UC. Studies for other conditions showed variable success ranging from 33% to 100%. **Table 4** shows clinical outcomes in CDI and IBD studies

Adverse events (AEs)

135 studies reported AEs for 4,493 patients, of which 36 studies experienced no AEs. The commonest AEs were related to the gastrointestinal system including diarrhoea (13.0%), abdominal distention/flatulence (11.6%), nausea/ vomiting (6.1%), abdominal pain (5.5%) and constipation (2.1%). Other common AEs included fever (2.7%), respiratory difficulty (2.4%), headache (1.5%) and fatigue (1.4%). 1.3% patients also experienced IBD flares or IBD-like symptoms. AE rates were generally higher in FMT by upper GI compared to lower GI or mixed routes, except for IBD-related AE like IBD flare, colectomy or colitis. IBD patients experienced more fever than CDI (7.9% vs 2.0%, p = 0.011) or patients with other, miscellaneous conditions (7.9% vs 1.7%, p = 0.0090). Otherwise, AE rates did not show significant difference between routes of FMT administration or disease indicating FMT. **Table 5** shows rates of commoner AEs for all patients, while **Appendix 12** shows rates of all AEs sorted by disease and route of FMT administration.

Discussion

To our knowledge, this systematic review of 168 FMT clinical studies offers the most comprehensive and updated insight into current global FMT practice. FMT protocols, specifically donor selection and testing varied substantially with geography and indications. FMT studies published has doubled in number over the past few years with an increasing number of studies from Asia which suggests global utilization of FMT. Thus a more efficient and standardized FMT strategy is essential.

We expanded on a recent systemic review that assess FMT methodology[21] and included an additional 78 FMT studies with detailed donor characteristics. We also contacted corresponding authors for clarification of data. Most studies preferred male donors whereas females may be frequently excluded for functional GI disorders including irritable bowel syndrome[22]. Males were more likely to provide larger stool mass. Most donors were in their early thirties, as co-morbidities and chronic medication – including antibiotics – render older population unsuitable donors. Intestinal microbiomes in children may be underdeveloped and hence less biodiverse, possibly explaining the lack of child

donors to date, but this requires further investigation[23].

Animal studies have shown microbiota from obese mice increases adiposity in recipient mice[24], but only small proportion of FMT studies have reported donor BMI, and some recruited donors with BMI exceeding 30[25-32]. Among studies that did not report the BMI of donors, three recipients had weight gain after FMT[33-34], although the cause for this is unclear. Most studies recruited unrelated donors, as relatives may acquire similar dysbiosis through common environmental exposures with the recipient[23].

Strikingly, we found that exclusion criteria and screening tests were heterogeneous and largely incomplete in over 50% of studies[5]. Most North American studies adopted questionnaires by Bakken et al[17] or AABB[19], which focuses on specific viruses and parasites but less on enteric pathogens, CMV or EBV. Giardia is the commonest intestinal parasite in the USA[35], whereas EBV-associated diseases, such as nasopharyngeal carcinoma or Burkitt's lymphoma are less endemic[36-37]. European studies screened less for antibiotic use, drug abuse, GI surgery, immune or neurological diseases[38-40] than North American or Asian studies. Asian studies mostly focused on the exclusion of locally endemic enteric pathogens[37-44], or the use of immunosuppressant and chemotherapy drugs, while placing less emphasis on viral or parasite screening. Screening was suboptimal for comorbidities, including diabetes mellitus[45], or drugs that can alter the microbiome like PPI[46]; less than one-third of studies screened H. pylori and antibiotic-resistant organisms like MRSA and VRE, which may pose a potential risk of resistant strains if such organisms undergo horizontal gene transfer in recipients[47]. The cut-off period for medication use in donors was mostly 3 months[48], while that for risk of sexually-transmitted or blood-borne diseases was 12 months to minimize risk of asymptomatic carriage or incubation[49-50], in line with recommendations of a recent FMT consensus for HIV and viral hepatitis[5]. Though certain bacteria (e.g. Bifidobacteria) can engraft for up to six months[51], few studies specified cut-off periods for probiotic use. It is advisable to include a minimum screening panel for which donors must absolutely be excluded, e.g. along the lines proposed by recent consensus reports[5].

