Gastroenterology Research and Practice

Recent Advances in Faecal Microbiota Transplantation

Lead Guest Editor: Fa-Ming Zhang
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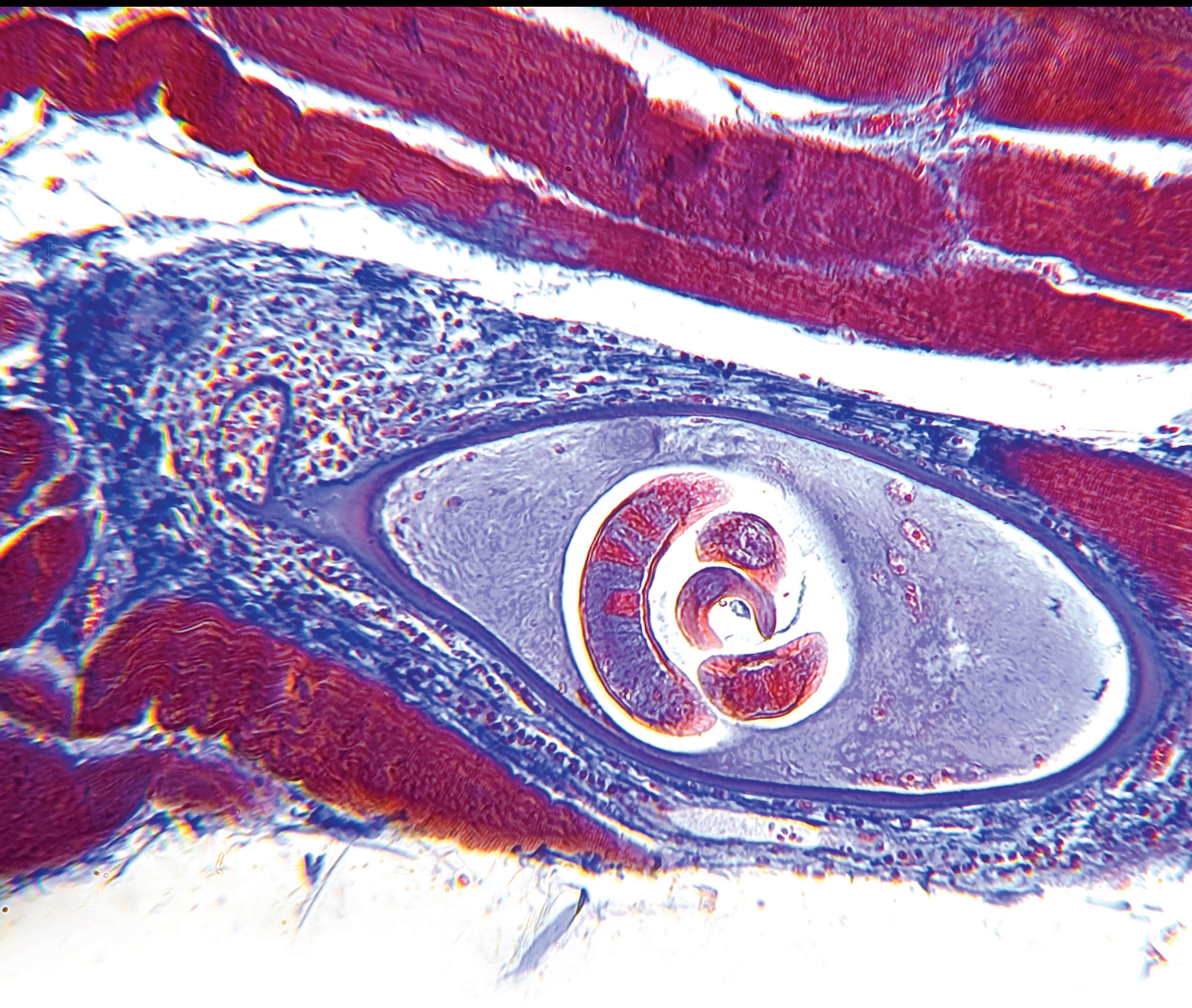

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Contents

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Review Article

Fecal Microbiota Transplantation as Therapy for Treatment of Active Ulcerative Colitis: A Systematic Review and Meta-Analysis

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Aim. Increasing evidence supports the role of the gut microbiota in the etiology of ulcerative colitis (UC). Fecal microbiota transplantation (FMT) is a highly effective treatment against recurrent Clostridium difficile infection; however, its efficacy in UC is still controversial. A systematic review and meta-analysis was conducted to evaluate the efficacy and safety of FMT for treatment of active UC. *Methods*. We searched Cochrane, Medline, Web of Science, and Embase from inception to February 2020. Randomized controlled trials (RCTs) recruiting adults with active UC, which compared FMT with controls, were eligible. The primary outcome was combined clinical remission with endoscopic remission/response. Secondary outcomes included clinical remission, endoscopic remission, and serious adverse events. Relative risk (RR) with 95% confidence interval (CI) is reported. *Results*. Five RCTs with 292 participants were eligible for inclusion. When data were pooled for all patients, FMT was associated with a higher combined clinical remission with endoscopic remission/response; the RR of combined outcome not achieving after FMT vs. control was 0.79 (95% CI 0.70-0.88). FMT delivered via lower gastrointestinal route was superior to upper gastrointestinal route with regard to combined clinical remission with endoscopic remission/response (RR = 0.79, 95% CI 0.70-0.89). FMT with pooled donor stool (RR = 0.69, 95% CI 0.56-0.85) and higher frequency of administration (RR = 0.76, 95% CI 0.62-0.93) may be more effective with regard to clinical remission. There was no statistically significant difference in serious adverse events with FMT compared with controls (RR = 0.98, 95% CI 0.93-1.03). *Conclusion*. FMT shows a promising perspective with comparable safety and favorable clinical efficacy for the treatment of active UC in the short term. However, further larger, more rigorously conducted RCTs of FMT in UC are still needed in order to resolve the controversial questions.

1. Introduction

Ulcerative colitis (UC) is characterized by chronic inflammation of the colon, as well as the periodicity of disease progression and remission [1]. The precise etiology of UC is unclear, which is thought to be multifactorial with the interaction of genetic susceptibility, environmental factors, gut microbiota, and dysregulated immune responses [2]. The imbalance of the gut microbiota has been suggested to markedly impact UC progression [3].

Fecal microbiota transplantation (FMT) refers to the therapeutic procedure of transplanting fecal bacteria from healthy persons into patients [4]. It is highly efficacious for the treatment of recurrent Clostridium difficile infection (CDI), with mean cure rates in the range of 87%-90% [5, 6]. Beyond CDI, FMT has been investigated as a treatment option in a variety of diseases, such as inflammatory bowel disease (IBD), irritable bowel syndrome (IBS), hepatic encephalopathy, autism, metabolic syndrome, and so on [7]. Since the first case of FMT for the treatment of UC was described by Justin Bennet in 1989 [8], there have been several case reports, case series, and randomized controlled trials (RCTs) in recent years on this topic. However, the efficacy and safety of FMT for treatment of UC is still controversial. Although there were meta-analyses examining this issue [9, 10], one of them by Narula et al. [9] did not identify the study by Crothers et al.

[11] which was available in abstract form. This meant that the data of this previous meta-analysis was absence from one RCT using upper gastrointestinal tract to administer FMT. The other meta-analysis by Tang et al. [10] did not differentiate patients with active UC and UC in remission. In order to evaluate the efficacy and safety of FMT in active UC and update the previous systematic reviews, we conducted a systematic review and meta-analysis using only high-quality evidence.

2. Materials and Methods

2.1. Literature Search Strategy. A systematic retrieval of records was performed in accordance with the PRISMA statement (Preferred Reporting Items for Systematic reviews and Meta-Analyses) and Cochrane guidelines. A literature search was performed using Cochrane, Medline, Web of Science, and Embase from inception to February 2020. We also searched by hand supplementary data and relative references for potentially eligible studies. The medical literature was searched using the following terms: {FMT or [(faecal or fecal or feces or faeces or stool) and (transplant or microbiota or transfusion or implant or instillation or donor or enema or reconstitution or infusion or transfer)] or bacteriotherapy} and [UC or (ulcerative colitis)]. Both free-text words and subject headings were searched. There were no language limits.

2.2. Inclusion/Exclusion Criteria. Studies included in this meta-analysis were required to meet the following criteria: (1) randomized controlled trial; (2) adult subjects (participants aged ≥ 18 years) with active UC assessed by clinical scores; (3) data of clinical efficacy, including clinical remission, endoscopic remission/response, and safety of FMT available; and (4) experimental group received donor FMT, and control group received placebo or an autologous FMT. Patients receiving FMT through different delivery routes (i.e., colonoscopy, nasojejunal tube, nasogastric tube, or enemas) were all eligible. Studies were excluded if they did not provide sufficient information, including data not obtained after contacting authors.

2.3. Outcome Assessment. The primary outcome was combined clinical remission with endoscopic remission/response within 12 weeks after FMT. Secondary outcomes included clinical remission, endoscopic remission/response, and safety of FMT which was assessed by serious adverse events (SAEs) during FMT. SAEs during FMT were defined as subjects with adverse events requiring treatment, hospitalization, surgery, or death during FMT procedure. Subgroup analyses of different delivery routes of FMT administration, number of donors, and frequency of FMT administration were also conducted.

2.4. Data Extraction and Quality Assessment. Two authors (L.X. and L.Y.) carried out literature search and data extraction independently. They reviewed all articles, initially by title and abstract, then by full text, to determine whether eligibility. When multiple publications related to the same patient group, the most complete data set was included. Disagreements were resolved by consensus with the senior author (C.M.). The Cochrane's risk of bias was used to evaluate the study quality of RCTs [12]. This assessment was based on seven criteria: (1) random sequence generation (selection bias), (2) allocation concealment (selection bias), (3) blinding of participants and personnel (performance bias), (4) blinding of outcome assessment (detection bias), (5) incomplete outcome data (attrition bias), (6) selective reporting (reporting bias), and (7) other sources of bias. The risk of bias was assessed as "low," "high," or "unclear." A quality score > 3 points (4-7 points) indicated a high-quality study.

2.5. Statistical Analysis. Data were pooled using a random-effects model, which can provide a more conservative estimate than a fixed-effects model when heterogeneity is present. The risk ratio (RR) with 95% confidence intervals (CI) was used to measure the effects in indirect comparisons, and a P value<0.05 was considered a statistically significant difference. We tested for heterogeneity using the chi-squared test and I^2 test. The chi-squared test suggests heterogeneity between studies with a $P < 0.10$. The I^2 test describes the percentage of variability in effect estimates that is due to heterogeneity rather than chance, used a cut off ≥ 50% to define a significant degree of heterogeneity [13]. For assessment of publication bias, we planned to perform funnel plots and calculated Egger's regression intercept for studies, if there were sufficient (≥10) eligible studies included in the meta-analysis [14]. Statistical analyses were performed using Review Manager Version 5.3 (RevMan for Windows 2014, the Nordic Cochrane Centre, Copenhagen, Denmark).

3. Results

3.1. Search Results and Study Characteristics. The search strategy identified a total of 3923 citations, which included 1336 duplicates. Titles and abstracts of 2587 citations were screened, and only 6 citations were deemed potentially eligible. After reviewing the full text carefully, 1 citation was excluded, because we failed to get the data from the authors. Finally, 5 studies [11, 15–18] were eligible for the meta-analysis (Figure 1).

All 5 eligible studies with 292 participants were prospective RCTs, which included 147 patients who received donor FMT and 145 patients who received placebo or an autologous FMT. All participants were patients with mild to moderate active UC. Two trials administered FMT through the upper gastrointestinal tract (naso-duodenal infusion or oral capsules) [11, 17], and three trials administered FMT through the lower gastrointestinal tract (colonoscopy infusion and enema) [15, 16, 18]. Three trials used pooled donors' stool (2-7 donors) for FMT preparation [11, 15, 16], and two trials used single donor's stool [17, 18]. Two trials [15, 17] used low frequency of FMT infusion (2-3 times total), and three trials used higher frequency of administration (6-84 times total) [11, 16, 18]. Two trials [15, 17] compared efficacy of donor FMT with autologous FMT, and three trials [11, 16, 18] compared FMT with placebo. Participants of two trials received preantibiotic and bowel lavage pretreatment [11, 17], two trials received bowel lavage but not preantibiotic pretreatment [15, 16], and one trial did not report pretreatment information [18]. Evaluation duration of the studies was

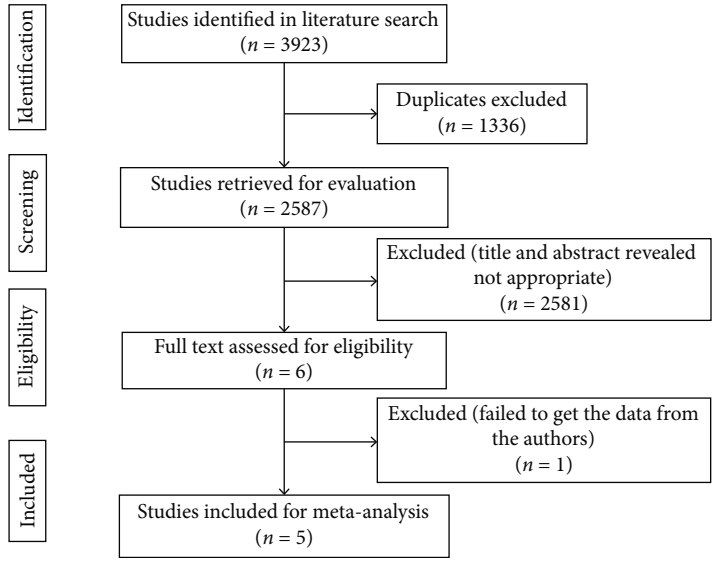

Figure 1: Flow diagram of search strategy.

between 7 and 12 weeks. All five trials provided dichotomous data for response or nonresponse to FMT. The characteristics of the included studies are summarized in Table 1.

3.2. Quality Assessment and Publication Bias. According to the Cochrane's risk of bias for assessing study quality, all studies we included were demonstrated as "high" rating (Figure 2). But there were too few studies to assess publication bias using funnel plot asymmetry.

3.3. Efficacy of Fecal Microbiota Transplantation in Ulcerative Colitis

3.3.1. Combined Clinical Remission with Endoscopic Remission/Response. All five trials provided dichotomous data for response or nonresponse to FMT. When data were pooled, there were 105 (71.4%) of 147 patients assigned to the FMT group who failed to achieve combined clinical remission and endoscopic remission/response, compared with 132 (91.0%) of 145 assigned to the control group. The pooled RR of combined outcome not achieving after FMT vs. control was 0.79 (95% CI 0.70-0.88, $P < 0.0001$), with a low risk of heterogeneity detected between studies ($\text{Chi}^2 = 1.34$, $I^2 = 0\%$, $P = 0.86$) (Figure 3).

We performed three subgroup analyses which are shown in Figures 3–5. Analysis according to the delivery route of administration demonstrated no benefit via the upper gastrointestinal tract in two pooled studies (RR = 0.79, 95% CI 0.58-1.09, $\text{Chi}^2 = 1.09$, $I^2 = 8\%$, $P = 0.30$) [11, 17], but a beneficial effect when the lower gastrointestinal tract was used when data were pooled from three studies (RR = 0.79, 95% CI 0.70-0.89, $\text{Chi}^2 = 0.24$, $I^2 = 0\%$, $P = 0.89$) [15, 16, 18]. When the number of donors' stools was studied, a beneficial effect was demonstrated in both pooled donor stool of three trials (RR = 0.76, 95% CI 0.65-0.89, $\text{Chi}^2 = 0.75$, $I^2 = 0$ %, $P = 0.69$) [11, 15, 16] and single donor stool of two trials (RR = 0.82, 95% CI 0.70-0.97, $\text{Chi}^2 = 0.15$, $I^2 = 0\%$, $P = 0.69$) [17, 18] compared with the control group. The same beneficial effect could also be seen in both higher frequency of administration of three trials (RR = 0.79, 95% CI 0.69-0.90, $\text{Chi}^2 = 0.84$, $I^2 = 0\%$, $P = 0.66$) [11, 16, 18] and lower frequency of two trials (RR = 0.79, 95% CI 0.65-0.96, $\text{Chi}^2 = 0.52$, $I^2 = 0\%$, $P = 0.47$) [15, 17] compared with the control group.

3.3.2. Clinical Remission. With regard to clinical remission, more patients receiving donor FMT achieved this outcome compared with those receiving control interventions, with the pooled RR of not achieving remission being 0.77 (95% CI 0.65-0.90, $\text{Chi}^2 = 3.84$, $I^2 = 0\%$, $P = 0.43$) (Figure 6). The pooled rate of clinical remission was 40.8% (60 of 147 patients) in the FMT group and 22.1% (32 of 145 patients) in the control group.

