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Safety comparison of single-donor and pooled fecal microbiota transfer product preparation in ulcerative colitis: systematic review and meta-analysis

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Abstract

Background Multiple studies have evaluated fecal microbiota transfer (FMT) in patients with ulcerative colitis (UC) using single-donor (SDN) and multidonor (MDN) products. Systematic review and meta-analysis were performed to compare the safety of SDN and MDN products.

Methods Systematic searches were performed in Web of Science, Scopus, PubMed, and Orbit Intelligence to identify studies that compared FMT products manufactured using SDN or MDN strategies against control treatment in patients with UC. Fifteen controlled studies were selected for meta-analysis (11 randomized controlled trials and 4 controlled cohort trials). Safety of each treatment type was assessed using the counts of adverse events and serious adverse events using fixed- and random-effects models. Significance of the indirect difference between FMT preparations was assessed using a network approach. Benefit-risk ratios were calculated by multiplicative utility model, incorporating geometric mean of risk ratios (RRs) of efficacy and safety.

Results Safety data was collected for a total of 587 patients (193 exposed to SDN products, 114 exposed to MDN products and 280 exposed to control treatment). The 12 studies showed similar overall safety event counts for MDN and SDN versus placebo (RRs: 0.90 and 1.09, respectively [$P=0.206$ and $P=0.420$, respectively]). Results indicated similar risk of safety events for MDN compared to SDN (RR: 0.83, $P=0.159$). Positive benefit-risk ratios were demonstrated for MDN and SDN versus placebo (RRs: 1.70 and 1.16, respectively [$P=0.003$ and $P=0.173$, respectively]). MDN had a greater benefit-risk ratio compared to SDN (RR: 1.46, $P=0.072$).

Conclusion Similar safety profiles were observed for MDN and SDN strategies. Alongside previously described superior efficacy, treatment with MDN has greater benefit-risk ratio than SDN in patients with UC. Further development of MDN FMT treatment for UC should be considered.

Keywords Ulcerative colitis, Fecal microbiota transfer, Pooling, Meta-analysis, Safety, Benefit-risk

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Background

Ulcerative colitis (UC) is a chronic, inflammatory bowel disease causing inflammation of the mucosal lining of the colon and rectum. While the exact cause remains unknown, research suggests that UC could be sustained by microbial antigens released by over-abundant pro-inflammatory bacteria triggering aberrant immune activation and chronic inflammation [1]. Randomized controlled trials (RCTs) evaluating the potential of fecal microbiota transfer (FMT) to restore microbial diversity [2] and gut homeostasis have shown promising results in UC. In line with this, previous meta-analyses support the use of FMT as an effective treatment to induce remission in UC patients [3, 4]. However, the difficulty in defining a "good" donor as well as the intrinsic variability of donor-derived products' taxonomic composition limits the translatability and reproducibility of these studies. Thus, the pooling of donors' feces has been proposed to homogenize product composition and achieve higher taxonomic richness and diversity. The superior efficacy of multidonor (MDN) compared to single-donor (SDN) products to treat UC patients has been demonstrated in several clinical studies [5]. Nevertheless, due to the multiplicity of source materials used, new safety concerns may be associated with MDN FMT, such as an increased risk of AEs or SAEs, especially infection by undetected pathogens.

The present systematic review with meta-analysis of RCTs builds on Levast 2023 to compare safety of MDN and SDN microbiotherapy products in UC patients and perform a benefit-risk assessment incorporating efficacy and safety data. The same literature search was performed, with the difference of collecting data up to a later cut-off date of 12 September 2022. In this study, the intervention was FMT, MDN product preparation strategy was defined as FMT product made from at least 2 different donors and SDN product preparation was defined as FMT product made from 1 donor.

Methods

The systematic review and meta-analysis were performed according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses method [6]. The protocol was registered with the Internation Prospective Register for Systematic Reviews (PROSPERO, registration number: CRD42020210649). The statistical analysis plan was locked before statistical analysis.

Search strategy and selection criteria

Two investigators (B.Le. and M.F.) independently performed a comprehensive literature research for articles, books, and abstracts related to the safety and efficacy of fecal microbiotherapies in inflammatory bowel disease, irrespective of language. The investigators searched for scientific articles containing clinical data on Scopus, PubMed, Web of Science, as well as Registers, and patents on Orbit Intelligence. They also identified records of interest by searching websites of organizations as well as through citation searching (see Supplemental Digital Content 1). Selected references were checked manually. The last search was performed on 12 September 2022.

B.Le. and M.F. independently assessed the abstracts of all selected articles for eligibility. Any disagreement was resolved by a third investigator (P.L.). Studies evaluating the safety and efficacy of fecal microbiotherapies in UC patients were selected. If several articles reported results associated with the same clinical trial, the article reporting the most extensive information was selected. Predesigned forms were used to independently assess eligibility of potentially relevant articles, according to predefined criteria. Studies were excluded after endpoint evaluation and because included patients did not meet the eligibility criteria for this analysis. Selected records were retrieved and further assessed for eligibility. A PRISMA flow diagram summarizing the selection process is presented in Fig. 1 [6].

All studies testing at least one efficacy endpoint and conformed to the following eligibility criteria were selected (Table 1):

1. All patients diagnosed with UC were selected. All variables such as follow-up duration, concomitant medication, sex, age or language were recorded and used as meta-regressive moderators;
2. Selected microbiotherapy interventions were FMTs administered at any individual treatment timepoint. FMT products were manufactured either with SDN or MDN strategies;
3. The following controls were considered as comparators in the study:Autologous FMT, defined as the patient's own stool collected and stored at study inclusion, reconstituted and administered to the patient at the treatment timepoint. This allows to perfectly control the stool process and the potential benefit of the administration of exogenous microbiota. Potential changes in patient's gut microbial composition between stool donation and autologous FMT treatment could introduce bias. In such case, the autologous FMT treatment could have a positive, negative, or neutral therapeutic effect on the patient;

    i. Autologous FMT, defined as the patient's own stool collected and stored at study inclusion, reconstituted and administered to the patient at the treatment timepoint. This allows to per-