Current donor selection protocols mostly target known diseases for exclusion, while selection criteria for "ideal" donor remains under development[52]. Recent studies show that besides clinical factors, stool microbiome changes, e.g. bacteriophages, may be transferable and affect treatment outcome[16]. Recruitment of donors for FMT is challenging with only a small percentage ultimately accepted as donors[31]. Pooling stool from universal donors with standard screening protocols, for example in stool banks, may improve efficiency and cost-effectiveness, and multi-donor transplants may be associated with higher microbial diversity[6]. FMT administration was variable, with fresh stool more commonly used than frozen stool[53]. Though freezing presented potential opportunities for storage of stool from universal donors, only nine studies explicitly mentioned doing so; similarly, only three studies utilized pooled, multi-donor stool for FMT so comparison of efficacy between single versus pooled donors FMT was not possible. Colonoscopy route and faecal enema were most commonly used to administer transplant[54], especially for large bowel diseases. FMT oral capsules were utilized in six studies, most of which were indicated for CDI.

Our final cure rate in FMT for CDI is consistent with that of other studies[13,54], but remission rates for IBD was more variable, possibly due to chronic, complex host immunity-microbiota interactions. This highlights the potential of donor-dependent efficacy of FMT in IBD[27]. Despite geographic variations, success rates across continents and route of FMT administration were comparable. The apparently higher primary remission rates in studies utilizing upper GI FMT as opposed to FMT by mixed routes may be an indirect result of studies changing the FMT route after failing the first FMT, hence their being designated as "mixed" route. Moreover, definitions of "response" as opposed to "remission" varied widely, and as most studies tended to experience patient relapses during follow-up, remission rates may decline in those with longer follow-ups[26]. Whether FMT success is dose-dependent remains to be determined.

AEs were infrequent and mostly self-limiting usually lasting for a few days after FMT[55]. Procedural complications were also rare, and the few fatalities occurred

in patients with serious co-morbidities, thus FMT is unlikely to be a significant factor. IBD flares post-FMT (1.3%), along with more frequent fevers in IBD patients, may be a result of microbial irritation of the hyperactive mucosa, or secondary to withdrawal of immunosuppressant to fulfil clinical trial protocol[56]. Long-term safety of FMT remains undetermined, as complications require longer follow up duration to develop. Currently the American Gastroenterology Association has developed the first and largest FMT registry to track several thousands of FMT for up to 10 years. Moreover, most studies lack comparator arms to assess frequency and type of AEs; and AEs from single case reports and letters to the editor were excluded from our systematic review. Nevertheless, it is to date reassuring to observe FMT has few directly attributable AEs.

Our study has some limitations. First, it contains reports from only 27 countries, and most studies were from USA with underrepresentation of studies from developing countries. In addition, reports from one region may generate publication bias, for example, Nanjing contributes 16 of 22 Chinese FMT studies. Thirdly, definitions of response from clinical studies were variable and lastly, most studies were descriptive and uncontrolled, and few included data on donor's clinical profile. Hence, a meta-analysis to derive clear correlations between donor characteristics, screening processes and clinical outcomes was not possible. IBD studies were also smaller in number and patient sample size than CDI studies, further constraining the range of analyses possible.

This review, to our knowledge, is the most comprehensive and up-to-date data on FMT procedures and applications for all indications. Previous systematic reviews and meta-analyses evaluated FMT's efficacy or safety[14-15] but did not provide details of donors and screening characteristics. We demonstrated variability in donor selection protocols. Future consensus on crucial elements in donor selection, stool processing, and administration method is important in the application of FMT, with at least a minimum panel of screening criteria to improve FMT safety. Ultimately, well-designed clinical trials that assessed donor characteristics and disease outcomes are needed to further elucidate donor effects on FMT outcome.

Figures legends: (see separate file)

Figure 1: Flowchart of study selection

Table 1: Clinical characteristics of donors reported across 117 studies

Table 2: Criteria for donor exclusion reported across 148 studies

Table 3: Donor screening tests across 159 studies

Table 4: Outcomes for CDI and IBD studies (Pooled data)

Table 5: Adverse events across 135 studies

Supplementary material: (see separate file)

Appendix 1: Inclusion and exclusion criteria, and detailed OvidSP search strategy for article selection (up to 29/08/2018)

Appendix 2a: Quality assessment of controlled, interventional studies

Appendix 2b: Quality assessment of pre-post studies with no control group

Appendix 2c: Quality assessment of cohort studies

Appendix 2d: Quality assessment of case series

Appendix 3: Publication information of manuscripts sorted by main disease of study