Subgroup analyses, according to the delivery route of administration, number of donors' stools, and frequency of FMT administration, were performed, which showed a significantly beneficial effect in the lower gastrointestinal tract subgroup (RR = 0.71, 95% CI 0.59-0.86, $\text{Chi}^2 = 0.97$, $I^2 = 0\%$, $P = 0.61$), pooled donor subgroup (RR = 0.69, 95% CI 0.56-0.85, $\text{Chi}^2 = 0.63$, $I^2 = 0\%$, $P = 0.73$), and higher frequency of administration subgroup (RR = 0.76, 95% CI 0.62-0.93, $\text{Chi}^2 = 0.43$, $I^2 = 0\%$, $P = 0.81$). But there were no significant benefits in the upper gastrointestinal tract subgroup (RR = 0.95, 95% CI 0.70-1.29, $\text{Chi}^2 = 0.46$, $I^2 = 0\%$, $P = 0.50$), single donor subgroup (RR = 0.88, 95% CI 0.69-1.13, $\text{Chi}^2 = 0.95$, $I^2 = 0\%$, $P = 0.33$), and lower frequency subgroup which had statistically significant heterogeneity (RR = 0.80, 95% CI 0.50-1.28, $\text{Chi}^2 = 3.38$, $I^2 = 70\%$, $P = 0.07$). The three subgroup analyses data are shown in Figures 6–8.

3.3.3. Endoscopic Remission. The pooled RR for not achieving endoscopic remission with donor FMT compared with controls was 0.91 (95% CI 0.84-0.99, $\text{Chi}^2 = 4.26$, $I^2 = 6\%$, $P = 0.37$) (Figure 9). The pooled rate of endoscopic remission

Table 1: Characteristics of randomized controlled trials of fecal microbiota transplantation vs. control in active ulcerative colitis.

Study	Country	No. patients (FMT/control)	Severity	Donor	Delivery route	Frequency	Dosage	Preantibiotic	Bowel lavage	Control intervention	Time of evaluation	Combined clinical and endoscopic improvement	Clinical remission	Endoscopic remission/response	Combined clinical remission and endoscopic remission/response (FMT/control)	Clinical remission (FMT/control)	Endoscopic remission (FMT/control)	Serious adverse events (FMT/control)
Costello 2019	Australia	38/35	Mild to moderate (Mayo score 3-10, with endoscopic subscore ≥ 2)	Healthy volunteers (pooled 3-4 donors' stool)	1 colonoscopy, 2 enemas	3 times over 7 days	Total stool weight 100 g	No	Yes	Autologous FMT	Week 8	A total Mayo score ≤ 2, with endoscopic subscore ≤ 1	SCCAI ≤ 2	Mayo endoscopic subscore < 1	12/3	18/6	4/0	3/2
Crothers 2018	USA	7/8	Mild to moderate (Mayo score: 4-10)	Healthy volunteers with high stool butyrate (pooled 2 donors' stool)	Daily FMT capsules	Daily	Capsule 0.375 g stool per time	Yes	Yes	Placebo	Week 12	A total Mayo score < 3, with decrease in Mayo endoscopic subscore ≥ 1	Mayo score < 3	Decrease in Mayo endoscopic subscore ≥ 1	3/0	2/1	3/0	0/0
Paramsothy 2017	Australia	41/40	Mild to moderate (Mayo score: 4-10)	Healthy volunteers (pooled 3-7 donors' stool)	1 colonoscopy, 40 enemas	1 colonoscopic infusion, followed by enemas 5 days per week for 8 weeks	37.5 g stool per time, 150 ml infusion volume	No	Yes	Placebo	Week 8	A total Mayo score ≤ 2, with all Mayo subscores ≤ 1 and reduction in endoscopic subscore ≥ 1	Mayo score < 3	Mayo endoscopic subscore = 0	11/3	18/8	5/3	2/1
Rossen 2015	Netherland	23/25	Mild to moderate (SCCAI 4–11, with endoscopic subscore ≥ 1)	Healthy partners, relatives, or volunteers (single donor's stool)	2 naso-duodenal infusions	2 times at week 0 and week 3	500 ml	Yes	Yes	Autologous FMT	Week 12	SCCAI score ≤ 2, decrease in Mayo endoscopic subscore ≥ 1	SCCAI score ≤ 2	Mayo endoscopic subscore = 0	7/5	7/8	2/2	2/2
Moayyedi 2015	Canada	38/37	Mild to moderate (Mayo score ≥ 4, with endoscopic subscore ≥ 1)	Healthy volunteers (single donor's stool)	6 retention enemas	Once per week for 6 weeks	50 g, 300 ml	NR	NR	Placebo	Week 7	A full Mayo score < 3 and endoscopic subscore = 0	Mayo score < 3	Mayo endoscopic subscore = 0	9/2	15/9	9/3	3/2

NR: not reported.

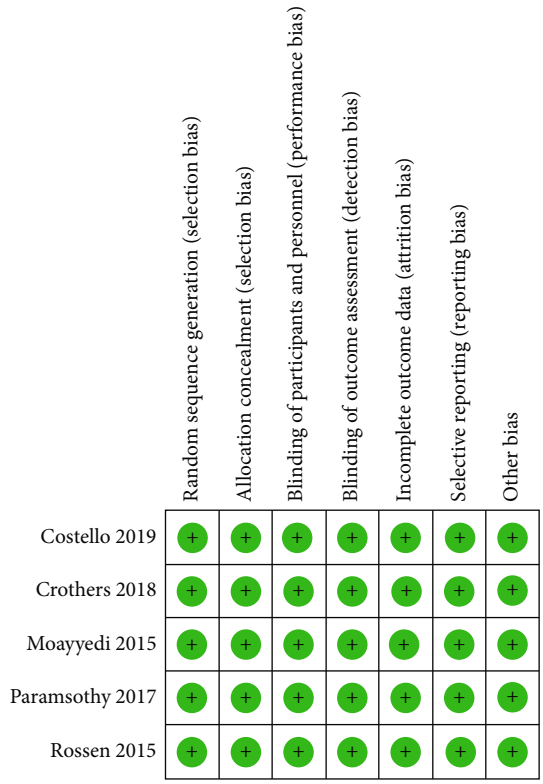

Figure 2: Risk of bias of included studies.

for patients who received donor FMT was 15.6% (23 of 147 patients) compared with 5.8% (8 of 137 patients) for patients in the control group.

Further subgroup analyses demonstrated a slightly beneficial effect in the lower gastrointestinal tract subgroup (RR = 0.90, 95% CI 0.83-0.98, $Chi^2 = 1.18$, $I^2 = 0\%$, $P = 0.55$). But there were no significant benefits when the FMT group compared with the control group in the upper gastrointestinal tract subgroup (RR = 0.82, 95% CI 0.47-1.45) with statistically significant heterogeneity ($Chi^2 = 3.19$, $I^2 = 69\%$, $P = 0.07$), the pooled donor subgroup (RR = 0.91, 95% CI 0.82-1.01, $Chi^2 = 2.31$, $I^2 = 13\%$, $P = 0.32$), the single donor subgroup (RR = 0.91, 95% CI 0.76-1.10, $Chi^2 = 1.95$, $I^2 = 49\%$, $P = 0.16$), the higher frequency subgroup (RR = 0.87, 95% CI 0.74-1.03, $Chi^2 = 3.15$, $I^2 = 36\%$, $P = 0.21$), and the lower frequency subgroup (RR = 0.93, 95% CI 0.84-1.02, $Chi^2 = 0.91$, $I^2 = 0\%$, $P = 0.34$). Relevant data are shown in Figures 9–11.

3.3.4. Safety of FMT in UC. SAE data were provided by all of the five trials. There were no significant differences between patients receiving donor FMT compared with control patients with regard to SAEs. When data were pooled from the five RCTs, there were 10 of 147 (6.8%) patients assigned to FMT who reported SAEs, compared with 7 of 145 (4.8%) allocated to the control group. The pooled RR was 0.98 (95% CI 0.93-1.03, $Chi^2 = 0.07$, $I^2 = 0\%$, $P = 1.00$) (Figure 12). Further subgroup analyses, including delivery routes, number of donors, and frequency of FMT administration, indicated no significant differences between FMT group and control group (Figures 12–14).

Individual SAEs included worsening colitis ($n = 3$) who needed admit to hospital for intravenous corticosteroid therapy or colectomy, C difficile colitis requiring colectomy ($n = 1$), pneumonia ($n = 1$), patchy inflammation of the colon and rectal abscess formation ($n = 2$), worsening abdominal discomfort tested positive for C difficile toxin ($n = 1$), small bowel perforation ($n = 1$), and abdominal pain ($n = 1$) in the FMT group. In the control group, individual SAEs included worsening colitis ($n = 4$), patchy inflammation of the colon and rectal abscess formation ($n = 1$), cytomegaloviruses (CMV) infection ($n = 1$), and cervix carcinoma ($n = 1$). Not all of them were related to FMT.

4. Discussion

This systematic review and meta-analysis evaluated the efficacy and safety of FMT for the treatment of active UC, synthesizing evidence from the available RCTs conducted to date. Five trials, of which one was abstract, fulfilling inclusion criteria were identified eligible. When data from all studies were pooled, there were significant improvements in the primary outcome (combined clinical remission with endoscopic remission/response) and secondary outcomes (clinical remission and endoscopic remission) when FMT vs. control. Our meta-analysis demonstrated that FMT is effective to mild to moderate active UC in the short term. Additionally, a recent pilot study showed maintenance FMT may help sustain clinical, endoscopic, and histological remission in patients with UC who are in clinical remission for a long term of 48 weeks [19], which meant FMT may also have beneficial effects in maintenance of UC.

With regard to delivery routes of FMT, our subgroup meta-analyses revealed better outcomes of lower gastrointestinal tract in both primary and secondary outcomes of FMT with controls. However, FMT via upper gastrointestinal tract did not show beneficial effects in any of the subgroup analyses when comparing FMT with controls. This result was not concordant with a recent study based on 134 UC patients, which proved no difference in efficacy between patients who received FMT from midgut and those from colonic transendoscopic enteral tubing (TET) [20]. The result also was not consistent with one of our recent prospective studies based on 9 UC patients, which showed no significant difference on the efficacy of FMT for treatment of UC between the nasojejunal tube and TET delivery routes [21]. The reason for these discordant results may be different patient inclusion criteria (mild to severely active UC patients were included in the two studies). With regard to the lower gastrointestinal route of FMT administration, the latest progress is colonic TET, which is a safe, convenient, and reliable procedure for FMT that results in a high degree of patient satisfaction [22, 23]. The experience of FMT through TET of patients with IBD leads them to maintain a positive attitude towards FMT [24]. Therefore, FMT delivery methods need to be rationally designed taking into account efficacy and recipient factors.

Another finding from our subgroup analyses was the apparently higher efficacy of pooled donor stools than single donor stools on clinical remission but not on combined

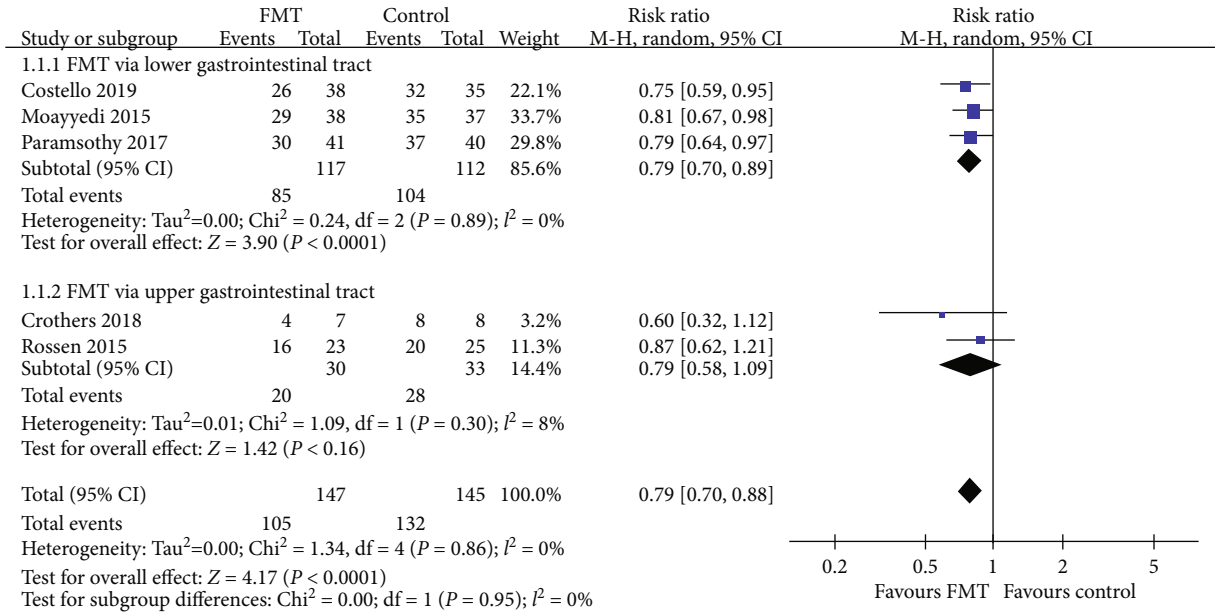

Figure 3: Forest plot of studies reporting combined clinical remission with endoscopic remission/response and subgroup analysis according to different delivery routes.

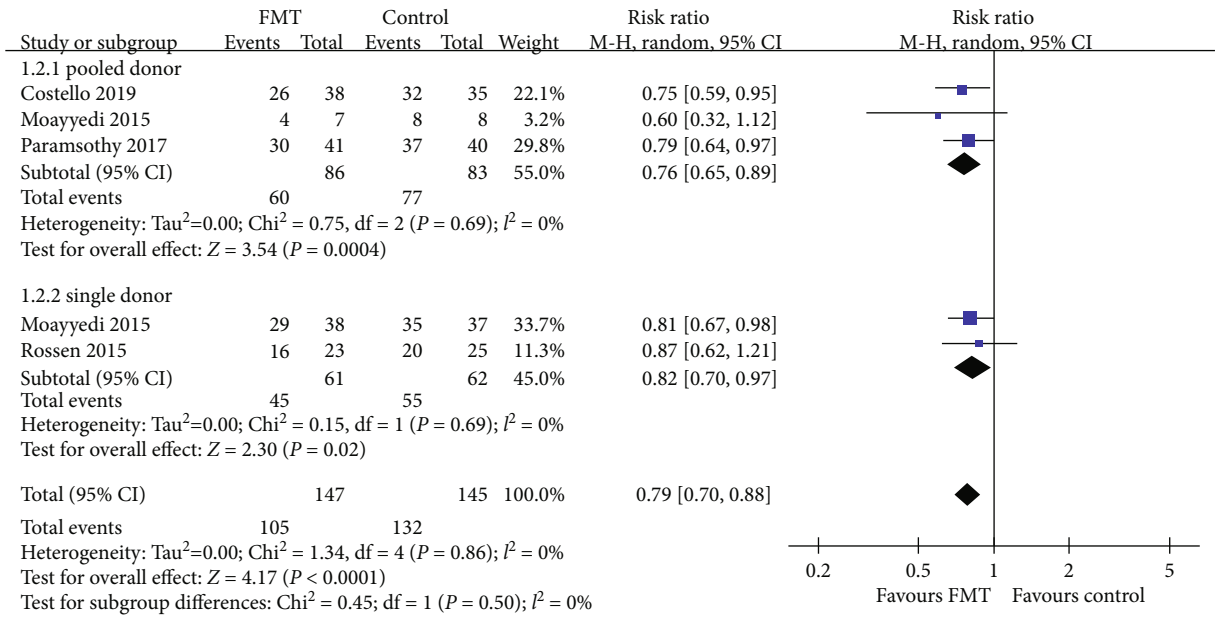

Figure 4: Forest plot of studies reporting combined clinical remission with endoscopic remission/response and subgroup analysis according to number of donors.

clinical remission with endoscopic remission/response and endoscopic remission. The result was consistent with a previous study which suggested that remission rates of UC patients could be enhanced by pooling stools from multiple donors to increase microbial diversity [16, 25]. Other studies also revealed the efficacy of FMT in UC was related to compositional and functional differences in the donor's and recipient's gut microbiota. For example, a previous small study including 8 refractory UC patients reported that higher bacterial species richness in donors was associated with successful transplantation [26]. Another study showed that sustained remission of UC patients was associated with butyrate-producing organisms, and relapse was associated with Proteobacteria and Bacteroidetes [27]. In addition, a recent prospective study demonstrated that the differences of the recipients' relative abundance in Eggerthella, Lactobacillus, and Ruminococcus between pre-FMT and 5 days post-FMT were remarkably correlated with the long-term clinical

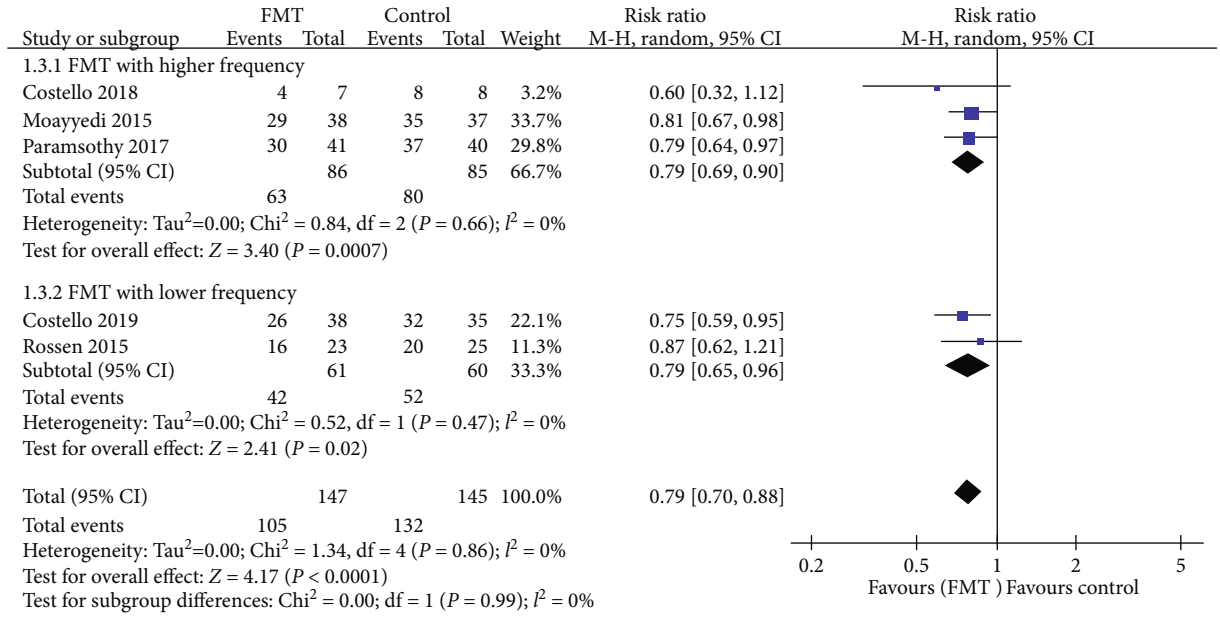

Figure 5: Forest plot of studies reporting combined clinical remission with endoscopic remission/response and subgroup analysis according to frequency of FMT administration.