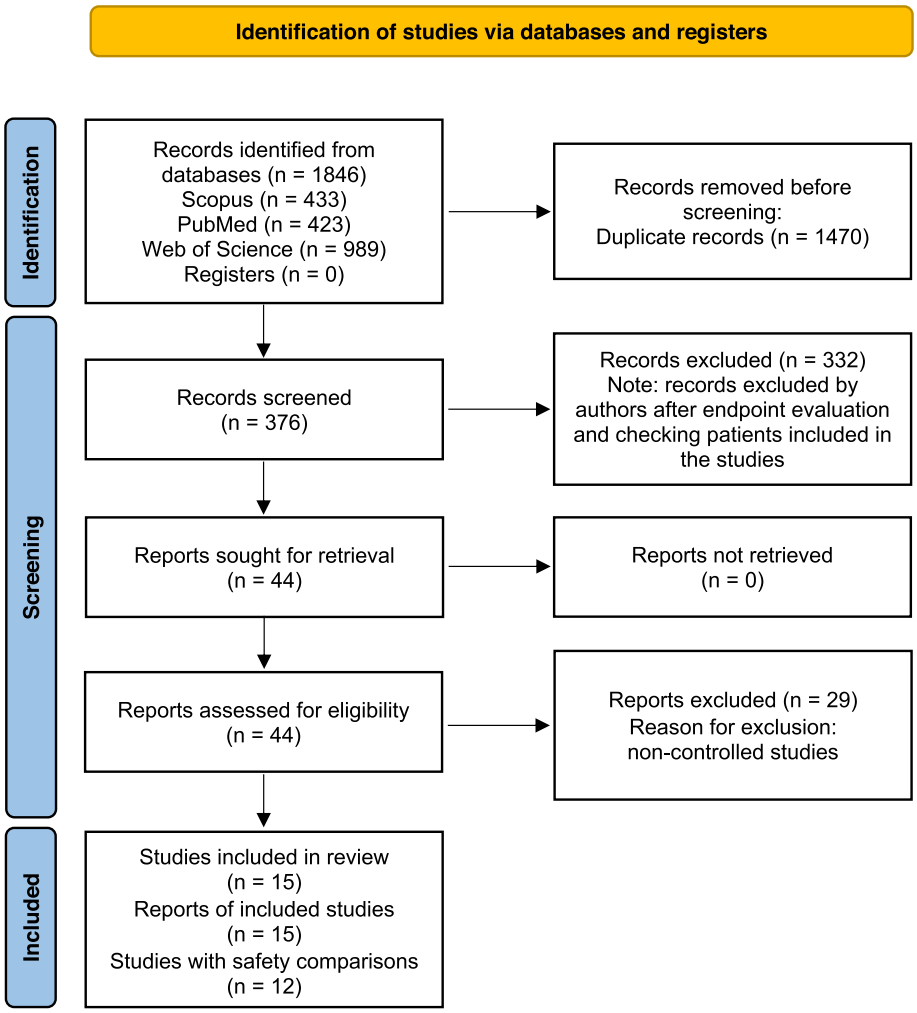

Fig. 1 PRISMA flow diagram showing the study selection process. n, number of records; PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses. From Page et al. [6]

fectly control the stool process and the potential benefit of the administration of exogenous microbiota. Potential changes in patient's gut microbial composition between stool donation and autologous FMT treatment could introduce bias. In such case, the autologous FMT treatment could have a positive, negative, or neutral therapeutic effect on the patient;

ii. Saline buffer, administered into the patient's gut, is the best inert control compared to FMT administration. While the quality of the blinding process is not as good for saline buffer as it is for autologous FMT, no activity bias is expected;

iii. Standard medical therapies (e.g., 5 aminosalicylic acid, topical steroids) or specific diet interventions (e.g., anti inflammatory or UC exclusion diets) used to treat UC are expected to have therapeutic effects and are important to consider in the benefit-risk assessment of microbiotherapy products.

Study selection and data collection are described in Supplementary Materials.

Outcome assessments

To assess the safety of FMT treatments against controls, the main safety endpoint was the count of adverse events (AEs) and serious adverse event (SAEs) occurring during the follow-up. The safety analysis was based on all selected studies which had AE or SAE comparison available. Separate analyses were conducted considering the count of:

Table 1 Characteristics of eligible studies

Study	Year	Studied treatment group[a]			Control group			Number of donors	Design	Mayo12	Antibiotic[b]	PEG[b]	Duration[c]	FMT[d]	Notes[e]	Route[f]
		Sample size	AE	SAE	Sample size	AE	SAE									
Paramsothy et al.	2017	41	32	2	40	33	1	2	RCT	8	0	0	8	40	2	1
Moayyedi et al.	2015	38	-	8	37	-	5	1	RCT	6.4	0	0	7	6	4	1
Costello et al.	2019	38	-	3	35	-	2	2	RCT	7	0	1	8	3	2	1
Sood et al.	2019	31	24	0	30	20	0	1	RCT	6	0	1	48	7	4	1
Rossen et al.	2015	23	-	2	25	48	2	1	RCT	6.4	0	0	12	2	4	2
Subhadra et al.	2016	26	-	-	24	-	-	2	CC	8	0	0	7	8		3
Kump-2 et al.	2017	17	-	-	10	-	-	1	CC	8	1	1	13	5		1
Scaldaferri et al.	2015	8	-	1	7	-	1	1	CC	6.4	0	0	12	3	3	1
Ishikawa et al.	2019	46	-	-	32	-	-	1	CC	8	1	0	4	1	3.4	1
Crothers et al.	2021	6	2	-	6	2	-	1	RCT	6.3/6.7	1	0	12	84		1 + 3
Haifer et al.	2021	15	10	2	20	17	2	1	RCT	5/7	1	0	8	56		3
Pai et al.	2021	12	-	5	12	-	1	1	RCT	PUCAI	0	0	6	12		1
Brezina et al.	2021	21	12	4	22	13	1	1	RCT	6	0	0	6	10		1
Sarbagili Shabat et al.	2022	19	13	-	15	4	-	1	RCT	6 (SCCAI)	0	1	2	3		1
Kedia et al.	2022	35	26	0	31	27	0	2–5	RCT	6 (SCCAI)	0	0	8	7	diet	1
Borody et al.	2003	6	2	-	-			1	C	6.4	1	1	6	5	4	1
Angelberger et al.	2013	5	5	0	-			1	C	7.3	1	0	12	3	4	2
Kump et al.	2013	6	1	0	-			1	C	8.9	0	0	12	1	4	1
Kunde et al.	2013	10	9	0	-			1	C	P	0	0	4	5		1
Ren et al.	2015	7	-	0	-			1	C	7.3	0	0	4–30	1	4	2
Suskind et al.	2015	4	3	-	-			1	C	P	1	0	12	1		2
Damman et al.	2015	7	7	0	-			1	C	6.2	0	0	12	1	4	1
Wei et al.	2016	20	3	0	-			1	C	5.8	1	1	12	1	4	1
Vermeire et al.	2016	8	-	4	-			1	C	8	0	0	8	2	4	2
Karakan et al.	2016	14	-	0	-			1	C	?	0	1	12	1–6		1
Mizuno et al.	2017	10	6	0	-			1	C	6.1	0	1	12	1	4	1
Nishida et al.	2017	41	0	0	-			1	C	6	0	1	8	1		1
Jacob et al.	2017	20	13	0	-			2	C	7.5	0	1	4	1	2	1
Uygun et al.	2017	30	7	-	-			1	C	10	0	0	12	1	4	1
Karolewska et al.	2017	8	3	0	-			1	C	PUCAI	0	0	4	8		2
Goyal et al.	2018	14	12	0	-			1	C	PUCAI	0	0	26	1		2
Tian et al.	2019	20	4	0	-			1	C	5	0	1	18	5	1	2
Steube et al.	2019	10	-	2	-			5	C	8.3	1	0	12	600	1.2	3
Cold et al.	2019	7	3	-	-			4	C	7	0	0	12	1250	2.4	3