Appendix 4: Donor characteristics of 117 studies, sorted by continents

Appendix 5: Donor exclusion criteria of 148 studies, sorted by continents

Appendix 6: Specification of selected exclusion criteria in 148 studies, sorted by continents

Appendix 7: Donor screening tests of 159 studies, sorted by disease under study

Appendix 8: FMT delivery methods of 168 studies, sorted by continents

Appendix 9: Outcomes of FMT for 132 CDI studies, sorted by route of FMT administration and type of CDI

Appendix 10: Outcomes of FMT for 10 CD studies, sorted by route of FMT administration

Appendix 11: Outcomes of FMT for 29 UC studies, sorted by route of FMT administration

Appendix 12: Adverse event rates in 135 studies, sorted by disease of study and route of FMT administration

Appendix 13: PRISMA checklist

References

1. Kelly CP, LaMont JT. Clostridium difficile--more difficult than ever. N Engl J Med. 2008;359(18):1932-1940.
2. Gough E, Shaikh H, Manges AR. Systematic review of intestinal microbiota transplantation (fecal bacteriotherapy) for recurrent Clostridium difficile infection. Clin Infect Dis. 2011;53(10):994-1002.
3. Borody TJ, Khoruts A. Fecal microbiota transplantation and emerging applications. Nat Rev Gastroenterol Hepatol. 2011;9(2):88-96.
4. Kakihana K, Fujioka Y, Suda W, et al. Fecal microbiota transplantation for patients with steroid-resistant acute graft-versus-host disease of the gut. Blood. 2016;128(16):2083-2088.
5. Konig J, Siebenhaar A, Hogenauer C, et al. Consensus report: faecal microbiota transfer - clinical applications and procedures. Alimentary Pharmacology & Therapeutics. 2017;45(2):222-239.
6. Paramsothy S, Kamm MA, Kaakoush NO, et al. Multidonor intensive faecal microbiota transplantation for active ulcerative colitis: a randomised placebo-controlled trial. Lancet. 2017;389(10075):1218-1228.
7. Smits LP, Bouter KE, de Vos WM, Borody TJ, Nieuwdorp M. Therapeutic potential of fecal microbiota transplantation. Gastroenterology. 2013;145(5):946-953.
8. Enforcement Policy Regarding Investigational New Drug Requirements for Use of Fecal Microbiota for Transplantation to Treat Clostridium difficile Infection Not Responsive to Standard Therapies. In: U.S. Department of Health and Human Services FaDA, Center for Biologics Evaluation and Research, ed. Rockville: U.S. Department of Health and Human Services, Food and Drug Administration; 2016:1-4.
9. Hoffmann D, Palumbo F, Ravel J, Roghmann MC, Rowthorn V, von Rosenvinge E. Improving regulation of microbiota transplants. Science. 2017;358(6369):1390-1391.
10. Cammarota G, Ianiro G, Tilg H, et al. European consensus conference on faecal microbiota transplantation in clinical practice. Gut. 2017;66(4):569-580.
11. Drekonja D, Reich J, Gezahegn S, et al. Fecal Microbiota Transplantation for

Clostridium difficile Infection: A Systematic Review. Ann Intern Med. 2015;162(9):630-638.
12. Kassam Z, Lee CH, Yuan Y, Hunt RH. Fecal microbiota transplantation for Clostridium difficile infection: systematic review and meta-analysis. Am J Gastroenterol. 2013;108(4):500-508.
13. Rossen NG, MacDonald JK, de Vries EM, et al. Fecal microbiota transplantation as novel therapy in gastroenterology: A systematic review. World J Gastroenterol. 2015;21(17):5359-5371.
14. Paramsothy S, Paramsothy R, Rubin DT, et al. Faecal Microbiota Transplantation for Inflammatory Bowel Disease: A Systematic Review and Meta-analysis. J Crohns Colitis. 2017;11(10):1180-1199.
15. Quraishi MN, Widlak M, Bhala N, et al. Systematic review with meta-analysis: the efficacy of faecal microbiota transplantation for the treatment of recurrent and refractory Clostridium difficile infection. Aliment Pharmacol Ther. 2017;46(5):479-493.
16. Zuo T, Wong SH, Lam K, et al. Bacteriophage transfer during faecal microbiota transplantation in Clostridium difficile infection is associated with treatment outcome. Gut. 2017.
17. Bakken JS, Borody T, Brandt LJ, et al. Treating Clostridium difficile infection with fecal microbiota transplantation. Clin Gastroenterol Hepatol. 2011;9(12):1044-1049.
18. Hamilton MJ, Weingarden AR, Sadowsky MJ, Khoruts A. Standardized frozen preparation for transplantation of fecal microbiota for recurrent Clostridium difficile infection. Am J Gastroenterol. 2012;107(5):761-767.
19. Full-Length Donor History Questionnaire (DHQ). 2016; v2.0:http://www.aabb.org/tm/questionnaires/Pages/dhqaabb.aspx. Accessed 21/1, 2018.
20. Study Quality Assessment Tools. (n.d.). Retrieved from https://www.nhlbi.nih.gov/health-topics/study-quality-assessment-tools
21. Bafeta A, Yavchitz A, Riveros C, Batista R, Ravaud P. Methods and Reporting Studies Assessing Fecal Microbiota Transplantation: A Systematic Review. Ann Intern Med. 2017;167(1):34-39.
22. Mayer EA, Naliboff B, Lee O, Munakata J, Chang L. Review article: gender-related differences in functional gastrointestinal disorders. Aliment