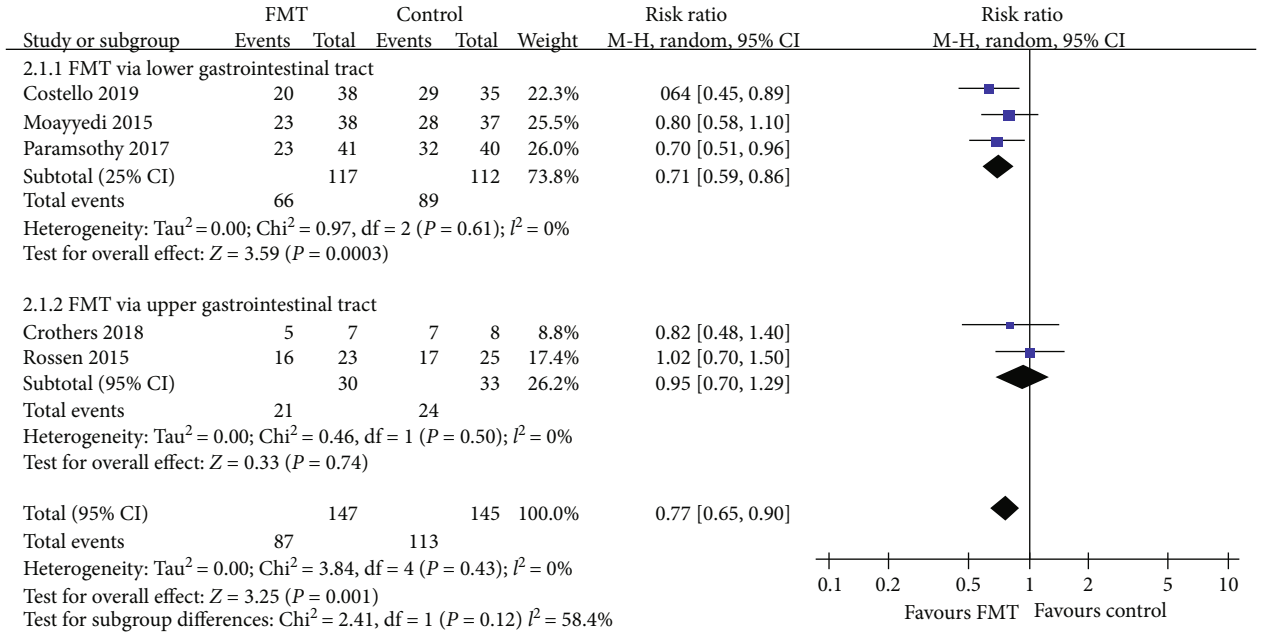

Figure 6: Forest plot of studies reporting clinical remission and subgroup analysis according to different delivery routes.

remission [28]. As a result, selecting donors based on microbial indicators and/or capability of the donor microbiota may be important for improved FMT efficacy [7].

Similar results were shown in the third subgroup analyses which revealed a beneficial effect of higher frequency of FMT administration than lower frequency on clinical remission but not on combined clinical remission with endoscopic remission/response and endoscopic remission. However, frequency of administration and optimal overall duration is still unclear as study parameters were not directly comparable across different studies [7]. Some authors considered higher frequency of administration as a high treatment burden that would likely limit applicability to practice [15]. Further studies should evaluate parameters such as dosage frequency and total treatment duration.

When data were pooled from studies reporting SAEs, although total SAEs were more frequent among FMT patients (10 patients) than among those assigned to control

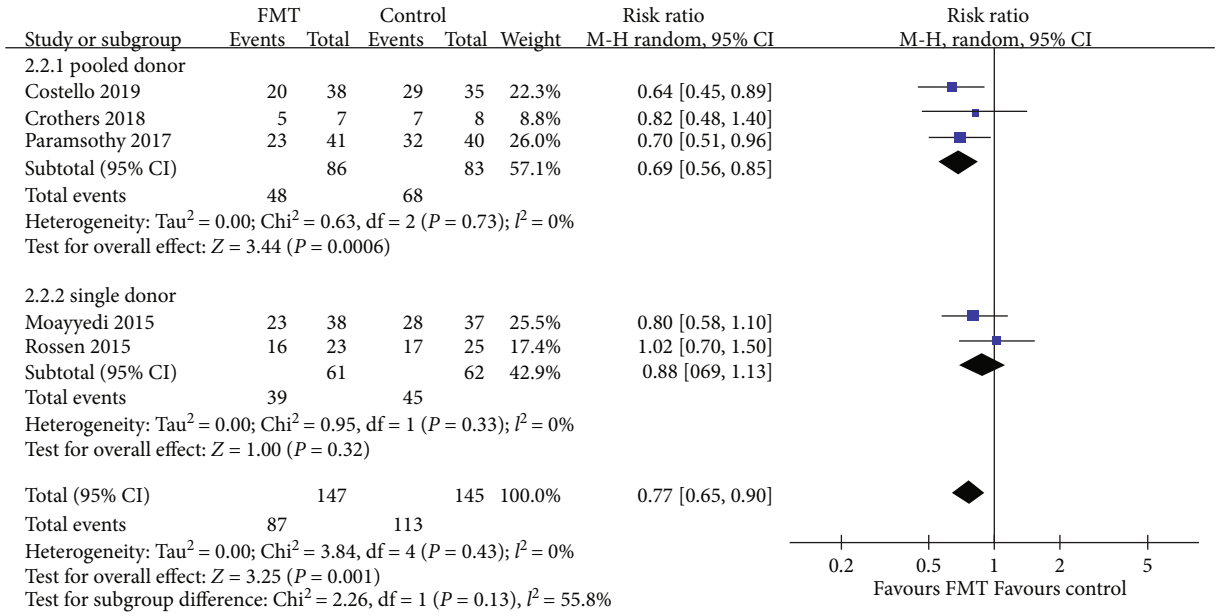

Figure 7: Forest plot of studies reporting clinical remission and subgroup analysis according to number of donors.

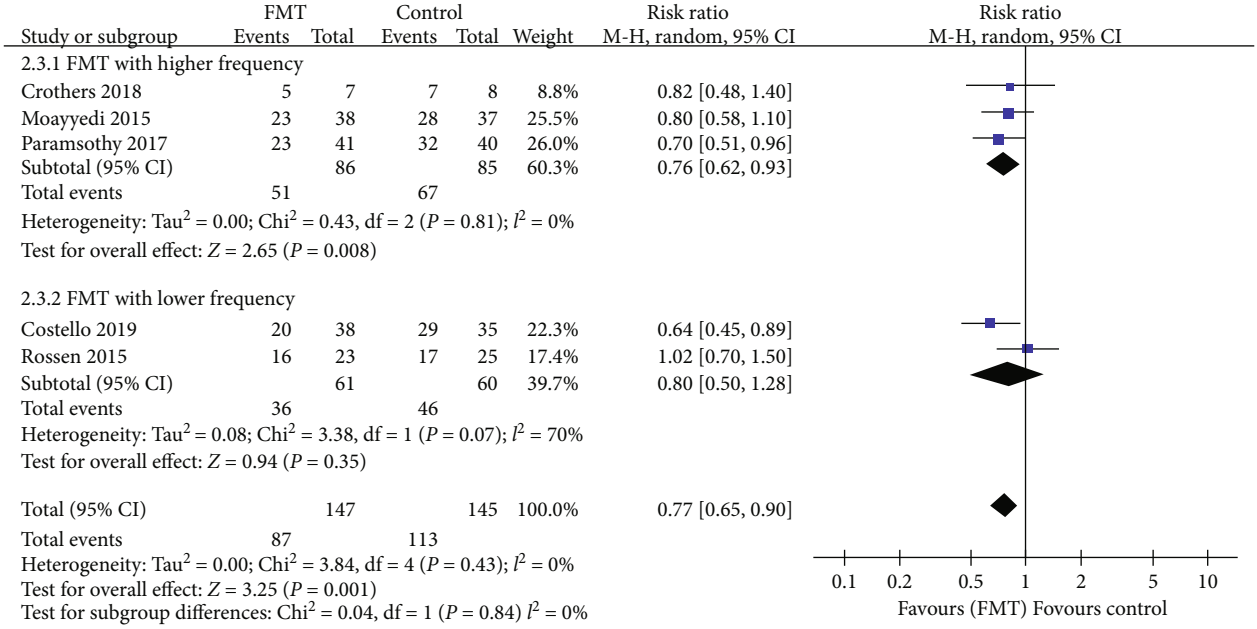

Figure 8: Forest plot of studies reporting clinical remission and subgroup analysis according to frequency of FMT administration.

group (7 patients), this difference was not statistically significant. It demonstrated that FMT is relatively safe for treatment of patients with active UC in the short term. A systematic review from this year revealed that FMT-related adverse events (AEs) were observed in 19% of FMT procedures, and diarrhea (10%) and abdominal discomfort/pain/cramping (7%) were most frequently reported. SAEs were reported in 1.4% of patients (0.99% microbiota-related SAEs), and 80% (4 of 5 patients) of FMT-related deaths were reported in patients receiving FMT via the upper gastrointestinal tract [29]. Another previous study analyzed the long-term safety of FMT in active UC with the follow-up ranged from 1 to 5 years [20]. They observed 17.4% (43/247) FMT-related AEs including one SAE. They also found that both the method of preparation of microbiota from stool using the automatic system (recently named as washed microbiota transplantation [30]) and the delivery method of colonic TET were associated with a lower rate of FMT-related AEs. All of these results demonstrated that FMT-related AEs were mild or moderate and self-limiting.

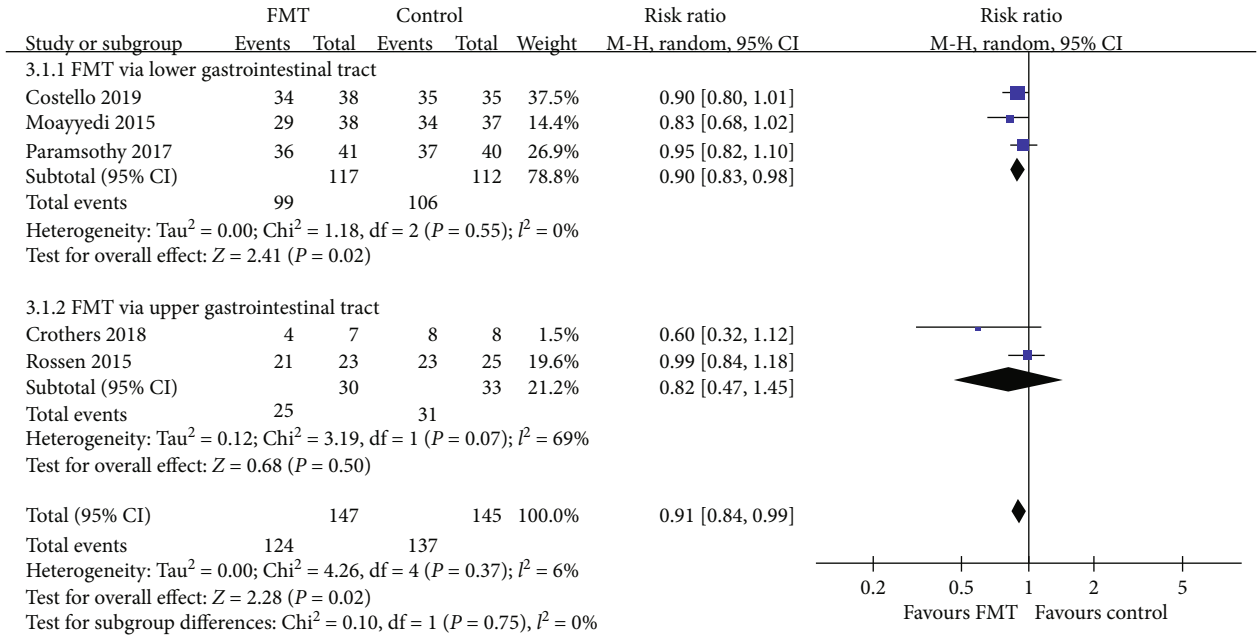

Figure 9: Forest plot of studies reporting endoscopic remission and subgroup analysis according to different delivery routes.

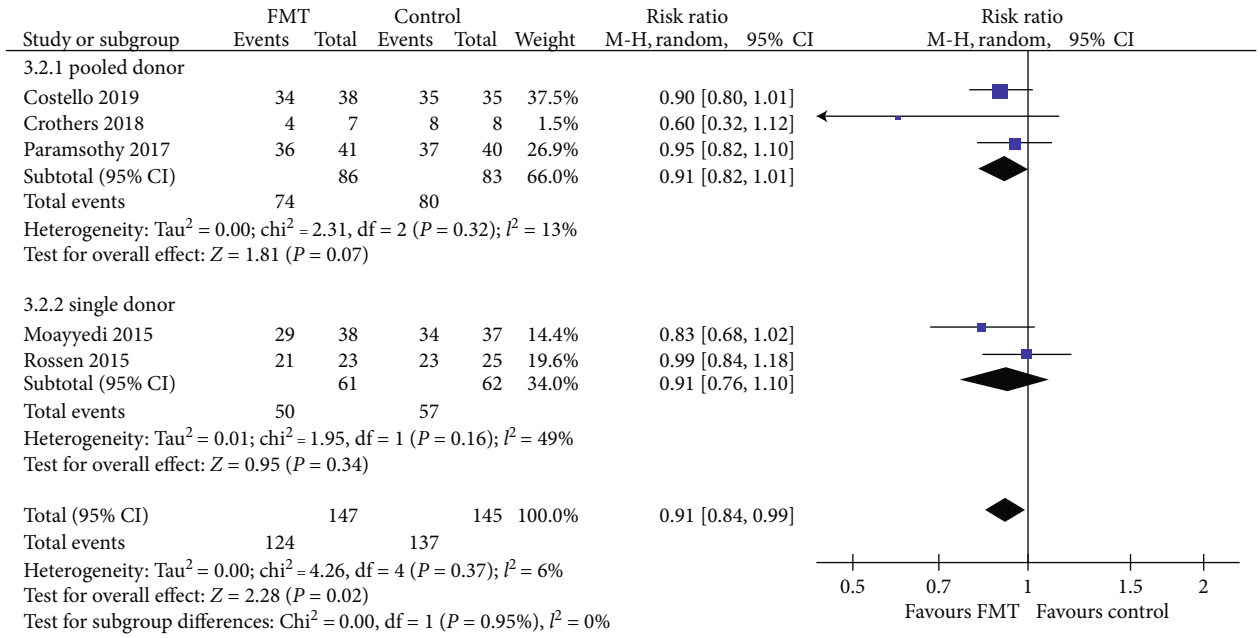

Figure 10: Forest plot of studies reporting endoscopic remission and subgroup analysis according to number of donors.