Table 1 (continued)

Study	Year	Studied treatment group[a]			Control group			Number of donors	Design	Mayo12	Antibiotic[b]	PEG[b]	Duration[c]	FMT[d]	Notes[e]	Route[f]
		Sample size	AE	SAE	Sample size	AE	SAE									
Ding et al.	2019	109	56	1	-			1	C	10.3	0	0	12	1–3	247	2
Adler et al.	2019	13	4	-	-			1	C	8	0	0	6	60		3
Sood-2 et al.	2019	36	17	-	-			2	C	8.9	0	0	14	7		1
Chen et al.	2020	44	3	0	-			1	C	5.9	0	1	12	3		1
Chen-2 et al.	2020	9	3	0	-			1	C	10.2	0	1	12	3		1 or 2
Dang et al.	2020	12	0	0	-			1	C	9.1 (SCCAI)	0	0	52	1		1
Ren-2 et al.	2021	20	31	-	-			1	C	9.58	0	1	18	2		1
Seth et al.	2022	27	18	-	-			1	C	6.4	0	1	12	3		1
Smith et al.	2022	22	83	1	-			1	C	6.5	1	0	6	6		1 + 3
Zhang et al.	2022	27	4	0	-			1	C	7	0	1	6	1		1

AE Adverse event, *SAE* Serious adverse event, *C* Cohort, *CC* Controlled cohort, *FMT* Fecal microbiota transfer, Mayo Score (range 0-12) at baseline, *PEG* (polyethylene glycol (bowel preparation), *PUCAI* Pediatric Ulcerative Colitis Activity Index, *RCT* Randomized controlled trial, *SCCAI* Simple Clinical Colitis Activity Index

[a] number of donors per FMT product (1 = SDN, > 1 = MDN)

[b] Antibiotic/PEG used: 0 (none); 1 (1 or more)

[c] Duration (follow up in weeks)

[d] Number of FMT administrations during the study

[e] 1: Mayo score replace by the probability of score < 2 (considered as relief). Pnorm(2,mean,sd)*n where n = number of patients. 2: Studied treatment where > 1 donor is considered as MDN; 3: AFM assimilated with placebo ST, standard therapy; 4: Baseline value was used. When not available, estimation based on protocol selection calculated as the truncated mean of distribution assumed to be N(6,2) following the expression sum(dnorm(q:12,6,2)*q:12)/sum(dnorm(q:12,6,2)), where M and m are the minimum and maximum values in the selection. Z < -seq(m,M,.01); sum(dnorm(z,6,2)*z)/sum(dnorm(z,6,2))

[f] 1 = Lower route; 2 = upper route; 3 = capsules

1. AEs only;
2. SAEs only;
3. AEs and SAEs.

The benefit/risk ratio (BRR) compared the overall efficacy and safety of SDN and MDN versus controls and was analyzed using all selected studies. BRR is the unweighted ratio of the risk ratios (RR) of efficacy (Binary therapeutic response) on safety (occurrence of AE and SAE) compared with control and log-transformed to preserve normality properties [7, 8].

Data availability

All data generated and analyzed during this study are included in this published article and its supplementary information.

Meta-analysis

All studies were analyzed for certainty of evidence based on the Grading of Recommendations, Assessment, Development, and Evaluations (GRADE) approach [9]. The Cochrane Collaboration's risk-of-bias tool [10] was used to evaluate bias and filled in by 3 reviewers on the 15 controlled studies, among which 12 had available safety comparison data and were included in the safety analyses (Table 2). Each study was separately analyzed for risk of bias or indirectness. Heterogeneity (using a random model), imprecision, and publication bias (using funnel plots) were evaluated at the meta-analysis level. Meta-regression was performed using evidence level as moderator. The significance of the indirect estimate of the difference between MDN and SDN treatments was sought through a frequentist network approach [11]. The risk ratio (RR) was calculated as the main calculation of effect size. A random-effects model was assumed to be most likely where difference may be expected among studies, and the fixed model was performed for sensitivity purposes. All results were compared with an alternative fixed statistical model, and heterogeneity tests were used. Correlated pairwise comparisons in multiarm studies were corrected by the weight reduction approach [12]. Model fit was assessed by generalized Cochrane Qt [13, 14]. Treatment ranking by P-scores measured the extent of certainty that any one treatment was better than another, averaged over all competing treatments [15]. Statistical analyses were performed using R statistical packages (version 3.2.4) and the meta-library Netmeta [16].

Data values provided as standard error of the mean (SEM) were converted into SDs as per the formula SD = SEM*sqrt(n). For endpoint calculation and effect size, given the heterogeneity of the studies in their clinical definition, the following transformations were also needed for direction and measurement.