Pharmacol Ther. 1999;13 Suppl 2:65-69.
23. Yatsunenko T, Rey FE, Manary MJ, et al. Human gut microbiome viewed across age and geography. Nature. 2012;486(7402):222-227.
24. Ridaura VK, Faith JJ, Rey FE, et al. Gut microbiota from twins discordant for obesity modulate metabolism in mice. Science. 2013;341(6150):1241214.
25. Webb BJ, Brunner A, Ford CD, Gazdik MA, Petersen FB, Hoda D. Fecal microbiota transplantation for recurrent Clostridium difficile infection in hematopoietic stem cell transplant recipients. Transpl Infect Dis. 2016;18(4):628-633.
26. Vaughn BP, Vatanen T, Allegretti JR, et al. Increased Intestinal Microbial Diversity Following Fecal Microbiota Transplant for Active Crohn's Disease. Inflamm Bowel Dis. 2016;22(9):2182-2190.
27. Vermeire S, Joossens M, Verbeke K, et al. Donor Species Richness Determines Faecal Microbiota Transplantation Success in Inflammatory Bowel Disease. J Crohns Colitis. 2016;10(4):387-394.
28. Lagier JC, Delord M, Million M, et al. Dramatic reduction in Clostridium difficile ribotype 027-associated mortality with early fecal transplantation by the nasogastric route: a preliminary report. Eur J Clin Microbiol Infect Dis. 2015;34(8):1597-1601.
29. Link A, Lachmund T, Schulz C, Weigt J, Malfertheiner P. Endoscopic peroral jejunal fecal microbiota transplantation. Dig Liver Dis. 2016;48(11):1336-1339.
30. Nishida A, Imaeda H, Ohno M, et al. Efficacy and safety of single fecal microbiota transplantation for Japanese patients with mild to moderately active ulcerative colitis. J Gastroenterol. 2017;52(4):476-482.
31. Paramsothy S, Borody TJ, Lin E, et al. Donor Recruitment for Fecal Microbiota Transplantation. Inflamm Bowel Dis. 2015;21(7):1600-1606.
32. Lopez-Sanroman A, Rodriguez de Santiago E, Cobo Reinoso J, et al. Results of the implementation of a multidisciplinary programme of faecal microbiota transplantation by colonoscopy for the treatment of recurrent Clostridium difficile infection. Gastroenterologia y Hepatologia. 2017;40(9):605-614.
33. Hagel S, Fischer A, Ehlermann P, et al. Fecal Microbiota Transplant in Patients With Recurrent Clostridium Difficile Infection. Dtsch Arztebl Int.