However, its methodology should be improved to reduce both delivery-related AEs and microbiota-related AEs [29].

Besides the above aspects, there were likely additional factors that could contribute to the accuracy of the final results, such as the transplantation stool dosage, the frequency of administration, pretreatment antibiotics use, bowel lavage, and so on. All of these factors remain ambiguous and controversial. Washed microbiota preparation, a recent named concept based on the automatic microfiltration machine (GenFMTer, Nanjing, China), makes delivering a precise dose of the enriched microbiota feasible, instead of using the weight of stool [30]. This method may resolve the bias between studies due to differences of stool dosage in the future. Additionally, a recent prospective study demonstrated that patients with UC should undergo the second course of FMT within 4 months after the first course of FMT for maintaining the long-term clinical benefits [28]. In terms of pretreatment antibiotics use, two trials [11, 17] had antibiotic pretreatment as part of their methods, two trials [15, 16] did not adopt the use of antibiotics prior to

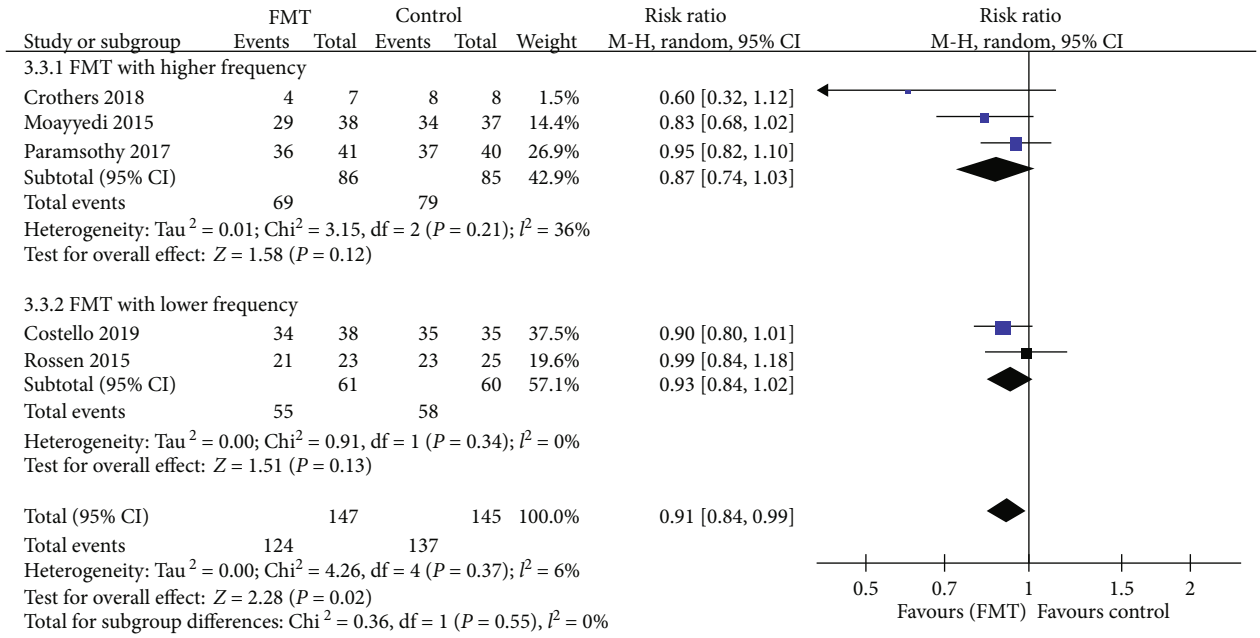

Figure 11: Forest plot of studies reporting endoscopic remission and subgroup analysis according to frequency of FMT administration.

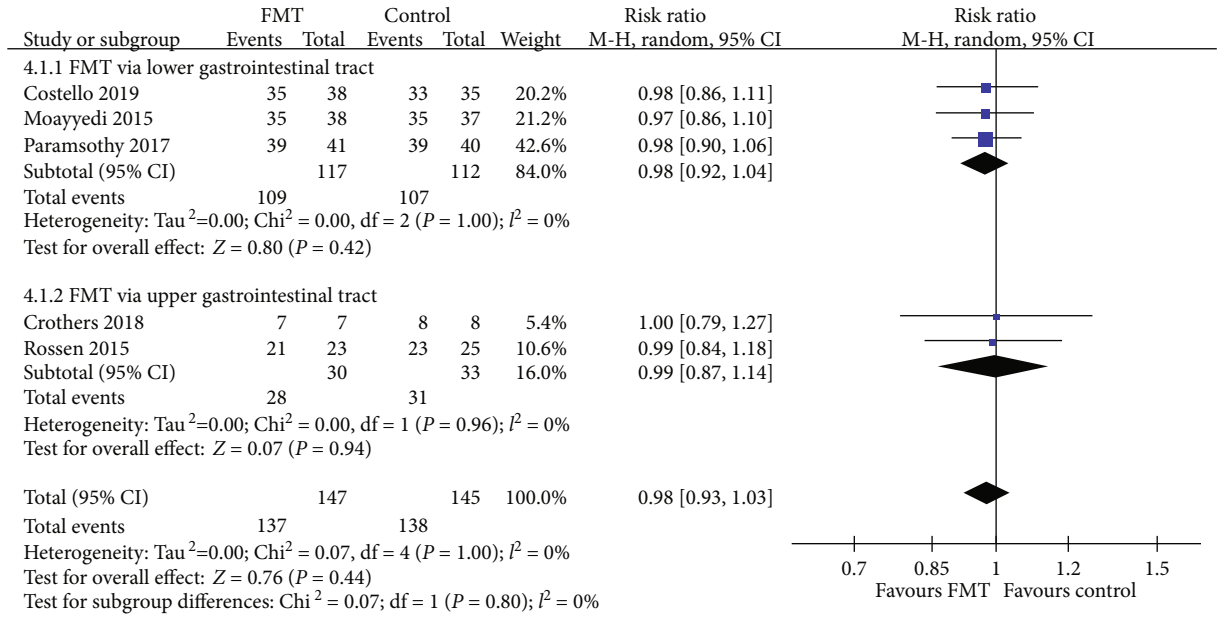

Figure 12: Forest plot of studies reporting serious adverse events and subgroup analysis according to different delivery routes.

FMT, and one trial [18] did not report this item. Although a recent study demonstrated that combination therapy of FMT and antibiotics was more effective than FMT therapy alone in restoring Bacteroidetes diversity in UC [31], antibiotic pretreatment remains controversial. The latest consensus in 2020 (Nanjing consensus on methodology of washed microbiota transplantation) stated that "Antibiotics should be stopped 12-48h before microbiota delivery" [32]. Future studies should specifically assess the role of antibiotics prior to FMT in different conditions and its cost-effectiveness [7].

Although FMT shows comparable safety and favorable clinical efficacy for the treatment of active UC in the short term, there were limitations of the included studies in our meta-analysis. All the included RCTs recruited patients with mild-moderate active UC, instead of serious conditions. However, patients with severely active UC were difficult to treat in clinic, and FMT was generally used to resolve these serious conditions. Most of patients with severely active UC were not suitable for RCT. As a result, further studies should pay more attention to these patients.

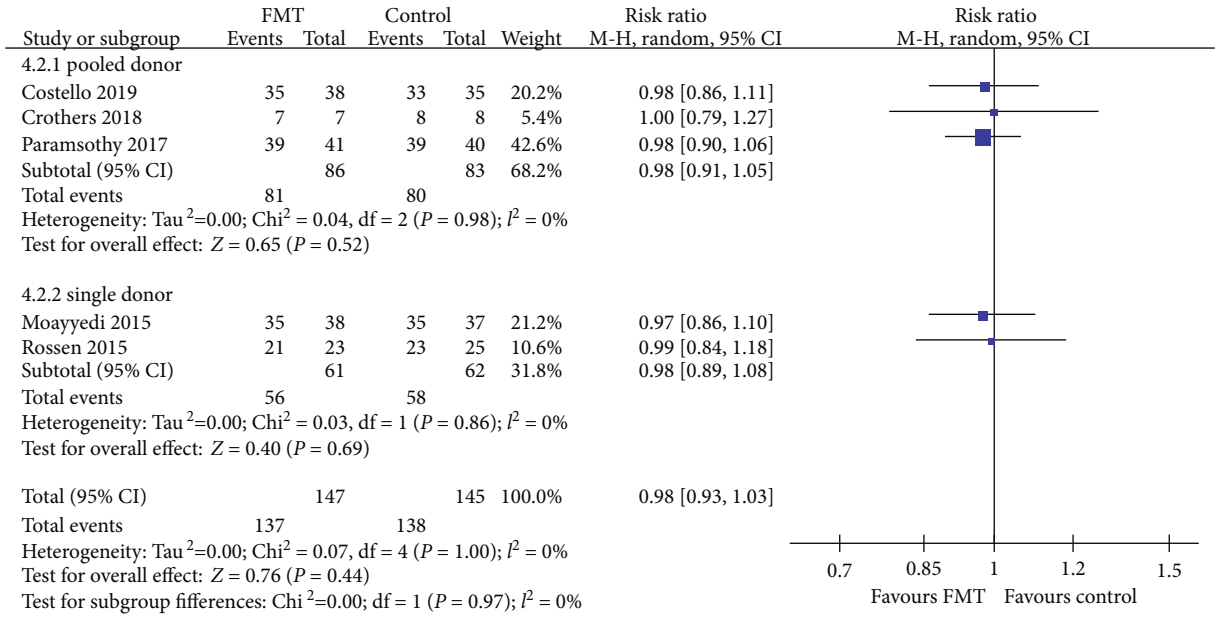

Figure 13: Forest plot of studies reporting serious adverse events and subgroup analysis according to number of donors.

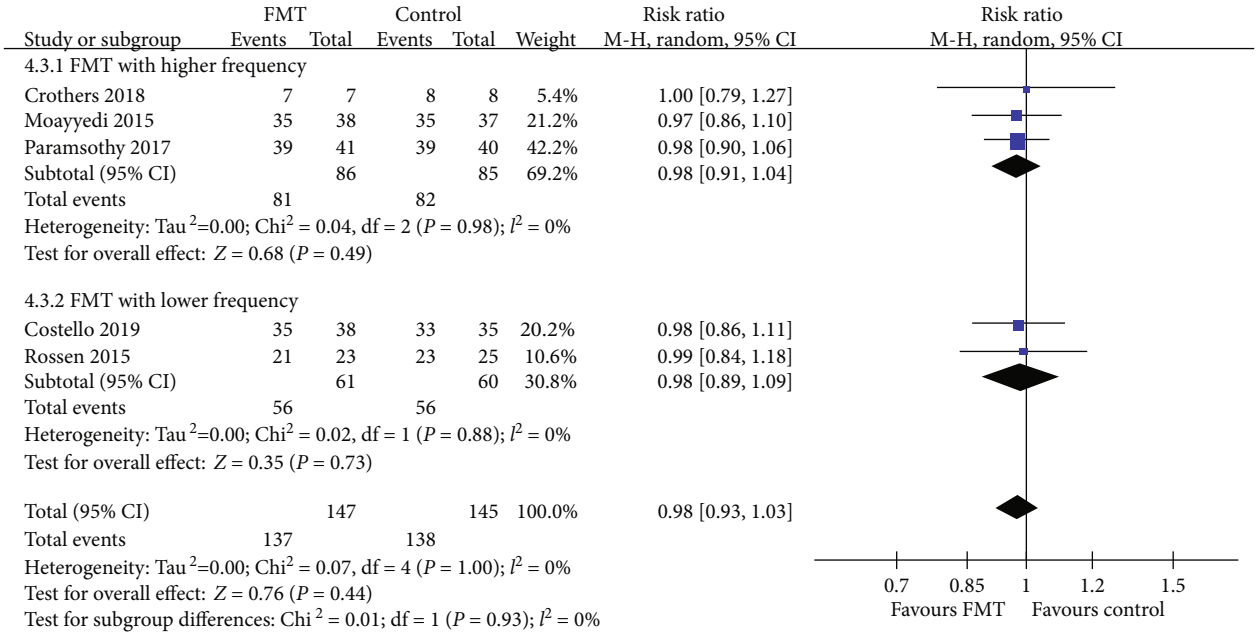

Figure 14: Forest plot of studies reporting serious adverse events and subgroup analysis according to frequency of FMT administration.

5. Conclusions

In conclusion, this systematic review and meta-analysis showed advantage of FMT over controls in clinical remission, endoscopic remission, and combined them together in patients with active UC when data from all RCTs were considered. In addition, the lower gastrointestinal route of delivery, pooled donor stool, and higher frequency of administration may be more effective. Meanwhile, no significant difference was noted on SAEs between FMT and the control group. Therefore, this meta-analysis demonstrated that short-term use of FMT is beneficial and safe for clinical and endoscopic improvements in patients with mild to moderate active UC. However, there have been only a few eligible RCTs conducted to date, so it is difficult to draw firm conclusions. Future RCTs are still required to address questions regarding donor selection, treatment prior to FMT, ideal stool or microbiota dosage, frequency of administration, predictors of patients most likely to respond, the most effective delivery route in different conditions, and cost-effectiveness, which remain controversial.

Data Availability

The retrospective data used to support the findings of this study are included within the article.

Additional Points

PRISMA 2009 Checklist Statement. The authors have read the PRISMA 2009 Checklist, and the manuscript was prepared and revised according to the PRISMA 2009 Checklist.

Conflicts of Interest

The authors declare that they have no conflict of interest.

Authors' Contributions

Min Chen and Yonquan Shi were responsible for the conception and design of the study, analysis and interpretation of data, and revising the article for important intellectual content. Xiaolei Liu and Yan Li were responsible for the acquisition of data, analysis and interpretation of data, and drafting the article. Kaichun Wu was responsible for revising the article for important intellectual content. Xiaolei Liu and Yan Li contributed equally to this work. Min Chen and Yongquan Shi were co-first corresponding authors. Xiaolei Liu and Yan Li were co-first authors, equally contributed to the article. All authors read and approved the final manuscript.

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Research Article

Gut Dysbiosis and Abnormal Bile Acid Metabolism in Colitis-Associated Cancer

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Background. Patients with prolonged inflammatory bowel disease (IBD) can develop into colorectal cancer (CRC), also called colitis-associated cancer (CAC). Studies have shown the association between gut dysbiosis, abnormal bile acid metabolism, and inflammation process. Here, we aimed to investigate these two factors in the CAC model. *Methods*. C57BL/6 mice were randomly allocated to two groups: azoxymethane/dextran sodium sulfate (AOM/DSS) and control. The AOM/DSS group received AOM injection followed by DSS drinking water. Intestinal inflammation, mucosal barrier, and bile acid receptors were determined by real-time PCR and immunohistochemistry. Fecal microbiome and bile acids were detected via 16S rRNA sequencing and liquid chromatography-mass spectrometry. *Results*. The AOM/DSS group exhibited severe mucosal barrier impairment, inflammatory response, and tumor formation. In the CAC model, the richness and biodiversity of gut microbiota were decreased, along with significant alteration of composition. The abundance of pathogens was increased, while the short-chain fatty acids producing bacteria were reduced. Interestingly, *Clostridium XlV* and *Lactobacillus*, which might be involved in the bile acid deconjugation, transformation, and desulfation, were significantly decreased. Accordingly, fecal bile acids were decreased, accompanied by reduced transformation of primary to secondary bile acids. Given bile acid receptors, the ileum farnesoid X receptor-fibroblast growth factor 15 (FXR-FGF15) axis was downregulated, while Takeda G-protein receptor 5 (TGR5) was overexpressed in colonic tumor tissues. *Conclusion*. Gut dysbiosis might alter the metabolism of bile acids and promote CAC, which would provide a potential preventive strategy of CAC by regulating gut microbiota and bile acid metabolism.

1. Introduction

Colorectal cancer (CRC) is one of the most common cancers worldwide [1] and can be identified as sporadic, hereditary CRC, or colitis-associated cancer (CAC) [2, 3]. Patients with long-term inflammatory bowel disease (IBD), especially ulcerative colitis, can develop into intestinal cancer, known as CAC. The risks of developing CAC in IBD patients were 2% by 10-year intervals, 8% by 20 years, and 18% by 30 years, as shown in a meta-analysis [4]. Specific factors of IBD patients can increase the prevalence of CAC, such as extensive mucosal involvement, the severity and duration of the disease, family history, primary sclerotizing cholangitis, and therapeutic effect of the disease [5, 6]. Factors involving CAC development include immune response, epigenetic modification, intestinal inflammatory response, and gut dysbiosis [7, 8].