1. Severity scores (higher values meaning higher severity) were converted into improvement scores;

Table 2 Selected studies

Study	Year	Design	Studied treatment group[a]			Control group			Treatment[b]
			Sample size	AE	SAE	Sample size	AE	SAE	
Moayyedi et al.	2015	RCT	38	-	8	37	-	5	SDN
Rossen et al.	2015	RCT	23	-	2	25	48	2	SDN
Scaldaferri et al.	2015	CC	8	-	1	7	-	1	SDN
Subhadra et al.	2016	CC	26	-	-	24	-	-	MDN
Paramsothy et al.	2017	RCT	41	32	2	40	33	1	MDN
Kump-2 et al.	2017	CC	17	-	-	10	-	-	SDN
Ishikawa et al.	2019	CC	46	-	-	32	-	-	SDN
Costelloet al.	2019	RCT	38	-	3	35	-	2	MDN
Sood et al.	2019	RCT	31	24	0	30	20	0	SDN
Brezina et al.	2021	RCT	23	12	4	22	13	1	SDN
Crothers et al.	2021	RCT	6	2	-	6	2	-	SDN
Haifer et al.	2021	RCT	15	10	2	20	17	2	SDN
Pai et al.	2021	RCT	12	-	5	12	-	1	SDN
Kedia et al.	2022	RCT	35	26	0	31	27	0	MDN
Sarbagili Shabat et al.	2022	RCT	19	13	-	15	4	-	SDN

AE Adverse event, *CC* Controlled cohort, *MDN* Multidonor, *SDN* Single donor, *RCT* Randomized controlled trial, *SAE* Serious adverse event

[a] Sample size and count of AEs and SAEs for the tested drug and the control groups

[b] Type of medication (SDN, MDN) for each study

2. Two alternative methods were used to aggregate scales based on quantitative values or proportions:

    i. Converting the proportions x/n into quantitative values in assimilating this value to a normal approximation of mean ($P = x/n$ and $SD = \sqrt{(P(1-P))/n}$);
    ii. Conversion of quantitative difference distributed according to a normal distribution $N(m,\sigma)$, assuming a success proportion of 0.5 for the tested drug.

The difference between SDN and MDN strategies was analyzed comparing all selected studies, and sensitivity analyses were performed by excluding studies with Moderate Certainty of Evidence (MCE) for each studied arm and for both arms as follows:

1. Analysis of studies with High Certainty of Evidence (HCE) for MDN and all studies of SDN;
2. Analysis of studies with HCE for SDN and all studies of MDN;
3. Analysis restricted to studies with HCE for both MDN and SDN.

The efficacy of MDN and SDN was also assessed separately vs placebo in discussing the heterogeneity and directness of the studies. Finally, meta-regressions were conducted by using the available baseline variables as potential moderators with the double purpose of assessing the confounding effect of publication date and type of study (RCT vs non-RCTs). For each covariate, a factorial model was used in testing the treatment effect, the covariate effect, and its possible interaction with the treatment. Given the low expected power of the interaction, a maximum P value of $P = 0.2$ was considered as significant.

Risk of bias and certainty of evidence

Risk of bias and certainty of evidence are tools that were used to measure the quality of selected studies and resulting meta-analytical results. They are detailed in [17–20]. We summarize them in adding quantification of some conditions (Supplementary Material).

Results

The search identified 1846 records in medical databases (376 records after deduplication) and 285 records from other sources (Fig. 1). After exclusion of 332 studies, 44 records were retrieved and assessed for eligibility. The main criteria for further excluding studies were nonpredominance of patients with UC over those with Crohn's disease or pouchitis and case reports with fewer than 4 patients. Four pediatric studies were included ($n = 48$ exposed to FMT). Only 1 report identified by the patient search tool could be retrieved and assessed for eligibility [21]; this was also identified in medical database search.

From the 44 studies identified (29 noncontrolled studies, 11 RCTs, and 4 controlled cohort [CC] studies), 15 studies with a control arm were selected as eligible [22]; 29 studies were non-controlled and were therefore excluded from the analyses. Of the 15 eligible studies, only 12 had safety comparison data available and were included in the safety analyses. Most patients in the selected studies were adults with UC; there were a few patients with Crohn's disease who were not considered in the calculation. Overall, 567 patients with UC were considered, with 173 exposed to SDN products, 114 exposed to MDN products and 280 exposed to controls. Disease history, grading, and follow-up were heterogenous between studies but documented in this work.

Main findings from individual studies

The variables were the number of AEs and SAEs reported in each study. There were 44 identified studies, 29 of them excluded and 15 deemed eligible. Characteristics of the 44 eligible studies are provided in Table 1 by study design, studied treatment group, sample size, number of AEs and treatment type (SDN or MDN) and for the 15 selected studies in Table 2. In the 15 selected studies, safety data considering between 1–84 FMT administrations over 2–48 weeks across countries in North America, Europe and Asia were evaluated. Most studies used the lower route of FMT administration, however the upper route and capsules were represented in 1 [23] and 2 [24, 25] studies, respectively. The MDN strategy was compared with controls in 3 RCTs [2, 22, 26], which reported 32 AEs and 2 SAEs in 41 patients, 3 SAEs in 38 patients and 26 AEs in 35 patients with FMT treatment and 33 AEs and 1 SAEs in 40 patients, 2 SAEs in 35 patients and 27 AEs in 31 patients with control treatments, respectively. The SDN strategy was compared with control treatments in 8 RCTs [23–25, 27–31] and 1 CC study [32].

Risk of bias and indirectness within studies

An acceptable risk of bias was determined for all controlled studies and is presented in Table 3. However, as a CC study was included in the meta-analysis, sensitivity analyses were needed to compare results. The selected studies were similar to routine medical practice in terms of patient population, interventions, comparators, and outcomes, confirming that there was no indirectness within this study. External validity is synonymous with indirectness when assessing whether the data include the population, intervention, comparator, and outcome in routine medical use.

Table 3 Summary of certainty of evidence, including risk of bias and directness for controlled studies

Study	Bias risk					Directness					ΣBD
	Blinding	ITT	Selected	Design	ΣBR	P	I	C	O	ΣD	
Rossen *et al.*	+	+	+	+	0	+	+	+	+	0	H
Moayyedi et al.	+	+	+	+	-1	+	-	+	+	0	H
Scaldaferri *et al.*	-	+	+	+	-1	+	+	+	+	-1	M
Kump-2 *et al.*	-	+	-	-	-1	+	+	+	+	-1	M
Ishikawa *et al.*	-	-	+	+	-1	+	+	+	+	-1	M
Sood *et al.*	+	+	+	-	-1	+	+	+	+	-1	M
Subhadra *et al.*	-	+	-	-	0	+	+	+	+	0	M
Paramsothy *et al.*	+	-	+	+	0	+	-	+	+	0	H
Costello *et al.*	+	+	+	+	0	+	+	+	+	0	H
Crothers *et al.*	+	+	+	-	-1	+	+	+	-	-1	M
Haifer *et al.*	+	+	+	+	0	+	+	+	+	0	H
Pai *et al.*	+	+	+	-	-1	+	+	+	-	-1	M
Brezina *et al.*	-	+	+	-	-1	+	+	+	+	0	M
Sarbagili Shabat *et al.*	-	+	+	+	-1	+	-	+	-	-1	M
Kedia *et al.*	-	+	+	+	-1	+	+	-	+	-1	M
Heterogeneity	I^2=0	Using a random model, a nonsignificant heterogeneity was demonstrated									H
Precision	OIS=492	Sample size (n=512) exceeds the OIS, and all results were significant									H
Publication bias	P=0.51	No asymmetry was observed on the Funnel plots									H
Total	Results with high level of certainty of evidence considered										H