2016;113(35-36):583-589.
34. Jiang ZD, Ajami NJ, Petrosino JF, et al. Randomised clinical trial: faecal microbiota transplantation for recurrent Clostridum difficile infection - fresh, or frozen, or lyophilised microbiota from a small pool of healthy donors delivered by colonoscopy. Alimentary Pharmacology and Therapeutics. 2017;45(7):899-908.
35. Kappus KD, Lundgren RG, Jr., Juranek DD, Roberts JM, Spencer HC. Intestinal parasitism in the United States: update on a continuing problem. Am J Trop Med Hyg. 1994;50(6):705-713.
36. Hjalgrim H, Friborg J, Melbye M. The epidemiology of EBV and its association with malignant disease. In: Arvin A, Campadelli-Fiume G, Mocarski E, et al., eds. Human Herpesviruses: Biology, Therapy, and Immunoprophylaxis. Cambridge2007.
37. Fulop T, Larbi A, Pawelec G. Human T cell aging and the impact of persistent viral infections. Front Immunol. 2013;4:271.
38. Aas J, Gessert CE, Bakken JS. Recurrent Clostridium difficile colitis: case series involving 18 patients treated with donor stool administered via a nasogastric tube. Clin Infect Dis. 2003;36(5):580-585.
39. Mattila E, Uusitalo-Seppala R, Wuorela M, et al. Fecal transplantation, through colonoscopy, is effective therapy for recurrent Clostridium difficile infection. Gastroenterology. 2012;142(3):490-496.
40. van Nood E, Vrieze A, Nieuwdorp M, et al. Duodenal infusion of donor feces for recurrent Clostridium difficile. N Engl J Med. 2013;368(5):407-415.
41. Kaakoush NO, Castano-Rodriguez N, Mitchell HM, Man SM. Global Epidemiology of Campylobacter Infection. Clin Microbiol Rev. 2015;28(3):687-720.
42. Bowen A. Shigellosis. Infectious diseases related to travel 2017; https://wwwnc.cdc.gov/travel/yellowbook/2018/infectious-diseases-related-to-travel/shigellosis. Accessed 31/8, 2017.
43. O'Reilly CEI, Martha; Griffin, Patricia M. Escherichia coli, Diarrheagenic. Infectious Diseases Related to Travel 2017; https://wwwnc.cdc.gov/travel/yellowbook/2018/infectious-diseases-related-to-travel/escherichia-coli-diarrheagenic. Accessed 31/8, 2017.
44. Wong KKB, Erin; Mintz, Eric D. Cholera. Infectious Diseases Related to

Travel 2017; https://wwwnc.cdc.gov/travel/yellowbook/2018/infectious-diseases-related-to-travel/cholera. Accessed 31/8, 2017.
45. Musso G, Gambino R, Cassader M. Obesity, diabetes, and gut microbiota: the hygiene hypothesis expanded? Diabetes Care. 2010;33(10):2277-2284.
46. Seto CT, Jeraldo P, Orenstein R, Chia N, DiBaise JK. Prolonged use of a proton pump inhibitor reduces microbial diversity: implications for Clostridium difficile susceptibility. Microbiome. 2014;2:42.
47. Modi SR, Collins JJ, Relman DA. Antibiotics and the gut microbiota. J Clin Invest. 2014;124(10):4212-4218.
48. Jernberg C, Lofmark S, Edlund C, Jansson JK. Long-term ecological impacts of antibiotic administration on the human intestinal microbiota. ISME J. 2007;1(1):56-66.
49. Nagy-Szakal D, Mir SA, Harris RA, et al. Loss of n-6 fatty acid induced pediatric obesity protects against acute murine colitis. FASEB Journal. 2015;29(8):3151-3159.
50. Anderson RM, Medley GF. Epidemiology of HIV infection and AIDS: incubation and infectious periods, survival and vertical transmission. AIDS. 1988;2 Suppl 1:S57-63.
51. Maldonado-Gomez MX, Martinez I, Bottacini F, et al. Stable Engraftment of Bifidobacterium longum AH1206 in the Human Gut Depends on Individualized Features of the Resident Microbiome. Cell Host Microbe. 2016;20(4):515-526.
52. Barnes D, Park KT. Donor Considerations in Fecal Microbiota Transplantation. Curr Gastroenterol Rep. 2017;19(3):10.
53. Lee CH, Steiner T, Petrof EO, et al. Frozen vs Fresh Fecal Microbiota Transplantation and Clinical Resolution of Diarrhea in Patients With Recurrent Clostridium difficile Infection: A Randomized Clinical Trial. JAMA. 2016;315(2):142-149.
54. Sha S, Liang J, Chen M, et al. Systematic review: faecal microbiota transplantation therapy for digestive and nondigestive disorders in adults and children. Aliment Pharmacol Ther. 2014;39(10):1003-1032.
55. Baxter M, Colville A. Adverse events in faecal microbiota transplant: a review of the literature. J Hosp Infect. 2016;92(2):117-127.
56. Kvasnovsky CL, Aujla U, Bjarnason I. Nonsteroidal anti-inflammatory drugs

and exacerbations of inflammatory bowel disease. Scand J Gastroenterol. 2015;50(3):255-263.