Previous studies have indicated the relevance between altered gut microbiota and risk of gastrointestinal diseases (such as IBD, CRC, and irritable bowel syndrome) [6, 9, 10]. Gut microbiota maintains host health by participating in immune modulation and host metabolism [8]. Moreover,

the presence of gut microbiota plays a crucial role in bile acid metabolism [9, 11]. Bile acids are synthesized by classical and alternative pathways in hepatocytes. The bile acids conjugated to either taurine or glycine are finally transported to the intestine. Bile salt hydrolase (BSH) containing bacteria can convert bile acids from conjugated to unconjugated, and bacteria that possess 7α-dehydroxylation activity can make primary bile acids transform into secondary bile acids. In the distal ileum, almost 95% of bile acids are returned to the liver [12, 13]. Physiologically, bile acids can regulate extensive metabolic and immune-related activities including glucose, lipid, and energy metabolism [14, 15]. Nevertheless, excessive bile acids in the intestine especially secondary bile acids have the capability of promoting CRC. Previous literature has shown that gut dysbiosis and bile acid metabolism disorder can promote CRC [10, 16]. However, the role of these two in the CAC progression is not fully understood.

Bile acids also exert metabolic effects by activating bile acid receptors, mainly the nuclear receptor farnesoid X receptor (FXR) and G-protein coupled receptor (TGR5) [17]. FXR, mainly expressed in the liver, kidney, and terminal ileum, has a significant influence on bile acids, liquid, and glucose metabolic homeostasis [15, 18–20]. Activation of intestinal FXR is responsible for bile acid reabsorption through the portal vein and limits the uptake of bile acids in the enterocytes [21, 22]. TGR5, highly expressed in the ileum, colon, and gallbladder, can regulate the energy homeostasis and bile acids, liquid and glucose metabolism, cell proliferation, and apoptosis and immune responses [23, 24]. It has shown that the TGR5 is highly expressed in esophageal and gastric adenocarcinoma [25, 26]; however, the role of TGR5 in CAC remains unclear.

We hypothesized that gut microbiota and bile acid metabolism could be involved in CAC development, and we chose the AOM/DSS model in the present study. Our results revealed gut dysbiosis during tumorigenesis, accompanied by abnormal bile acid metabolism. In addition, FXR and TGR5, the two main bile acid receptors, were also involved in CAC. These results provide a better understanding of CAC, suggesting that the regulation of gut microbiota and bile acids might be a guiding therapeutic strategy for CAC.

2. Materials and Methods

2.1. Animals and Induction of CAC. In the present study, we chose the AOM/DSS-induced CAC model, which had the advantages of reproducibility, simplicity, affordability, and mainly invading the colon, similar to human sporadic CRC [27]. Twenty female C57BL/6 mice aged 7 weeks were obtained from Beijing Huafukang Bioscience Co. Inc. (Beijing, China) and acclimatized 1 week before the experiment. They were randomly divided into the AOM/DSS and control groups with 10 mice, respectively. All mice were maintained in a specific pathogen-free (SPF) condition with the 12 : 12 light-dark cycle. The mice were fed a diet of AIN-93M rodents and free to eat and drink. According to our previous study and literatures [28–30], intraperitoneal injection of 10 mg/kg azoxymethane (AOM) (Sigma, USA) was applied to the AOM/DSS group, while the control group was intraperitoneally injected with sterile isotonic saline on day 1. After seven days, the AOM/DSS group was given 1.5% dextran sodium sulfate (DSS) (MP Biomedicals, USA) in drinking water on days 8–13, 27–32, and 46–51, and each cycle of DSS treatment was followed by 14-day drinking water. Mice were euthanized by CO_2 asphyxiation on day 70 (Figure 1(a)). Animal experiments were performed following the experimental regulations of the Animal Ethics Committee.

2.2. Tissue and Feces Collection. All mice were observed every day and weighed weekly. The general condition and defecation of the mice were recorded during AOM/DSS treatment. On days 0 and 70, each mouse was individually housed in a clean cage for two hours to collect feces. Then, mice were sacrificed with measurement of colon length and spleen weight. The intestine was washed with ice PBS and dissected longitudinally. The location, size, and numbers of intestinal tumors were observed. Tumor load refers to the sum of the tumor diameters of each mouse. Intestinal tissues (ileum and colon) were stored at -80°C for subsequent study. The colon was rolled and embedded in paraffin for further pathological and immunohistochemistry analysis.

2.3. Pathology and Immunohistochemistry. Colon tissue was cut into sections (5 μm), and then, hematoxylin and eosin (H&E) staining was applied to colon sections for assessment of tumor and inflammatory cell infiltration. In addition, colon sections were deparaffinized and rehydrated for immunohistochemistry to detect the expression of TGR5. Slides were incubated with rabbit monoclonal TGR5 antibody (1 : 100, Abcam, MA, USA) at 4°C overnight, followed by corresponding secondary antibody. Then, the sections were treated with horseradish peroxidase- (HRP-) streptavidin solution. Finally, 3,3′-diaminobenzidine was applied for counterstaining and further observation. At least five areas from each single section were observed under light microscope DM5000B (Leika, Germany).

2.4. Immunofluorescent Staining. Colon sections were incubated with primary antibody ZO-1 (Abcam, MA, USA) in a humid chamber for 12 h at 4°C. Subsequently, after washing with PBS slightly, the fluorescently conjugated secondary antibody was applied. And this incubation process lasted for 1 h at room temperature. After DAPI reaction and seal, the slides were observed with a fluorescence microscope, and then, we obtained DAPI and FITC images of a unified area.

2.5. Real-time PCR Analysis. Total RNA was extracted from the intestinal tissues (ileum and colon) by a RNeasy mini kit. Reverse transcription of cDNA was performed with the TIANScript RT Kit. Real-time PCR analysis was performed by the Applied Biosystems StepOnePlus™ Real-time PCR System. Each run consisted of 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 60°C for 60 s, and then 95°C for 15 s, 60°C for 60 s, and 95°C for 15 s in a 20 ml volume. The levels of mRNA were analyzed by the $\Delta\Delta$Ct method. The oligonucleotide primer sequences are listed in Table 1.

2.6. Gut Microbiota Analysis. The 16S rRNA sequencing was performed using the Illumina HiSeq PE250. DNA was

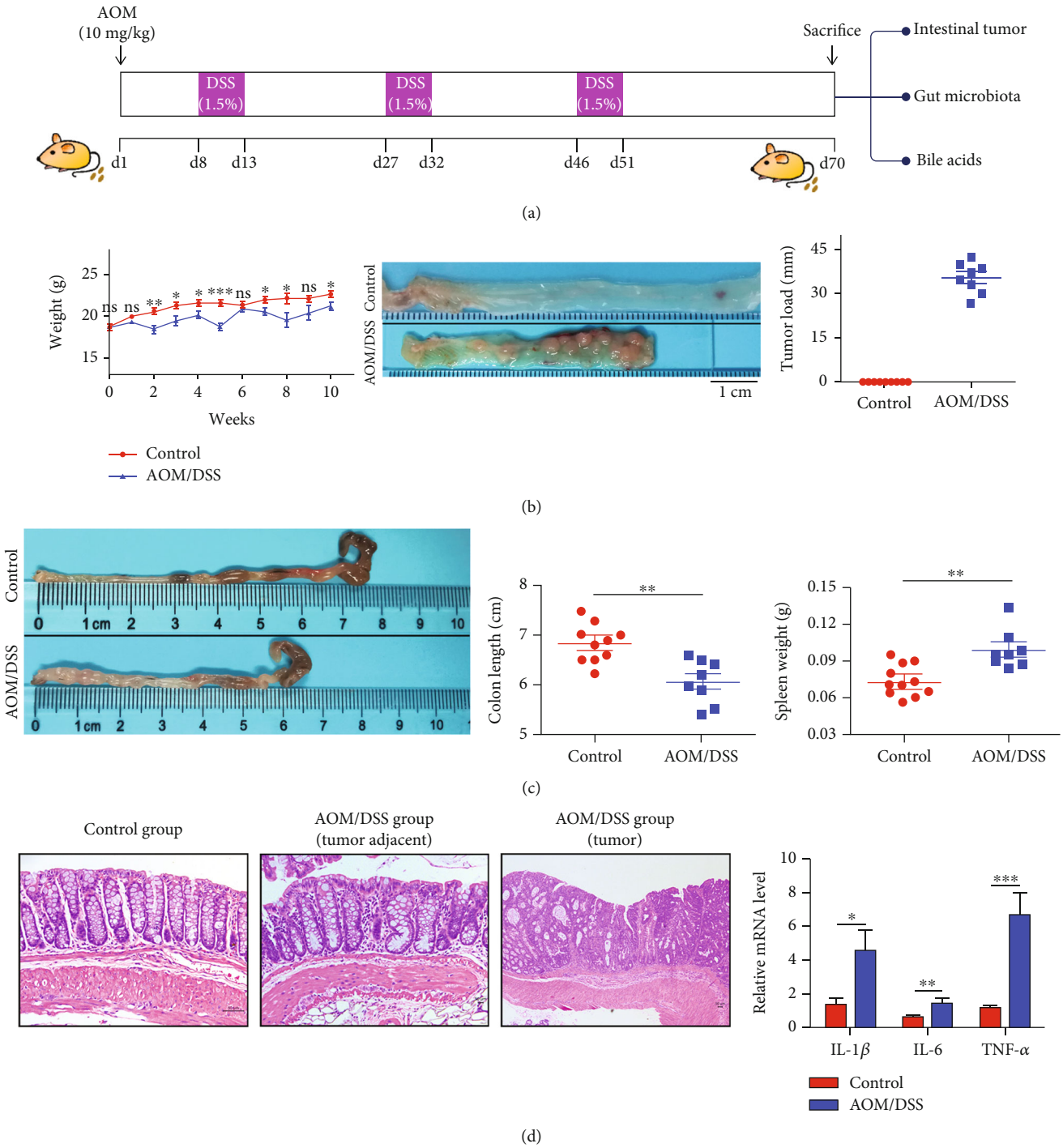

Figure 1: Tumor formation and severe inflammatory response in the colon of mice treated with AOM/DSS. (a) Mice received intraperitoneal injection of 10 mg/kg AOM on day 1 and followed by three circles of 1.5% DSS drinking water in the AOM/DSS group. And all mice were killed on day 70. (b) Body weight, colon lumen appearance image, and tumor load of the two groups. (c) AOM/DSS treatment shortened the colon length and increased the weight of the spleen. (d) H&E staining revealed colon tumor formation and inflammatory cell infiltration in the AOM/DSS group. Real-time PCR showed the increased levels of several inflammatory cytokines (IL-1β, IL-6, and TNF-α) in the colon. $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$. $n = 8$-10.

extracted with the QIAamp DNA Stool Mini kit (Qiagen, Germany). Then, the primer F341 (5′-ACTCCTACG GGRSGCAGCAG-3′) and R806 (5′-GGACTACVVGGGT ATCTAATC-3′) were designed for 16S rRNA gene (V3-V4 region) amplification. The sequences from samples of the two groups were clustered to generate operational taxonomic units (OTUs) at the 97% identity using Usearch. The representative sequence of each OTU was classified using the

Table 1: The oligonucleotide primer sequences used in the experiments.

Primers	Sequence
GAPDH	Forward 5′-TGTGTCCGTCGTGGATCTGA-3′ Reverse 5′-CCTGCTTCACCACCTTCTTGA-3′
TNF-α	Forward 5′-ACTCCAGGCGGTGCCTATG-3′ Reverse 5′-GAGCGTGGTGGCCCCT-3′
IL-1β	Forward 5′-GTGGCTGTGGAGAAGCTGTG-3′ Reverse 5′-GAAGGTCCACGGGAAAGACAC-3′
IL-6	Forward 5′-CCAGTTGCCTTCTTGGGACT-3′ Reverse 5′-GGTCTGTTGGGAGTGGTATCC-3′
ZO-1	Forward 5′-GGGCCATCTCAACTCCTGTA-3′ Reverse 5′-AGAAGGGCTGACGGGTAAAT-3′
Occludin	Forward 5′-CGGTACAGCAGCAATGGTAA-3′ Reverse 5′-CTCCCCACCTGTCGTGTAGT-3′
Claudin1	Forward 5′-AGACCTGGATTTGCATCTTGGTG-3′ Reverse 5′-TGCAACATAGGCAGGACAAGAGTTA-3′
Claudin3	Forward 5′-CCTGTGGATGAACTGCGTG-3′ Reverse 5′-GTAGTCCTTGCGGTCGTAG-3′
FXR	Forward 5′-GGACGGGATGAGTGTGAAG-3′ Reverse 5′-TGAACTTGAGGAAACGGGAC-3′
FGF15	Forward 5′-TGAAGACGATTGCCATCAAGG-3′ Reverse 5′-GGATCTGTACTGGTTGTAGCC-3′
ASBT	Forward 5′-AGGAATACTGTACCAAAGTGCC-3′ Reverse 5′-TTTCCAAGGCTACTGTTCGG-3′
OSTα	Forward 5′-TGCTCACCTCCCTACTCTTC-3′ Reverse 5′-AACAAGCCTCATACCCAACC-3′
OSTβ	Forward 5′-GCTTTGGTATTTTCGTGCAGAAG-3′ Reverse 5′-GTTTCTTTGTCTTGTGGCTGC-3′
TGR5	Forward 5′-AAAGGTGTCTACGAGTGCTTC-3′ Reverse 5′-TGCATTGGCTACTGGTGTG-3′

RDP database. Alpha diversity and beta diversity were performed using QIIME.

2.7. Measurement of Bile Acids in Feces. The liquid chromatography-mass spectrometry (LCMS) method was applied to measure fecal bile acid concentration. As reference standards, cholic acid (CA), chenodeoxycholic acid (CDCA), and lithocholic acid (LCA) were purchased from Aladdin, with α-muricholic acid (α-MCA) and β-muricholic acid (β-MCA) from Toronto Research Chemicals, and deoxycholic acid (DCA) from Sigma. And they were added to fecal samples for preliminary measurement by an external standard method. Each fecal sample was suspended in 5 ml of chromatographic ethanol and then ultrasonically extracted for 60 min at 30°C. After 10 minutes of centrifugation (10,000 rpm, 4°C), the supernatant (4 ml) was aspirated and dried under nitrogen. The samples were redissolved with methanol and went through a 0.22 μm filter. Finally, bile acids were analyzed using the Agilent 1260 Series liquid chromatograph combined with a 6120B mass spectrometer. The concentrations of bile acids were determined based on the peak areas [31, 32].

2.8. Statistical Analysis. The data were described as the mean ± SEM. Differences between the two groups were determined by Student's t-test. GraphPad Prism 5.01 and SPSS 22.0 were applied for data analysis. $P < 0.05$ was considered significant.

3. Results

3.1. General Characteristics of CAC Mouse Model. Mice in the control group grew well, while two mice in the AOM/DSS group died while receiving DSS. Mice in the AOM/DSS group showed noticeable weight loss, accompanied by hematochezia during each cycle of DSS treatment. At 10 weeks, tumor load of the AOM/DSS group was 35.41 ± 1.901 mm, characterized by shortened colon length 6.06 ± 0.158 *vs.* 6.83 ± 0.125 cm and increased spleen weight 0.10 ± 0.006 *vs.* 0.07 ± 0.004 g (Figures 1(b) and 1(c). H&E staining revealed significant inflammatory cell infiltration and intramucosal tumor in the colon of the AOM/DSS group, and the mRNA levels of inflammatory cytokines (IL-1β, IL-6, and TNF-α) were significantly increased (Figure 1(d)).