Risk of bias tools and indirectness are reported for each study. Risk of bias tools (blinding, ITT: intention to treat, selection, and design) are coded as Low (+), Unclear (0), or High (-). The light-blue colored column ΣBR summarizes the decision on risk of bias (0 = low risk of bias, -1 = downgraded of one level). Directness quantifies how well the results of the study are generalizable for the Population (P), Intervention (I), Comparator (C) and Outcome (O). The light blue colored column ΣD summarizes the decision on directness. The column ΣBD is the summary of final decision from the Biases and Directness (H=High quality, M=Medium quality). The three last rows refer to the meta-analysis, and not each study in particular. Heterogeneity refers to the interpretability of results of important differences in the effects of interventions across studies. Precision measures how accurate is the effect estimation, confirmed when the MAL sample sized exceeds the optimal information size (OIS) or the confidence interval excludes important benefit or harm. n: number of patients. Publication bias assesses the exhaustivity of the available studies estimated by the significance of the asymmetry observed in funnel plot. The green shaded column summarizes the decision based on heterogeneity, precision and publication bias, and the yellow cell summarizes the decision for the three items.

Based on risk of bias and directness, 5 studies were considered as HCE (Rossen, Moyaedi, Paramsothy, Costello and Haifer), the 10 others associated as MCE (Table 3).

Synthesis of results: safety assessment

The sample size and number of AEs and SAEs for each treatment group in each selected studies are summarized in Table 1.

The 12 selected studies had HCE/MCE and compared MDN or SDN with control treatment. No substantial differences between fixed- and random-effects models were observed in all safety analyses, henceforth, results of the random-model are presented. Risk of AE/SAE occurrence tended to be reduced with FMT treatment, as indicated by risk ratios (RRs; 95% confidence intervals [CIs]) for comparisons of MDN and SDN versus control, 0.90 ([0.77; 1.06], $P=0.206$) and 1.09 ([0.88; 1.34], $P=0.421$), respectively (Fig. 2). Furthermore, a tendency towards lower risk of AE and SAE occurrence was observed in MDN versus SDN (RR [95% CI]): 0.83 ([0.64; 1.08], $P=0.159$), with considerable homogeneity shown across all studies (generalized Cochran for homogeneity, $P=0.480$, $I^2=0\%$). P-scores associated with control, SDN and MDN were 0.446, 0.145 and 0.909, respectively for both the fixed- and random-effects models.

For sensitivity purposes the analysis was performed individually for AEs and SAEs, on studies with available

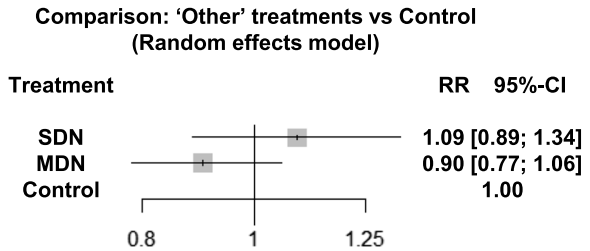

Fig. 2 Forest plot of safety analysis for all safety events. CI, confidence interval; MDN, multidonor; RR, risk ratio; SDN, single donor. Comparison of SDN and MDN was performed by using placebo as the null reference. RR SDN/placebo and MDN/placebo

comparison with control treatment. Considering the 7 studies with available AE comparison, similar results were estimated for comparative risk (RRs [95% CIs]) of AEs occurring with MDN and SDN versus control, 0.90 ([0.72; 1.12], $P=0.333$) and 1.05 ([0.83; 1.35], $P=0.655$), respectively. MDN tended to have a reduced risk of AE occurrence versus SDN (RR [95% CI]): 0.85 ([0.65; 1.12], $P=0.244$). Regarding the 8 studies with available SAE comparison, results indicate a similar risk (RRs [95% CIs]) of SAE occurrence with both MDN and SDN versus control, 1.56 ([0.39; 6.25], $P=0.533$) and 1.77 ([0.90; 3.49], $P=0.100$), respectively. The risk of SAE occurrence in MDN and SDN was similar (RR [95% CI]): 0.88 ([0.19; 4.14], $P=0.871$).

Benefit-risk evaluation

Benefit-risk ratios were calculated by multiplicative utility model, incorporating geometric mean of RRs of efficacy (binary therapeutic response, Levast 2023) [5] and safety (occurrence of AEs/SAEs). Tests of heterogeneity within designs and inconsistency between designs were performed and demonstrate a non-significant study effect ($P=0.18$). Positive benefit-risk ratios [95% CI] calculated for both MDN and SDN versus control were 2.61 ([1.26; 3.95], $P \leq 0.001$) and 1.43 ([0.67; 2.18], $P \leq 0.001$), respectively (Fig. 3). MDN had a higher benefit-risk ratio compared to SDN [95% CI]: 1.18 ([-0.36; 2.72], $P=0.133$).

Discussion

Through systematic review and meta-analysis, this study has collected data from studies of FMT treatments to compare the safety of FMT products prepared by single and multi-donor strategies in patients.

With the implementation of FMT as a well-recognized, approved, and life-saving therapy for the management of recurrent *Clostridioides difficile* infection, the short-term outcome and safety of FMT has been well documented [33]. When guidelines for the screening of donors and fecal material are followed, FMT treatment appears to be highly safe [34], with only transient, mainly gastrointestinal side effects and risks attributable to the method of administration (endoscopic procedures) rather than FMT itself [35, 36]. In the 15 studies selected for this analysis, most commonly reported AEs following FMT were mild and transient, consisting of abdominal discomfort, bloating, flatulence, diarrhea, nausea and low-grade fever. Most studies report that FMT-related AEs happened within hours following FMTs and were usually resolved under 48 h, in line with literature reports [37]. The study by Sood and colleagues [25] is the only study where the occurrence of AEs was followed through time, after each FMT administration. In this study, no significant difference in the number of AEs was observed between the placebo and FMT groups over time. In all other studies, AEs numbers were reported as the total of AEs that occurred during the follow-up period ranging from 2 to 48 weeks (Table 1), regardless of the number of FMT administrations. Only few or no SAE were documented in these studies, mostly consisting in colitis, worsening of UC disease activity or appearance of new conditions that were deemed unrelated to treatments. In particular, similar AEs and SAEs were reported in studies using either SDN or MDN products. These results are in line with the most common AEs described in the literature in response to FMT procedure, occurring in roughly 29% of patients and usually resolved under 48 h [37].