Statement of Interests: The authors have no conflicts of interest to declare. There was no grant support or writing assistance.

Table 1: Clinical characteristics of donors reported across 117 studies

Characteristics of donor, N=1504	Results
Male, N (%)	485 (58.35)
Donor age, mean (range, years)	34.25 (5-94)
Donor BMI, mean (range)	21.55 (13.3-37.2)
Donor relationship to recipient, n (%)	
First degree	286 (21.70)
Second degree	28 (2.12)
Universal	943 (71.55)

Table 2: Criteria for donor exclusion reported across 148 studies

Donor exclusion criteria	Studies, N (%); N=148
Recent travel to high risk or diarrhoea-endemic areas	53.4 (79)
Recent hospitalization	23.7 (35)
Incarceration	36.5 (54)
Hospital or healthcare workers	7.4 (11)
Exposure to HIV or viral hepatitis	39.2 (58)
High risk sex or behaviours	51.4 (76)
Needle-stick injury	17.6 (26)
Reception of blood products	21.6 (32)
Vaccinations	8.8 (13)
Tattoos or piercings	41.9 (62)
Grafts or transplant reception	6.1 (9)
Recent antibiotics	93.9 (139)
Probiotics	10.8 (16)
Laxatives	12.8 (19)

Antineoplastic drugs	31.8 (47)
Immunosuppressive drugs (calcineurin inhibitors, exogenous glucocorticoids etc.)	43.9 (65)
Chronic ongoing medication	14.9 (22)
Over the counter aids for digestion	12.2 (18)
Drug abuse/illicit drugs	39.9 (59)
PPI	3.4 (5)
Communicable diseases	57.4 (85)
GI surgery (e.g. gastric bypass)	56.8 (84)
GI morbidities in general	54.7 (81)
Chronic diarrhoea	62.8 (93)
Chronic constipation	64.9 (96)
IBS and associated symptoms	71.0 (105)
IBD	69.6 (103)
Indications of liver disease	4.1 (6)
GI endoscopy history	3.4 (5)
Systemic autoimmunity (multiple sclerosis, connective tissue disease etc.)	51.4 (76)
Allergy or atopy of the donor (asthma, eczema etc.)	36.5 (54)
Chronic pain syndromes (fibromyalgia, chronic fatigue syndrome)	29.7 (44)
Ingestion of recipient's allergen	22.3 (33)
Metabolic syndrome (obesity, poor OGTT etc.)	40.5 (60)
DM	20.3 (30)
BMI requirements	32.4 (48)
Malignancy	62.2 (92)
Polyposis	31.8 (47)
Malaria	5.4 (8)

Babesiosis	4.7 (7)
Chagas disease	4.7 (7)
Smallpox contact	4.1 (6)
Variant Creutzfeldt-Jakob disease risk	30.4 (45)
Psychiatric or neurological illness	29.1 (43)
Age requirement	12.2 (18)
Hypertension	4.1 (6)
Cardiovascular diseases	3.4 (5)
Pregnancy	3.4 (5)

Note:

The studies by Cohen N.A. et al (2016) and Fischer M. et al (2017) are split into 2 as they involve 2 separate medical centres

For the listed selective criteria, unmentioned tests were considered as not performed

Table 3: Donor screening tests across 159 studies

Screening tests		**Studies performing screening tests, %**
Bacteria	C. Difficile	95.0
	H. pylori	32.1
	Aeromonas	4.4
	VRE	20.1
	MRSA	13.8
	CRE	3.1
Viruses	Rotavirus	23.3
	Norovirus	22.6
	Adenovirus	16.4
Enteric	E. Coli O157	38.4

pathogens	Yersinia	41.5
	Campylobacter	52.8
	Salmonella	53.5
	Shigella	53.5
	Vibrio	24.5
	Listeria	13.2
	Enteric pathogens in general	47.8
Parasites	Parasites/ova	93.7
	Giardia	49.1
	Cryptosporidium	51.6
	Isospora	30.8
	Cyclospora	27.7
	Microsporidia	20.1
	Dientamoeba fragilis	15.7
	Blastocystis hominis	14.5
	Helminths	3.8
Others	Faecal occult blood	3.1
	Calprotectin	3.1
Bacteria	Syphilis (treponema pallidum)	83.7
Viruses	HIV	98.1
	HAV	85.5
	HBV	98.1
	HCV	98.1
	HEV	15.1
	HTLV	27.7
	CMV	31.5
	EBV	28.3
	Herpes Simplex	4.4
	Varicella Zoster	4.4
	Cysticercus cellulosae	8.8
	Strongyloides stercoralis	10.7
Blood analysis	Liver enzymes	27.0