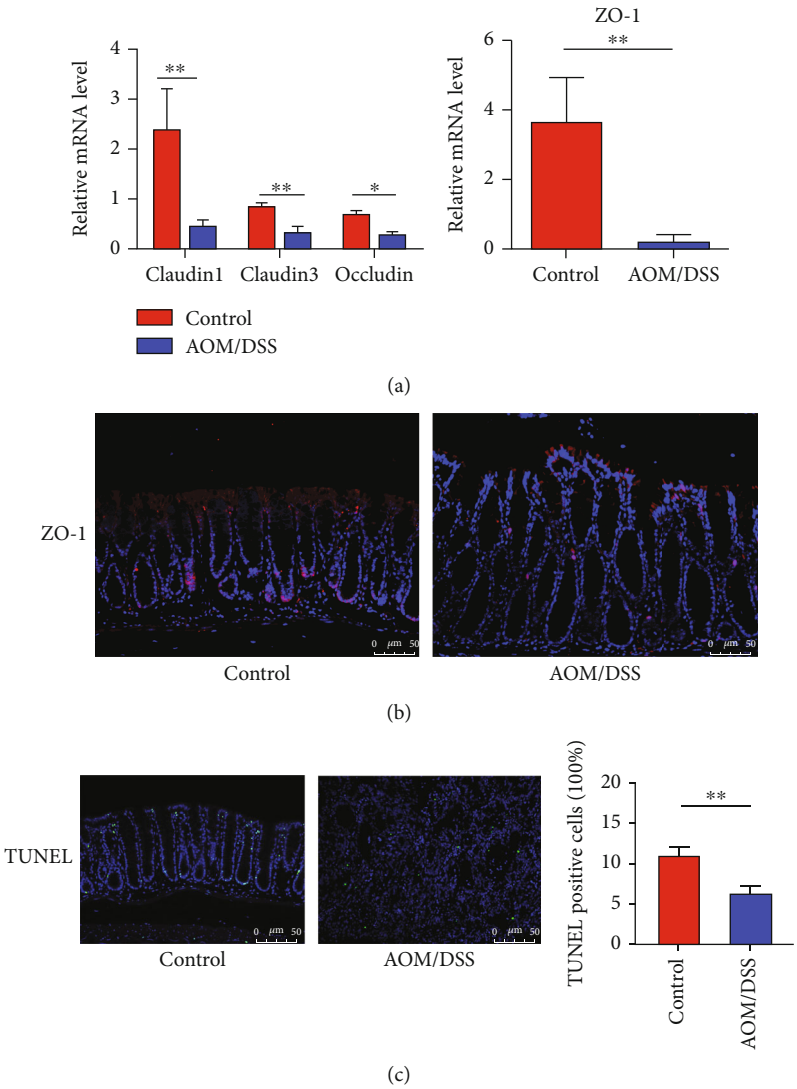

Figure 2: Intestinal barrier was disrupted in the CAC model. (a) The mRNA level of Occludin, Claudin1, Claudin3, and ZO-1 was reduced in the colon after AOM/DSS treatment. (b) Immunofluorescent staining for ZO-1 in colon tissues of the control and AOM/DSS group. (c) Colon sections from the two groups were stained with TUNEL. Data were quantified as the mean percentage of positive-stained cells in five randomly selected fields in each sample. Scale bars, 50 μm. $^{*}p < 0.05$, $^{**}p < 0.01$. $n = 8$-10.

3.2. Intestinal Barrier Disruption and Apoptosis Inhibition after AOM/DSS Treatment. The intestinal barrier exists as an effective defense system to maintain homeostasis of the host. Tight junctions including ZO-1, Claudins, and Occludin are critical in preventing the penetration of pathogenic microorganisms. The mRNA expression of ZO-1, Occludin, Claudin1, and Claudin3 was significantly reduced in the colon of the AOM/DSS group (Figure 2(a)), indicating that the mucosal barrier was disrupted in the development of CAC. Additionally, the expression of ZO-1 in immunofluorescence was decreased after AOM/DSS treatment (Figure 2(b)). The AOM/DSS group showed significantly decreased apoptotic cells than the control group (6.24 ± 0.82 *vs.* 10.95 ± 1.08, $P < 0.01$, Figure 2(c)), hinting the inhibition of cell apoptosis in the CAC model.

3.3. Decreased Gut Microbiota Diversity in the Development of CAC. A total of 372 and 353 OTUs were detected in the AOM/DSS group, while the control group was 358 and 379 OTUs at 0 and 10 weeks (Figure 3(a)). At the phylum level, compared with the control group, the abundance of *Firmicutes* increased (17.3% *vs.* 19.8%) in the AOM/DSS group at 10 weeks, and the *Bacteroidetes* decreased (79.7% *vs.* 72.1%, Figure 3(b)). Since there was no statistical difference in α-diversity between the two groups at 0 weeks ($P > 0.05$), the observed species, chao1, and Shannon index were significantly reduced in the AOM/DSS group after 10 weeks ($P < 0.05$), suggesting a decreased of gut microbiota richness and diversity in the CAC model (Figure 3(c)).

3.4. Alteration of Gut Microbiota Composition in the Development of CAC. The principal coordinate analysis (PCoA) plot showed that the microbial composition among the two groups was similar at 0 weeks and especially distinct after 10 weeks (Figure 4(a)). Unweighted Unifrac Anosim analysis showed that the R value was 0.636 and the P value

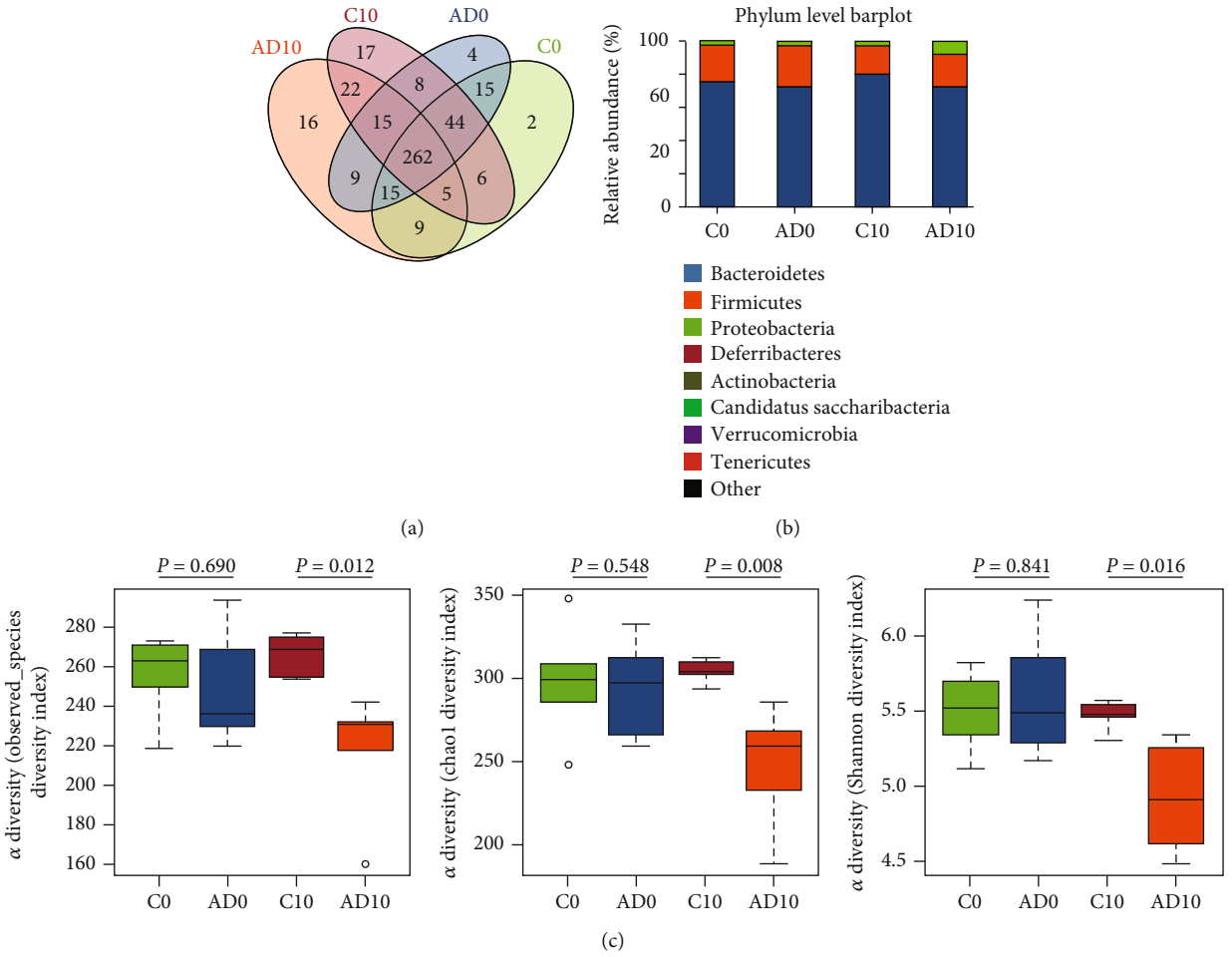

Figure 3: The gut microbiota composition at 0 and 10 weeks. (a) Venn diagram in the control and AOM/DSS group. (b) The abundance of *Bacteroidetes* decreased in the AOM/DSS group at 10 weeks, and the *Firmicutes* increased. (c) The α-diversity (observed species, chao1, and Shannon) showed no significant distinction between the two groups at 0 weeks, but it was dramatically reduced in the AOM/DSS group compared with the control group at 10 weeks. C0 and C10: control group at 0 and 10 weeks. AD0 and AD10: AOM/DSS group at 0 and 10 weeks. $n = 5$ in each group.

was 0.007, which indicated pronounced differences in species diversity between the two groups at 10 weeks (Figure 4(b)). The LefSe analysis was applied to evaluate the differential abundant species of the two groups at different levels (Figure 4(c)). The fecal microbiota results at week 10 showed a high abundance of the family *Bacteroidaceae*, *Eubacteriaceae*, and *Helicobacteraceae* in the AOM/DSS group and low abundance of *Clostridiaceae 1*, *Porphyromonadaceae*, and *Rikenellaceae*. At the genus level, the abundance of pathogens *Helicobacter* and *Streptococcus* was increased in the CAC model, and the short-chain fatty acids (SCFAs) producing bacteria including *Alistipes*, *Lachnospiracea_incertae_sedis*, and *Odoribacter* were decreased. Interestingly, the abundance of *Clostridium XlV* and *Lactobacillus*, which might be engaged in the metabolic process of bile acids, was decreased in the AOM/DSS group.

3.5. Fecal Bile Acid Profile in the CAC Model. To investigate bile acid metabolism during CAC development, the concentration in feces was tested by LCMS. After AOM/DSS treatment, levels of CA, DCA, and LCA in the feces were significantly reduced ($P < 0.05$, Figure 5(a)). Importantly, the ratio of DCA/CA and LCA/CDCA also decreased in the AOM/DSS group, indicating an impaired conversion from primary bile acids to secondary bile acids (Figure 5(b)). As previously mentioned, the abundance of *Clostridium XlV* and *Lactobacillus*, which were associated with bile acid metabolism, was reduced in the AOM/DSS group. Thus, the ability to bile acid deconjugation, transformation, and desulfation might be impaired after AOM/DSS treatment.

3.6. Bile Acid Receptors FXR and TGR5 in CAC Development. Bile acid receptors FXR and TGR5 can be activated by bile acids. Real-time PCR showed decreased levels of ileum FXR and fibroblast growth factor 15 (FGF15) in the AOM/DSS group (Figure 6(a)). Consistent with this, the expression of organic solute transporter subunits α and β (OSTα and OSTβ) was reduced, while the apical sodium-dependent bile acid transporter (ASBT) was highly expressed, which led to the accumulation of bile acids in enterocytes and limited the return to the liver (Figure 6(b)). Moreover, our results also showed that TGR5 mRNA expression was higher in

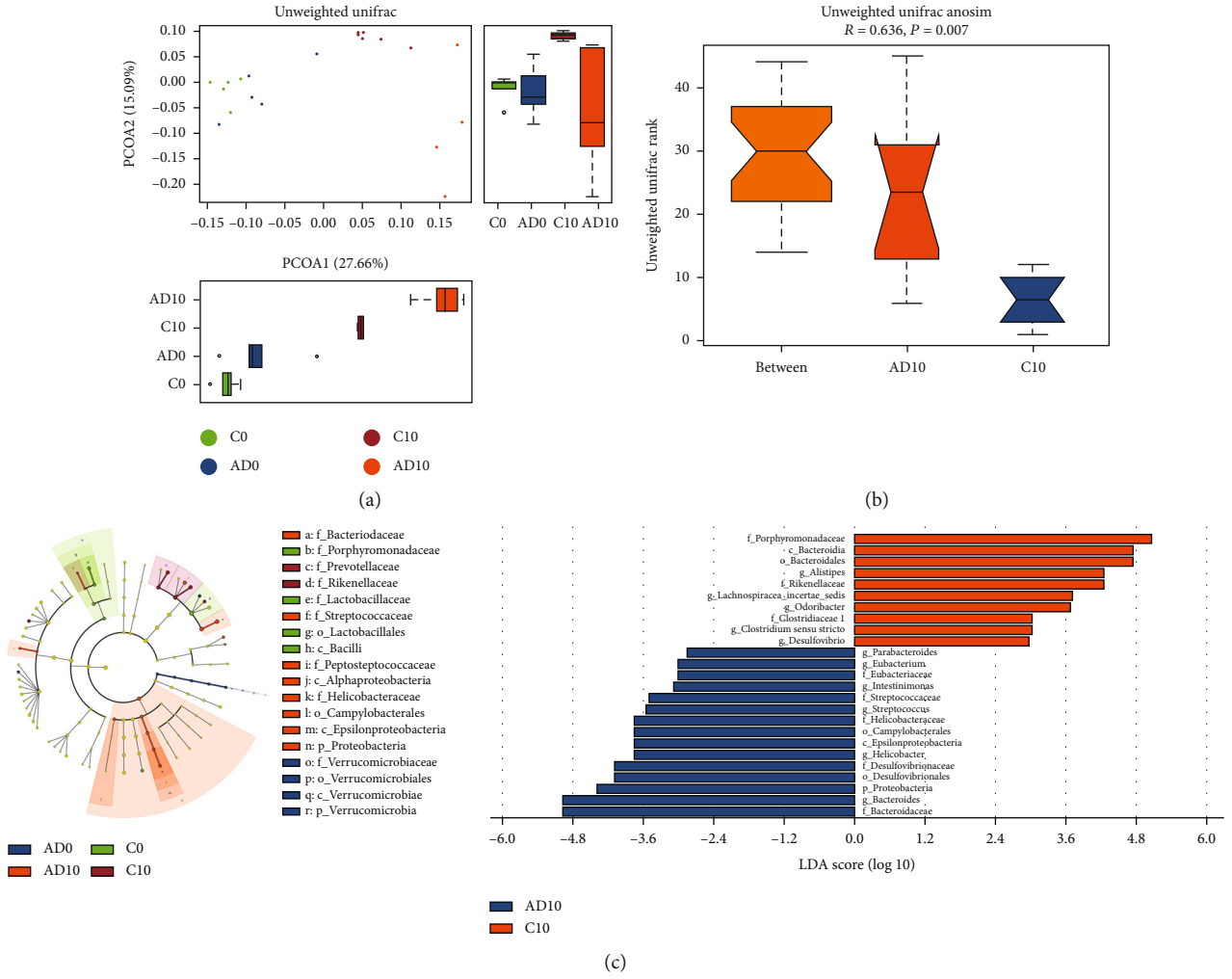

FIGURE 4: The alteration of gut microbiota during colitis-associated cancer development. (a) The PCoA plot showed a significant distinction in microbial composition among the two groups at 10 weeks. (b) Unweighted Unifrac Anosim analysis suggested a reasonable grouping after AOM/DSS treatment. (c) The LefSe analysis listed bacteria with significant differences at different levels in each group. C0 and C10: control group at 0 and 10 weeks. AD0 and AD10: AOM/DSS group at 0 and 10 weeks. $n = 5$ in each group.

the colon of mice after AOM/DSS treatment than in the control group (Figure 6(c)). Simultaneously, immunohistochemical staining confirmed a high expression of TGR5 in the AOM/DSS group (Figure 6(d)).

4. Discussion

It has been pointed out that 18% of IBD patients may develop CRC 30 years after colitis is diagnosed, known as CAC [4]. Substantial evidence has demonstrated that gut dysbiosis and abnormal bile acid metabolism exist in many diseases such as CRC, nonalcoholic fatty liver disease, and diabetes. Our previous studies have reported that bile acid-induced dysbiosis promoted intestinal tumorigenesis in *Apc*$^{min/+}$ mice [32, 33]. Our results in the present study showed destroyed intestinal barrier and colon tumor formation after AOM/DSS treatment. Meanwhile, the abundance of Helicobacter and Streptococcus, known as pathogens, was increased. Interestingly, the BSH containing bacteria *Clostridium XlV* and *Lactobacillus* were reduced with the decreased conversion of primary bile acids to secondary bile acids. Furthermore, the bile acid receptor FXR-FGF15 axis was downregulated. Our results suggested that gut dysbiosis inhibited the bile acid metabolism, led to the accumulation of bile acids in enterocytes, and promoted tumorigenesis in the CAC model (Figure 7). Taken together, it will provide a new insight that gut dysbiosis and abnormal bile acid metabolism play a crucial role in CAC development.