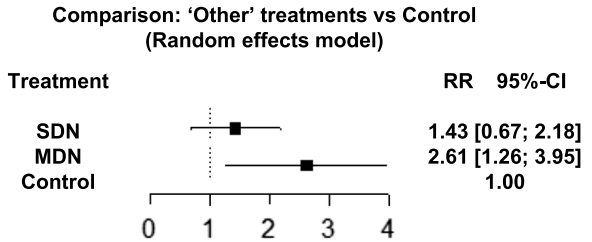

Fig. 3 Forest plot of benefit-risk analysis. CI, confidence interval; MDN, multidonor; RR, risk ratio; SDN, single donor. Comparison of SDN and MDN was performed by using placebo as the null reference. RR SDN/placebo and MDN/placebo

This analysis demonstrates similar safety profiles of SDN and MDN strategies with regards to AE/SAE counts. Weighing greater efficacy and comparable safety, an appreciable benefit-risk ratio of MDN FMT preparation over SDN was observed. In the context of an unmet medical need for relapsing UC and the relatively recent knowledge about fecal microbiota, this study provides a good overview of what a pooling strategy can bring to healthcare providers both in term of safety profile and treatment efficacy.

Despite promising results, the translatability and reproducibility of FMT treatment in UC patients remains limited due to the intrinsic variability of taxonomic composition of donor-derived products [38]. Because the objective of FMT is to restore a healthy microbial ecosystem in patients, the richness and diversity of FMT products is critical. Indeed, differences in donor stool composition translate into major changes in engraftment dynamics, directly affecting treatment efficacy. Moreover, microbial diversity was identified as a reliable predictor of FMT success [39–41]. In this respect, MDN microbiotherapies were designed to homogenize product composition, achieve higher taxonomic richness, and enrich specific bacterial genera with health benefits such as butyrate-producing bacteria to improve patient care [42]. In line, preclinical research has demonstrated the superiority of MDN microbiotherapies compared to corresponding SDN products to protect against infectious diseases in mice [43]. Similarly, prior research shows significant clinical benefit for UC patients treated with MDN and published meta-analysis indicates increased efficacy over SDN [5]. Enhanced efficacy may depend on donors possessing microbial taxa complementary to those lacking in receiver patients, conceivably easier to achieve with pooled FMT from multiple donors [40, 41]. Standardization of FMT preparation permits consistency between FMT treatments of a batch and can be used to

generate product with greater certainty of safety and efficacy [44].

Alongside the advantage of using multiple donors to maximize microbial diversity of the prepared FMT product, importance must be placed upon the effect the recipient has on the outcome of FMT treatment. The interaction between the recipient's uniquely composed endogenous microbiota and the bacteria introduced by FMT will influence both efficacy and safety of the treatment [45, 46], thus a personalized treatment would be ideal to maximize clinical benefit.

Although this study shows comparable safety of MDN and SDN strategies, pooled treatments have some hypothetical limitations. A greater number of donors contributing to a FMT preparation increases the chance of altering the recipient's gut microbiome to the extent that they become prone to developing chronic conditions (e.g., autoimmune disorders) [47]. Additionally, an AE resulting from a donor's sample would be challenging to determine the source donor and to correct the FMT product [42]. These potential issues emphasize the importance of donor selection and the processing and screening of samples in the manufacture of FMT product.

Efforts have been made to improve the preparation of FMT; washed microbiota transplantation, involving optimized automatic purification and centrifugation of microbiota has been shown to significantly reduce the rate of related AEs versus manual methods of FMT preparation [48, 49]. The washing method reliably reduced incidence of FMT-related AEs due to the improved intestinal mucosal permeability and decreased levels of pro-inflammatory metabolites of the washed preparation. Further refinement of standardized FMT processing may improve patient safety.

This study has limitations, and the meta-analysis should be verified by large-scale dedicated RCT. Because of the lack of standard protocol or regulatory framework to standardize FMT administration procedures, the studies described here are representative of the high heterogeneity of FMT-related clinical trials found in the literature. Differences in the dose or number of FMTs could affect the outcomes of the studies, as repeated administrations have been linked with higher response rates in patients with irritable bowel syndrome [50]. Of note, other factors can also influence the study results, such as the mode of delivery or the administration of concomitant treatments (Table 1). As such, the MDN FMT strategy is one of many ways to improve FMT procedures through standardization of microbial taxonomic composition and richness. A variety of control types were observed across the selected studies: autologous FMT, placebo (saline buffer) and standard medical therapy. All control types may each generate a different patient response to treatment, thereby affecting comparability and introducing a potential risk of bias to the meta-analysis. Meta-regressions on predictors at baseline, considering the therapeutic response were presented in Levast 2023 and indicated strong clinical benefit of the capsule formulation of FMT and antibiotic gut preparation before FMT treatment. These meta-regressions could be calculated using safety outcome data to estimate if the same predictors have a similar positive impact on the number of AEs and SAEs experienced by patients treated with FMT. Capsules have also been identified as a route of FMT treatment worthy of pursuit in recently published review [3] and a study for the indication of *C. difficile infection* found FMT capsules to have fewer AEs compared to lower route of administration [51]. The meta-analysis considered the count of AEs and SAEs; a detailed analysis of the nature of individual events could further inform the benefit-risk assessment of FMT treatments.

The variability of how AEs and SAEs are defined in each included study is also a concern that is inherent to meta-analysis. For example, a higher proportion of SAEs in patients receiving active FMT treatment was reported in [29] compared to other trials, probably due to a less stringent definition of SAEs [29]. Indeed, patient hospitalization was always classified as a SAE, even if this was necessary solely to initiate intravenous steroid treatments for active colitis. Development of *C. difficile* infection was also classified as a SAE, despite being unable to differentiate a recurrent infectious episode from an inflammatory UC flare. Most but not all selected studies had a description of AEs and SAEs, and the exact proportion of the symptoms contributing to the total reported AEs was also not provided in all studies. Thus, it was not possible to define and use our own criteria to stratify AEs and SAEs more accurately. Nevertheless, an analysis accounting for Common Terminology Criteria for Adverse Events (CTCAE) grading, relatedness to treatment, and system organ class analysis would provide this additional insight.

Conclusions

In conclusion, this systematic review and meta-analysis reveals similar safety profiles of MDN and SDN strategies in UC patients. Combined with favorable efficacy, MDN has a slightly higher benefit-risk ratio compared to SDN. Although these results support further development of pooled microbiotherapies in UC patients, they must be interpreted with caution due to the aforementioned limitations, and should be tested in RCT studies including a higher number of patients. Alternatively, a study comparing SDN and MDN treatments could be of great interest to resolve this question and improve UC patients care.