	Full blood count	27.0
	C-reactive protein	17.6
	Renal function tests (including urea, creatinine)	10.7
	Albumin	3.8
	Lipids	7.6
	Electrolytes	5.7
	IgA, IgG, IgM profiles	3.1
	Fluorescent antinuclear antigen/antibody	6.9
	Fasting plasma glucose	4.4
	ESR	3.8

Note: studies by Cohen N.A et al (2015), Fischer M. et al (2017 and 2016) are split into 2 as 2 separate screening protocols were involved for each.

CMV, cytomegalovirus; CRE, carbapenem-resistant Enterobacteriaceae; EBV, Epstein-Barr virus; ESBL, extended spectrum beta-lactamase producing bacteria; ESR, erythrocyte sedimentation rate; HTLV, human T-lymphotropic virus; MRSA, methicillin-resistant S. aureus; TSH, thyroid-stimulating hormone; VRE, vancomycin-resistant Enterococci

Table 4: Outcomes for CDI and IBD studies (Pooled data)

	Clinical Outcomes*
Clostridium difficile infections (CDI)	
Total number of study, n	132
Total initial patient population, n	4609
Follow up, months (range)	7.7 (0-364)
Primary cure rate, % (95% CI)	87.7 (84.4-90.7)
Final cure rate, % (95% CI)	95.6 (93.9-97.1)
Mean number of FMT per subject, n (range)	1.20 (1 to 6)

Crohn's disease (CD)	
Total number of study, n	10
Total initial patient population, n	115
Follow up, months (range)	6.1 (1-15)
Primary remission rate, % (95% CI)	52.3 (33.5-70.8)
Final remission rate, % (95% CI)	47.5 (29.4-65.8)
Mean number of FMT per subject, n (range)	1.01 (1-2)
Ulcerative colitis (UC)	
Total number of study, n	29
Total initial patient population, n	545
Follow up, months (range)	7.8 (1-66)
Primary remission rate, % (95% CI)	34.0 (21.3-47.8)
Final remission rate, % (95% CI)	39.6 (25.4-54.6)
Mean number of FMT per subject, n (range)	7.02 (1-41)

Note: studies by Li N. et al (2017) and Oprita R. et al (2016) were split into 2 as they have separate recurrent CDI and IBD arms.

* Only study reporting corresponding outcomes were included

Table 5: Adverse effects across 135 studies

Adverse event		**% of total patient population (N = 4493)**
Mild GI side effects	**Abdominal pain**	5.52
	Abdominal distention	11.60
	Constipation	2.07
	Diarrheal	13.04
	Nausea or vomiting	6.05
	Non-specified GI symptoms	0.11
Systemic flu-like signs/symptoms	**Fever**	2.67
	Chills	0.07
	Headache	1.47

	Fatigue	1.40
	Malaise	1.22
	Non-specified flu-like symptoms	0.09
	Non-specified infection	0.49
Procedure-related	**Leakage/intolerance of FMT**	0.18
	Dysphasia	0.18
	Respiratory difficulty	2.36
	Venting	0.49
	Nasopharyngitis	0.16
	Discomfort during tube insertion	0.24
	Sore throat after tube insertion	0.91
Serious	**Aspiration or aspiration pneumonia**	0.16
	Sedation complications	0.02
	Bowel perforation	0.07
	Sepsis	0.07
	Hospitalization	0.02
	Death	0.13
IBD-related	**IBD flare, IBD-like signs/symptoms**	1.27
	Perianal or rectal abscess	0.13
	Proceeded to colectomy	0.27
	Bloody stool	0.16
	Colitis	0.38

Note: studies by Li N. et al (2017) and Oprita R. et al (2016) are split into 2 as they have separate CDI and IBD arms. 2 studies report only total AE number, they were excluded from analysis as aforementioned. Studies by Lagier J.C. et al (2015) and Vaughn B.P. et al (2016)

were also excluded from analysis as the number of certain AEs were indeterminable.

Figure 1: Flowchart of study selection