Firmicutes and *Bacteroidetes* are the dominant phylum bacteria in the intestine. Our data revealed the decreased abundance of *Bacteroidetes* and increased *Firmicutes* in the CAC model. At the genus level, the *Lachnospiracea_incertae_sedis*, *Alistipes*, and *Odoribacter*, known as the short-chain fatty acids (SCFAs) producing bacteria, were decreased after AOM/DSS treatment. As a vital source of energy, SCFAs can provide energy for colonic epithelial cells. Simultaneously, they are responsible for epithelial barrier enhancement and gastrointestinal immunological regulation [34]. Therefore, the dysbiosis impaired the production and protective effect of SCFAs. Besides, previous studies had shown a

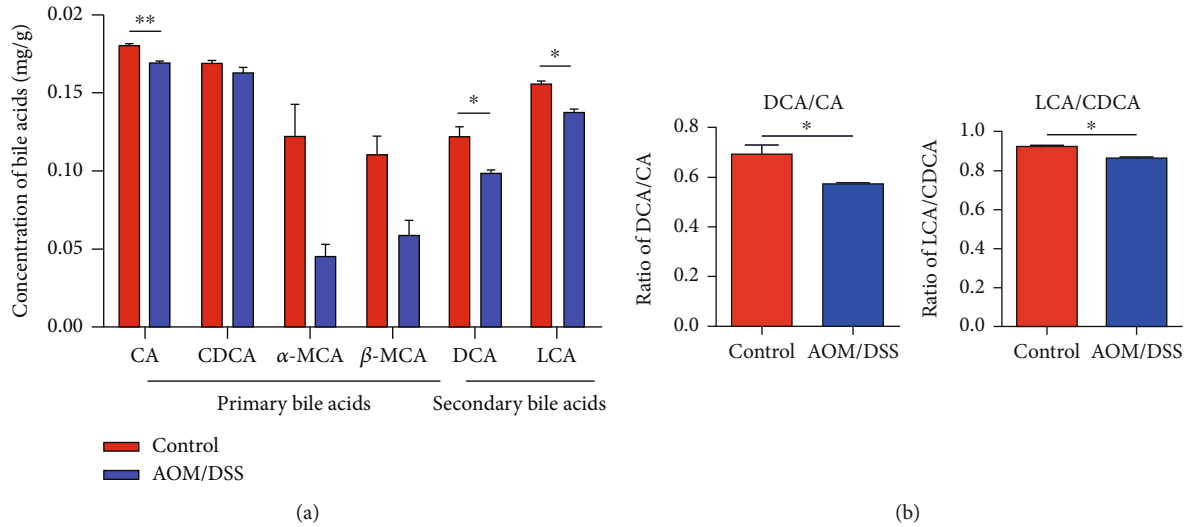

FIGURE 5: The bile acid profile alteration in feces after AOM/DSS treatment. (a) Levels of fecal bile acids including CA, DCA, and LCA were reduced in the AOM/DSS group. (b) The conversion of primary bile acids to secondary bile acids was reduced in the AOM/DSS group, as evidenced by a decreased ratio of DCA/CA and LCA/CDCA. $^{*}p < 0.05$, $^{**}p < 0.01$. $n = 5$ in each group.

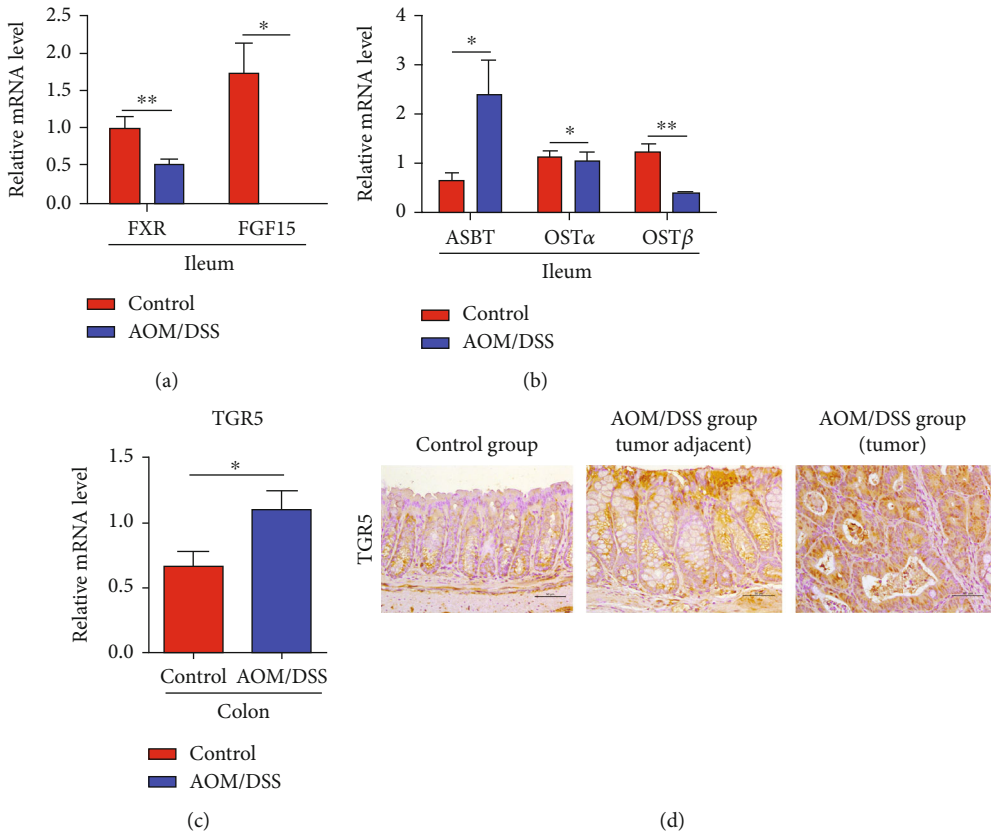

FIGURE 6: The expression of bile acid receptors in the colitis-associated cancer model. (a) The mRNA expression of ileum FXR and FGF15 was reduced in the AOM/DSS group. (b) Real-time PCR showed an increased expression of ASBT and a decreased level of OSTα and OSTβ in the ileum of the AOM/DSS group. (c) The mRNA expression of colon TGR5 was elevated in the tumor of the AOM/DSS group by Real-time PCR. (d) Immunohistochemical staining showed higher expression of TGR5 in mice with AOM/DSS treatment than in the control group. ASBT: apical sodium-dependent bile acid transporter. OSTα and OSTβ: organic solute transporter subunit α and β. Scale bars: 50 μm. $^{*}p < 0.05$, $^{**}p < 0.01$. $n = 8$-10.

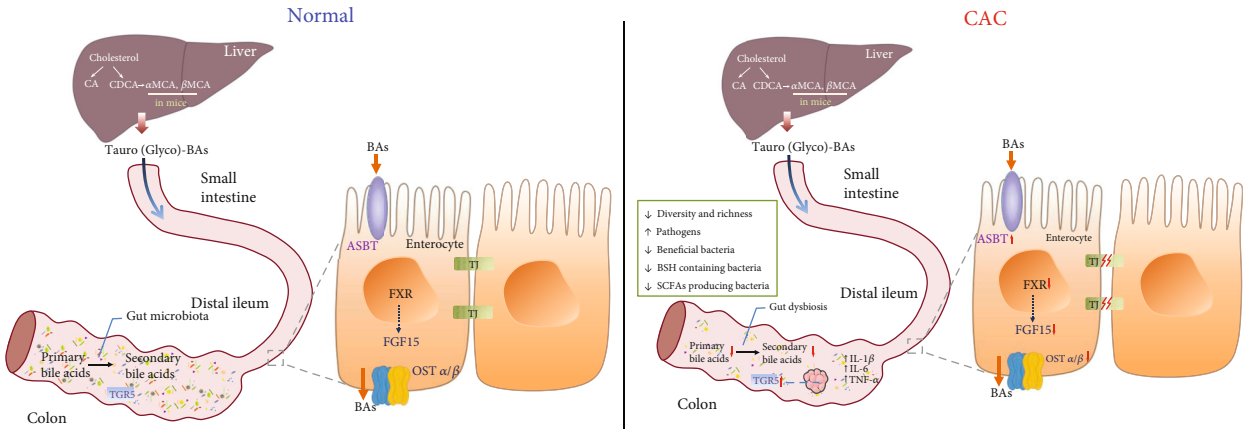

FIGURE 7: Gut dysbiosis and abnormal bile acid metabolism in colitis-associated cancer. Synthesized in the liver, bile acids are transported to the intestine in the form of conjugated bile acids. In the distal ileum, most of them are reabsorbed and conveyed to the liver in the presence of the FXR-FGF15 axis and bile acid transport receptors. In the colon, the gut microbiota promotes bile acid deconjugation and the conversion of primary bile acids to secondary bile acids. After AOM/DSS treatment, the BSH containing bacteria is reduced. Thus, dysbiosis inhibits bile acid metabolism and induces decreased secondary bile acids. Considering the bile acid receptors, the expression of FXR-FGF15 axis, OSTα, and OSTβ is decreased, while ASBT is increased, which limits the reabsorption of bile acids and leads to the accumulation of bile acids in enterocytes. The colon of the CAC model shows a severe inflammatory response, disrupted barrier function, and elevated expression of TGR5 in tumor tissues. BAs: bile acids; ASBT: apical sodium-dependent bile acid transporter; FXR: farnesoid X receptor; FGF15: fibroblast growth factor 15; TGR5: G-protein coupled receptor; OSTα and OSTβ: organic solute transporter subunit α and β; BSH: bile salt hydrolase; CAC: colitis-associated cancer; TJ: tight junction.

reduced abundance of *Alistipes* in IBD patients [35], and *Alistipes* was reported to play a role in alleviating colitis [36]. Thus, in the CAC model, the reduction of *Alistipes* might diminish its protective effect. On the contrary, the level of pathogens such as *Helicobacter* and *Streptococcus* increased. In addition, *Parabacteroides* and *Bacteroides* have been reported to have higher levels in CRC patients [37], which also remarkably increased in the CAC model in our study, suggesting that *Parabacteroides* and *Bacteroides* are involved in intestinal tumorigenesis of CAC. The above results indicated a pronounced increase in pathogens and reduction in beneficial bacteria during CAC progression.

We also observed a decreased output of fecal bile acids after AOM/DSS treatment. The ratios of DCA/CA and LCA/CDCA, which represent the conversion of primary bile acids to secondary bile acids, were also reduced. Secondary bile acids have been reported to have anti-inflammatory effects. For example, DCA can inhibit TNF-α production [38] and LCA can downregulate NF-κB activity in colon cells [39]. Additionally, DCA and LCA restrained the IL-8 secretion and exerted anti-inflammatory effects on Caco-2 cells [40]. Alternatively, genus *Clostridium XlV* and *Lactobacillus* were reduced in CAC. It is well known that *Clostridium XlV* and *Lactobacillus* have bile salt hydrolase (BSH) activity, and *Clostridium XlV* also possesses 7α-dehydroxylation and bile acid sulfatase activity [12, 13]. The reduction may account for the impaired ability of bile acid deconjugation, transformation, and desulfation activity in CAC. An intriguing study has similar results in IBD, which showed higher levels of fecal sulfated and conjugated bile acids in IBD patients than the healthy subjects [40]. Similarly, a recent study found the reduction of LCA and DCA and the relative overabundance of primary bile acids in IBD subjects by detecting the metabolomic profiles of stool samples [41]. Thus, the reduction of secondary bile acids might be one of the causes of CAC.

Bile acids can directly regulate gut microbiota or through the bile acid receptors FXR and TGR5 [42, 43]. Bile acids are regarded as FXR agonists, and the order of activation effect is CDCA > DCA > LCA > CA [44]. The reduction of DCA, LCA, and CA levels in our study led to the inactivation of FXR. Moreover, a decreased abundance of *Lactobacillus* caused the accumulation of conjugated bile acids, such as T-β-MCA, which has been reported as the FXR antagonist [45], so a high level of T-β-MCA may be involved in the decreased expression of FXR [46]. The downregulation of the FXR-FGF15 axis decreased the bile acid efflux transporters and affected the reabsorption of bile acids. In our study, the expression of FXR, OSTα, and OSTβ was decreased, while ASBT was increased, which resulted in the accumulation of bile acids in the enterocytes. These data revealed that abnormal bile acid metabolism was involved in CAC development. Several studies have found that the FXR mRNA expression is inversely correlated with CRC progression, and FXR deficiency increased the tumor load in $Apc^{min/+}$ mice and xenograft tumor model [47–49]. Moreover, mice lacking FXR showed disrupted intestinal epithelium integrity and an overgrowth of intestinal bacteria [50]. It has been found that FXR activation reduced intestinal inflammation and syndrome and improved intestinal mucosal barrier in the DSS-induced colitis model [51]. In esophageal and gastric adenocarcinoma, the expression of TGR5 elevates remarkably [25, 26]. Furthermore, TGR5 has been found increased in an inflammatory state of the colitis model and Crohn's disease patients [52, 53]. Moreover, TGR5 agonist has been reported to ameliorate colitis [54]. We found a higher level of TGR5 in the tumor tissues of CAC. Thus, targeting bile acid receptors FXR and TGR5 would be a promising approach against CAC.

5. Conclusion

These data suggested that gut dysbiosis might affect the bile acid metabolism during the development of CAC, and the reduced production of secondary bile acids with anti-inflammatory effects could promote tumorigenesis. Our study revealed a pivotal role of gut microbiota and bile acids in CAC progression, which may provide a new preventive strategy against CAC.

Data Availability

The related data underlying the findings of this research are freely available. And readers can make requests for access to these data to the corresponding author via email.

Disclosure

This study was presented as a poster in Digestive Disease Week 2019 and abstract in the *Journal of Digestive Diseases* in 2019.

Conflicts of Interest

There are no potential conflicts of interest regarding the publication of this paper.

Authors' Contributions

Li Liu, Min Yang, and Wenxiao Dong contributed equally to this work.

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Research Article

Colonic Transendoscopic Enteral Tubing: Route for a Novel, Safe, and Convenient Delivery of Washed Microbiota Transplantation in Children

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Aim. Colonic transendoscopic enteral tubing (TET) has been used for delivering fecal microbiota transplantation by washed preparation since 2015, which was recently named as washed microbiota transplantation (WMT). However, there are few reports available regarding the feasibility and safety of these studies in low-age population. This study is aimed at evaluating the safety, feasibility, and value of colonic TET in 3-7 years old children. *Methods*. All patients aged 3-7 years who underwent colonic TET in our center for WMT or medication were prospectively evaluated. The feasibility and safety of TET were evaluated. A questionnaire was completed by the children's parents to evaluate the children's response to the colonic TET as well as the parent's satisfaction. *Results*. Forty-seven children were included (mean age 5 years). TET was implemented into the colon of all the patients, and the success rate of the procedure was 100%. The median retention time of TET tube within the colon was 6 (IQR 5-7) days in 45 patients with tube falling out spontaneously, and the maximum retention time was up to 21 days. Multivariate analysis demonstrated that endoscopic clip number ($P = 0.009$) was an independent contributing factor for the retaining time of tube. With increase in the number of large clips, the retention time of TET tube was prolonged. No discomfort was reported during injection of the microbiota or medication suspension through the TET tube. During the follow-up, no severe adverse events were observed. All children's parents were satisfied with TET. Interestingly, the proportion of children's parents choosing TET as the delivery way of WMT increased from 29.79% before to 70.21% after TET ($P < 0.001$). *Conclusions*. This study, for the first time, demonstrates that colonic TET is a novel, safe, and convenient colonic delivery way for WMT and medication in children aged 3-7 years.

1. Introduction

The value of fecal microbiota transplantation (FMT) has grown exponentially in recent years. FMT has already been explored in the treatment of a variety of illnesses in children, other than recurrent *Clostridioides difficile* infection (CDI) [1], such as inflammatory bowel diseases (IBD) [2–4], allergic colitis [5], and gut-brain axis disease like autism [6] and epilepsy [7]. Along with studies on FMT in children, there are increasing number of studies highlighting the involvement of gut microbiota in various nongastrointestinal chronic disease like asthma, type 1 diabetes, Tourette's syndrome, etc. [8–10]. Similar to gut-brain-axis, another term called gut-skin-axis was recently termed for involvement of gut microbiota in skin disorders like atopic dermatitis [11, 12]. Although the evidence for FMT in children was mostly limited to case series and individual reports, FMT in pediatrics is important and promising.

The improved methodology of FMT based on the automatic washing process [13] and the related delivering consideration was coined as washed microbiota transplantation (WMT) by the consensus statement from the FMT-standardization Study Group in 2019 [14]. However, to deliver WMT in low-age children is more challenging than

in adults, especially for those who are chronically ill and mentally immature, such as IBD and autism patients. There are three routes of delivering WMT, i.e., the upper gut, midgut, and lower gut [15, 16]; each method has its advantages and its limitations. Depending on the age, simple oral capsule administration is convenient for older children and adolescents but may not be feasible for young children [17]. Importantly, asphyxia may occur in children by oral capsules. WMT via colonoscopy is a typical choice, but patients cannot endure frequent bowel preparation and colonoscopy over a short period of time. Enema is an easy way of delivering fecal microbiota, but the access only arrives at the rectum and the sigmoid colon, making it difficult for children to hold the delivered microbiota for enough time. Therefore, in order to meet the needs of patients with multiple fresh WMTs or whole-colon administration of medications with one to two weeks, we developed a colonic delivery method for long-term maintenance of an indwelling, colonoscopically placed transanal enteral tube, which was called colonic transendoscopic enteral tubing (TET) [15, 18].