Abbreviations

AE	Adverse event
CC	Controlled cohort
CI	Confidence interval
FMT	Fecal microbiota transfer
GRADE	Grading of Recommendations, Assessment, Developments, and Evaluations
HCE	High certainty of evidence
MCE	Medium certainty of evidence
MDN	Multidonor
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
PROSPERO	International Prospective Register of Systematic Reviews
RCT	Randomized controlled trial
RR	Risk ratio
SAE	Serious adverse event
SD	Standard deviation
SDN	Single donor
SEM	Standard error of the mean
UC	Ulcerative colitis

Supplementary Information

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Supplementary Material 1 [52].

Supplementary Material 2.

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Authors' contributions
B.La., B.Le. and M.F. performed the literature review. P.L. designed the study, calculated the meta analysis, performed all statistical tasks, scored the GRADE and quality of studies and participated in the data monitoring committee. B.Le. scored the GRADE and quality of the studies. B.La. wrote the manuscript. S.N. and J.D. contributed to discussions and reviewed the manuscript. All authors contributed to discussions, reviewed the manuscript and approved the final version of the article. Editorial support was provided by Veristat.

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Data availability
All data generated and analyzed during this study are included in this published article and its supplementary information.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
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References

1. Yang H, Mirsepasi-Lauridsen HC, Struve C, et al. Ulcerative Colitis associated E. coli pathobionts potentiate colitis in susceptible hosts. Gut Microbes. 2020;12(1):1847976.
2. Paramsothy S, Kamm MA, Kaakoush NO, et al. Multidonor intensive faecal microbiota transplantation for active ulcerative colitis: A randomised placebo-controlled trial. The Lancet. 2017;389(10075):1218–28.
3. Huang T, Xu J, Wang M, et al. An updated systematic review and meta-analysis of fecal microbiota transplantation for the treatment of ulcerative colitis. Medicine. 2022;101(30):e29790.
4. Wei Z-J, Dong H-B, Ren Y-T, Jiang B. Efficacy and safety of fecal microbiota transplantation for the induction of remission in active ulcerative colitis: A systematic review and meta-analysis of randomized controlled trials. Ann Transl Med. 2022;10(14):802–802.
5. Levast B, Fontaine M, Nancey S, Dechelotte P, Doré J, Lehert P. Single-donor and pooling strategies for fecal microbiota transfer product preparation in ulcerative colitis: A systematic review and meta-analysis. Clin Transl Gastroenterol. 2023;14(5):e00568.
6. Page MJ, McKenzie JE, Bossuyt PM, et al. The PRISMA 2020 statement: An updated guideline for reporting systematic reviews. BMJ. 2021;372:n71.
7. Lehert P, Falissard B. Multiple treatment comparison in narcolepsy: a network meta-analysis. Sleep. 2018;41(12):zsy185. https://doi.org/10.1093/sleep/zsy185. PMID: 30239930; PMCID: PMC6289237.
8. Bouyssou D. Some remarks on the notion of compensation in Multiple criterion Decision Making MCDM. Eur J Oper Res. 1986;26(1):150–60.
9. Guyatt G, Oxman AD, Akl EA, et al. GRADE guidelines: 1. Introduction-GRADE evidence profiles and summary of findings tables. J Clin Epidemiol. 2011;64(4):383–94.
10. Higgins JPT, Altman DG, Gøtzsche PC, et al. The Cochrane Collaboration's tool for assessing risk of bias in randomised trials. BMJ. 2011;343:d5928.
11. Rucker G, Schwarzer G. Reduce dimension or reduce weights? Comparing two approaches to multi-arm studies in network meta-analysis. Stat Med. 2014;33(25):4353–69.
12. Lumley T. Network meta-analysis for indirect treatment comparisons. Stat Med. 2002;21(16):2313–24.
13. Salanti G. Indirect and mixed-treatment comparison, network, or multiple-treatments meta-analysis: Many names, many benefits, many concerns for the next generation evidence synthesis tool. Res Synth Methods. 2012;3(2):80–97.
14. Rucker G. Network meta-analysis, electrical networks and graph theory. Res Synth Methods. 2012;3(4):312–24.
15. Rucker G, Schwarzer G. Ranking treatments in frequentist network meta-analysis works without resampling methods. BMC Med Res Methodol. 2015;15(1):58.
16. Rucker G, Schwarzer G, Krahn U, et al. Package Netmeta Version 0.8–0, Network Meta-Analysis Using Frequentist Methods. R Library, 2015, pp 23–34. Repository CRAN 06–26.
17. Guyatt G, Oxman AD, Akl EA, et al. GRADE guidelines: 1. Introduction-GRADE evidence profiles and summary of findings tables. J Clin Epidemiol. 2011;64(4):383–94.
18. Guyatt GH, Oxman AD, Vist G, et al. GRADE guidelines: 4. Rating the quality of evidence-study limitations (risk of bias). J Clin Epidemiol. 2011;64(4):407–15.
19. Schünemann H, Brożek J, Guyatt G, et al. Grade handbook: Introduction to GRADE Handbook. https://gdt.gradepro.org/app/handbook/handbook.html. Accessed 21 Feb 2024.
20. Higgins JPT, Thomas J, Chandler J, et al. Cochrane Handbook for Systematic Reviews of Interventions version 6.2. www.training.cochrane.org/handbook. Cochrane 2021. Accessed 21 Feb 2024.
21. Subhadra B. Devices, Systems and Methods for the Production of Humanized Gut Commensal Microbiota. United States patent 2018/0030403 A1.

22. Kedia S, Virmani S, Vuyyuru SK, et al. Faecal microbiota transplantation with anti-inflammatory diet (FMT-aid) followed by anti-inflammatory diet alone is effective in inducing and maintaining remission over 1 year in mild to moderate ulcerative colitis: A randomised controlled trial. Gut. 2022;71(12):2401–13.
23. Rossen NG, Fuentes S, van der Spek MJ, et al. Findings from a randomized controlled trial of fecal transplantation for patients with ulcerative colitis. Gastroenterology. 2015;149(1):110–8.e4.
24. Haifer C, Paramsothy S, Kaakoush NO, et al. Lyophilised oral faecal microbiota transplantation for ulcerative colitis (LOTUS): A randomised, double-blind, placebo-controlled trial. Lancet Gastroenterol Hepatol. 2022;7(2):141–51.
25. Sood A, Mahajan R, Singh A, et al. Role of faecal microbiota transplantation for maintenance of remission in patients with ulcerative colitis: A pilot study. J Crohn's Colitis. 2019;13(10):1311–7.
26. Costello SP, Hughes PA, Waters O, et al. Effect of fecal microbiota transplantation on 8-week remission in patients with ulcerative colitis: A randomized clinical trial. JAMA. 2019;321(2):156–64.
27. Moayyedi P, Surette MG, Kim PT, et al. Fecal microbiota transplantation induces remission in patients with active ulcerative colitis in a randomized controlled trial. Gastroenterology. 2015;149(1):102–9.e6.
28. Crothers JW, Chu ND, Nguyen LTT, et al. Daily, oral FMT for long-term maintenance therapy in ulcerative colitis: Results of a single-center, prospective, randomized pilot study. BMC Gastroenterol. 2021;21(1):281.
29. Pai N, Popov J, Hill L, et al. Results of the first pilot randomized controlled trial of fecal microbiota transplant in pediatric ulcerative colitis: Lessons, limitations, and future prospects. Gastroenterology. 2021;161(2):388–93.e3.
30. Březina J, Bajer L, Wohl P, et al. Fecal microbial transplantation versus mesalamine enema for treatment of active left-sided ulcerative colitisresults of a randomized controlled trial. J Clin Med. 2021;10(13):2753.
31. Sarbagili Shabat C, Scaldaferri F, Zittan E, et al. Use of faecal transplantation with a novel diet for mild to moderate active ulcerative colitis: The CRAFT UC randomised controlled trial. J Crohn's Colitis. 2022;16(3):369–78.
32. Scaldaferri F, Pecere S, Bruno G, et al. An open-label, pilot study to assess feasibility and safety of fecal microbiota transplantation in patients with mild-moderate ulcerative colitis: Preliminary results. J Crohn's Colitis. 2015;9:S278.
33. Ooijevaar RE, Terveer EM, Verspaget HW, Kuijper EJ, Keller JJ. Clinical application and potential of fecal microbiota transplantation. Annu Rev Med. 2019;70:335–51.
34. Marcella C, Cui B, Kelly CR, Ianiro G, Cammarota G, Zhang F. Systematic review: the global incidence of faecal microbiota transplantation-related adverse events from 2000 to 2020. Aliment Pharmacol Ther janv. 2021;53(1):33–42.
35. Baxter M, Ahmad T, Colville A, Sheridan R. Fatal aspiration pneumonia as a complication of fecal microbiota transplant. Clin Infect Dis. 2015;61(1):136–7.
36. Merrick B, Allen L, Masirah M, Zain N, Forbes B, Shawcross DL, Goldenberg SD. Regulation, risk and safety of faecal microbiota transplant. Infect Prev Pract. 2020;2(3):100069.
37. Baxter M, Colville A. Adverse events in faecal microbiota transplant: a review of the literature. J Hosp Infect fevr. 2016;92(2):117–27.
38. Nicco C, Paule A, Konturek P, et al. From donor to patient: collection, preparation and cryopreservation of fecal samples for fecal microbiota transplantation. Diseases. 2020;8(2):9.
39. Kump P, Wurm P, Gröchenig HP, et al. The taxonomic composition of the donor intestinal microbiota is a major factor influencing the efficacy of faecal microbiota transplantation in therapy refractory ulcerative colitis. Aliment Pharmacol Ther. 2018;47(1):67–77.
40. Vermeire S, Joossens M, Verbeke K, et al. Donor species richness determines faecal microbiota transplantation success in inflammatory bowel disease. J Crohn's Colitis. 2015;10(4):387–94.
41. Wilson BC, Vatanen T, Cutfield WS, O'Sullivan JM. The super-donor phenomenon in fecal microbiota transplantation. Front Cell Infect Microbiol. 2019;9:2.
42. Kazerouni A, Wein LM. Exploring the efficacy of pooled stools in fecal microbiota transplantation for microbiota-associated chronic diseases. PLoS ONE. 2017J 9;12(1):e0163956.
43. Reygner J, Delannoy J, Barba-Goudiaby MT, Gasc C, Levast B, Gaschet E, et al. Reduction of product composition variability using pooled microbiome ecosystem therapy and consequence in two infectious murine models. Appl Environ Microbiol. 2024;90(5):e0001624.
44. Malard F, Loschi M, Huynh A, Cluzeau T, Guenounou S, Legrand F, et al. Pooled allogeneic faecal microbiota MAAT013 for steroid-resistant gastrointestinal acute graft-versus-host disease: A single-arm, Multicentre phase 2 trial. eClinicalMedicine. 2023;62:102111.
45. Rees NP, Shaheen W, Quince C, et al. Systematic review of donor and recipient predictive biomarkers of response to faecal microbiota transplantation in patients with ulcerative colitis. EBioMedicine. 2022;81:104088.
46. Okahara K, Ishikawa D, Nomura K, et al. Matching between donors and ulcerative colitis patients is important for long-term maintenance after fecal microbiota transplantation. J Clin Med. 2020;9(6):1650.
47. Smith MB, Kelly C, Alm EJ. Policy: How to regulate faecal transplants. Nature. 2014;506(7488):290–1.
48. Lu G, Wang W, Li P, Wen Q, Cui B, Zhang F. Washed preparation of faecal microbiota changes the transplantation related safety, quantitative method and delivery. Microb Biotechnol. 2022;15(9):2439–49.
49. Zhang T, Lu G, Zhao Z, Liu Y, Shen Q, Li P, et al. Washed microbiota transplantation vs. manual fecal microbiota transplantation: Clinical findings, animal studies and in vitro screening. Protein and Cell. 2020;11(4):251–66. https://doi.org/10.1007/s13238-019-00684-8.
50. El-Salhy M, Hausken T, Gunnar HJ. Increasing the dose and/or repeating Faecal Microbiota Transplantation (FMT) Increases the Response in Patients with Irritable Bowel Syndrome (IBS). Nutrients. 2019;11(6):1415.
51. Kao D, Roach B, Silva M, Beck P, Rioux K, Kaplan GG, Chang HJ, Coward S, Goodman KJ, Xu H, Madsen K, Mason A, Wong GK, Jovel J, Patterson J, Louie T. Effect of oral capsule- vs colonoscopy-delivered fecal microbiota transplantation on recurrent clostridium difficile infection: a randomized clinical trial. JAMA. 2017;318(20):1985–93.
52. Egger M, Davey Smith G, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. BMJ. 1997;315(7109):629–34.

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