TET as a procedure has been reported as a safe and convenient procedure for multiple WMTs and colonic medication administration with a high degree of satisfaction among adult patients [15, 19–22]. The TET device (FMT Medical, Nanjing, China) was approved by National Medical Products Administration for endoscopic use since 2017. Allegretti et al. states that the TET is considered as a promising approach for FMT [23]. Recently, colonic TET has been recommended by the latest consensus from FMT-standardization Study group in Asia in 2019 [14] and an international FMT expert group in 2020 [24]. This method may be less psychologically challenging for patients than delivery of WMT via the upper and middle gut. The recent study reported that two to four large endoscopic clips could be recommended to maintain the TET tube within the colon for over 7 days in adults [18]. Our recent randomized controlled trial indicates that cap-assisted colonoscopy can reduce the time of second incubation of colonoscope in those colonoscopies with difficulty and decrease abdominal pain during endoscopy [25]. However, there were few data available regarding the feasibility and safety of these studies in low-age population. This study is aimed at evaluating the safety and feasibility of using colonic TET in pediatric patients aged 3-7 years, as well as evaluation of the possible affecting factors on the procedure. Furthermore, the perception and response of the children's parents related to the different delivery way of WMT have been assessed.

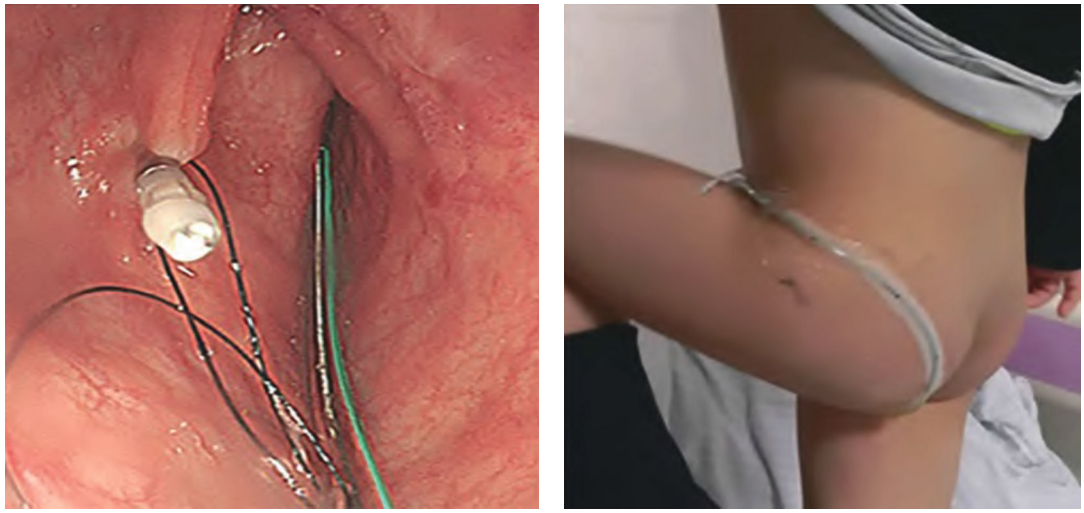
2. Patients and Methods

2.1. Patients. A prospective observational study was conducted at the Second Affiliated Hospital of Nanjing Medical University from May 2017 to January 2020. All patients met the inclusion criteria: age 3 to 7 years, suitability for endoscopy, and with parents' consent to undergo WMT and TET for children's diseases. Patients were excluded if they had severe intestinal stenosis, fistula, and risk of perforation during endoscopy; complication with serious anus lesions which might affect endoscopy; and no proper mucosa for endoscopic tissue clip fixation, the parents of the patients disagreed for the survey, or lost contacts. This study was approved by the Institutional Ethical Review Board of the Second Affiliated Hospital of Nanjing Medical University (2015KY042).

2.2. Colonic TET Procedure. Regular colonoscopy, using a colonoscope with working channel diameter ≥ 3.2 mm, was performed under intravenous anesthesia. After complete evaluation of the colon, a soft TET tube (outer dimeter 2.7 mm, FMT medical, China) was inserted into the colon via the paraffin-lubricated colonoscope channel. Once the TET tube reached the target location (such as cecum), the colonoscope was carefully withdrawn, while keeping the tube in place. Then, the colonoscope was reinserted up to the target location, and the tube was fixed onto the wall with 1-4 endoscopic clips (ROOC-D-26-195-C, ≥10 mm, Nanjing Microtech Co.; HX-610-135 L, 135°, Olympus) along the three sites (named "the first site," "the second site," and "the third site," each separated by 10 cm) on the distal part of the tube (Figure 1(a)). Generally, 1-2 clips at the first site and 0-2 clips at the second and/or the third site (as possibly required) were used. The location and number of the clips used for fixing the tube were chosen based on the mucosal folds, disease severity, and the duration for which the tube needs to be retained. The tube was secured with a medical tape on the right hip for easy access during the WMT administration (Figure 1(b)). The TET device was approved by China National Medical Products in 2017. The number, type, and location of the clips and procedure-related adverse events (AEs) were recorded for every patient. The TET tube retention time and method of tube expulsion were also recorded for statistical analysis.

2.3. WMT or Medication Delivery. Based on our previous reports on donor screening protocol for donors and automatic purification system (GenFMTer, FMT Medical, Nanjing, China) for microbiota from donated stool in a special lab [22] and the one-hour WMT protocol for WMT [7], the fecal microbiota suspension or medication suspension was delivered into the colon through TET tube. The right lateral position is recommended when delivering WMT or medication (such as mesalazine suspension). The microbiota (15-50 mL of suspension according to age in 1-2 min) or medication (e.g., mesalazine) solution should be injected at the temperature of 37°C. Patients are recommended to lay in 10° Trendelenburg position for 30 minutes after infusion and then in the supine position in order to prolong the retention of the infused fluid [19]. About 5 mL of saline is used to flush the tube after infusion. Retention of the microbiota suspension for over 1 hour indicates successful delivery of the microbiota through colonic TET.

2.4. Questionnaire. A questionnaire (Supplementary file available here), also approved by the Institutional Ethical Review Board of our hospital, was retrospectively given to the parents to evaluate their perspectives on colonic TET before and after the procedure, as well as to evaluate their



(a) (b)

Figure 1: The procedure of colonic transendoscopic enteral tubing (TET). Under endoscopic guidance, the TET tube was fixed onto the mucosal fold of the colon with endoscopic clips (a). Nonrestricted leg movement of a 3-year-old child with a TET tube fixed onto the hip (b).

children's responses to the colonic TET. The preferred delivery way of WMT before and after the procedure, parents' concerns prior to the procedure, parents' satisfaction, postprocedural change in motility/activity of the child, and child's toleration for TET were noted. Overall behavior of the patients was evaluated based on the parent's description. Among the five options offered, gastroscopy, colonoscopy, midgut TET, colonic TET, and enema, the parents were further asked which transplant route they preferred.

2.5. Clinical Evaluation of Colonic TET. The purpose, the success rate of the procedure, the fixation location, and the retaining time of the TET tube, as well as the type and number of endoscopic clips used were recorded. The retaining time is defined as the time from the implantation to natural shedding of the TET tube. Adverse events and the parents' satisfaction during and after TET were also recorded. Safety was evaluated in all patients by recording adverse events throughout long-term follow-up using the China microbiota transplantation system (http://www.fmtbank.org).

3. Statistical Analysis

The data were analyzed by using SPSS 21.0 (Chicago, IL, USA). Continuous variables were expressed using median and interquartile range. Categorical variables were summarized using absolute numbers and percentages. When the normality of the distribution of variables was acceptable, independent sample t-test was used. Comparisons of categorical variables between groups were performed using the chi-squared test. The relation between the retaining time and the endoscopic clips was evaluated using univariate and multivariable logistic regression analysis. A value of $P < 0.05$ (two-tailed) was considered significant.

Table 1: Characteristics of 47 patients who underwent colonic TET.

Items	Results
Patients, *n*	47
Age, years, median (IQR)	5 (4–6)
Gender, male, *n* (%)	42 (89.36%)
Disease type, *n* (%)	
Autism	21 (44.68%)
Ulcerative colitis	6 (12.77%)
Clostridioides difficile infection	2 (4.26%)
Crohn's disease	1 (2.12%)
Others*	17 (36.17%)
Disease duration, years, median (IQR)	2 (1–3.5)
Success rate of TET, %	100%
Location for fixing distal tube, *n* (%)	
Ileocecal	29 (61.70%)
Transverse colon	12 (25.53%)
Ascending colon	6 (12.77%)
Endoscopic clip type, *n* (%)	
Small endoscopic clip	12 (25.53%)
Large endoscopic clip	35 (74.47%)
Retaining time of TET tube, days, median (IQR)	6 (5-7)
Removal of tube, *n* (%)	
Naturally fell out	45 (95.74%)
Actively pulled out	2 (4.26%)
Satisfaction, %	100%
Purpose of TET, *n* (%)	
WMT	45 (95.74%)
WMT and medical administration	2 (4.26%)

WMT: washed microbiota transplantation; TET: transendoscopic enteral tubing. *Four cases with constipation, four with antibiotics-related dysbiosis, three with epilepsy, two with Tourette syndrome, two with atopic dermatitis, and two with allergic colitis.

Table 2: Univariate analysis for the retaining time of TET tube.

Items	Total	Short-retaining (≤6 days)	Long-retaining (>6 days)	*P* value
Patients, *n*	45	29	16	—
Gender, male, *n*	40	27	13	0.226
Age, years, mean ± SD	5.36 ± 1.25	5.59 ± 1.12	4.94 ± 1.39	0.196
Disease duration, years, median (IQR)	2 (1–3.5)	2 (1–4)	1.5 (1–3)	0.176
Fixed position	45	29	16	0.277
Ileocecal	28	18	10	
Nonileocecal	17	11	6	
Endoscopic clip type	45	29	16	0.222
Large endoscopic clip	33	23	10	
Small endoscopic clip	12	6	6	
Endoscopic clip number	2 (1.75-3)	2 (1–2)	3 (2–4)	0.006

SD: standard deviation; IQR: interquartile range.

4. Results

4.1. Characteristics of Patients. A total of 47 patients were included in this prospective study: 42 males and 5 females aged 3 to 7 years. As shown in Table 1, 45 (45/47, 95.74%) patients used TET for multiple WMTs and two (2/47, 4.26%) for WMT and intracolonic medication administration.

4.2. Feasibility of Colonic TET in Children. The colonic TET was successful performed in all 47 cases (100%). In 29 cases (61.70%), the tip (closed to mouth direction) of the TET tube was fixed in ileocecal region, transverse colon in 12 patients (25.53%), and the ascending colon in 6 patients (12.77%). Large clips were used on the sites of the TET tube in 35 cases during our preliminary observational period, 11 cases had one clip, 19 had two clips, three had three clips, and two had four clips. In the remaining 12 cases, small clips were used on the sites. In all cases, WMT or medication administration through colonic TET was successful. Two patients with UC were injected with mesalazine and steroids, respectively, through the TET tube after WMT until the TET tube fell off. After the treatment was completed, the TET tube naturally shed off in 45 patients (95.74%), and the median retaining time was 6 (IQR 5–7) days. The maximum retention time of the TET tube was up to 21 days.

4.3. Analysis on Retention Time of TET Tube. Of all the patients, 45 patients experienced natural expulsion of the TET tube. They were divided into the short-retaining time group (≤6 days) and the long-retaining time group (>6 days), considering 6 days as median retention time. As shown in Table 2, significant difference was observed between TET retaining time and the endoscopic clip number ($P = 0.006$) in the univariate analysis. Multivariate analysis demonstrated that only endoscopic clip number ($P = 0.009$) was an independent factor for affecting the retaining time. In patients with large endoscopic clips, we found that the number of endoscopic clips used significantly affected their retaining time ($P = 0.006$) (Table 3). In patients with small endoscopic clips, the retaining time of the TET tube significantly increased with the increased number of endoscopic clips ($P = 0.025$).

Table 3: Correlation between the endoscopic clip number and TET retaining time.

	Endoscopic clip number	*N*	TET retaining time	*P* value
Small endoscopic clip	3	8	6 (5-7)	
	4	4	8 (5.5-15)	0.025
Large endoscopic clip	1	11	5 (4-6)	
	2	17	6 (6-7)	
	>2	5	8 (7-10)	0.006

4.4. Optimal Methods of Performing WMT. Among the five options offered for delivering WMT, the parents of the pediatric patients were asked which route of transplantation they would have preferred before and after the TET procedure. All the delivering ways were explained to the parents in detail, along with the pros and cons of each procedure. As shown in Figure 2, the most preferred choice for delivery of WMT before the procedure was enema (51.06%). This was obvious, given that enema is the least invasive procedure. Whereas after the colonic TET procedure, the colonic TET was the most preferred choice (70.21%) for the parents. The percentage of the first choice for colonic TET after the TET procedure was much higher than that before the TET procedure (29.79% vs. 70.21%, $P < 0.001$). Meanwhile, there were no parents who changed from the original acceptance attitude for colonic TET to not accepting it.

4.5. Safety and Satisfaction of the Colonic TET. During injection of the washed microbiota or medication suspension, through the TET, no mild to severe abdominal pain or diarrhea was reported. No severe AEs were observed during and after colonic TET. Among all patients with colonic TET, four parents (8.51%) complained that the tube affected their children's activities significantly during its retention period, and they (three of them were 3 years old) could not tolerate this change. This discomfort was largely due to the patients being too young, so we classified it as mild adverse events, definitely related to TET. All parents (100%) were satisfied with the colonic TET.

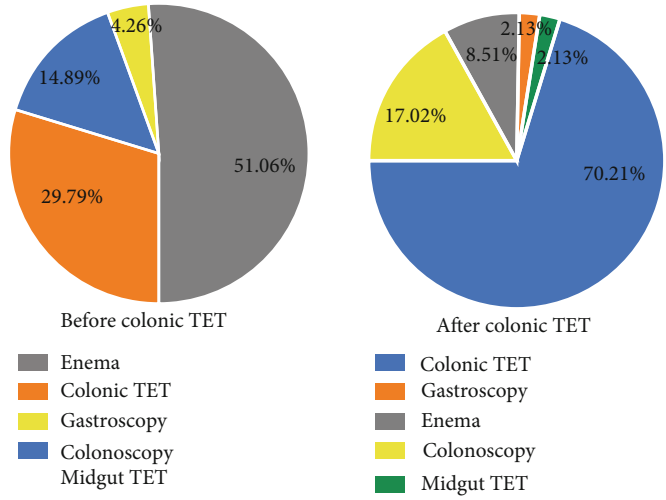


Figure 2: The most preferred delivery way of washed microbiota transplantation (WMT) by the children's parents before and after the procedure.

5. Discussion

WMT has shown a promising prospect for the treatment of dysbiosis-related diseases in children, but it is more challenging for them to undergo WMT or whole-colon medication. When compared to the adult population, repeated anesthesia, endoscopy, or enema, within short intervals, put children at greater risk; hence, we urgently need to explore a more convenient and safe delivery method. Colonic TET, as a new approach for colon-targeted drug delivery, was published for the first time in 2016 and has since been used in hospitals in China mainland [15, 19, 26–28] and China Taiwan since then [20]. In the present study, TET and WMT were successfully performed in all cases, and WMT or medicine retention time was longer than 1 hour. This indicates that colonic TET should be a feasible procedure in children.

In the present study, we found that the retaining time of colonic TET tube was significantly correlated with the number of endoscopic clips in children. Our results showed prolonged retention time of the TET tube with the increase of the number of large endoscopic clips. The retention time of the TET tube is related to the clinician's decision on the patient's condition. When multiple WMTs or a long-term intracolonic administration of medications is required, the TET tube should be retained for as long as possible, and it should be fixed with more endoscopic clips. However, the relationship between the type and number of endoscopic clips and the retention time should be evaluated in a larger sample size.

In previous studies, oral capsules or repeated endoscopic operation was the options for WMT in children [6, 29]. However, because of their young age and the psychological impact of long-term illness, it is difficult for children to cooperate with doctors to complete treatment. They cannot tolerate repeated invasive operation and swallow too many capsules. In a recent study about FMT-related adverse events, colonic TET was the route with the lowest incidence of delivery-related adverse events, at 6% [30]. In comparison, the incidence of delivery-related adverse events with FMT capsules was 29% [30]. Capsulized FMT has helped to overcome concerns of invasive administration but not other drawbacks [23], such as biting capsule, aspiration into trachea, and difficulty for taking too much. Thus, more research for capsulized FMT is required. Moreover, the effectiveness from a single WMT might be limited in severe and refractory microbiota-related conditions [22]. The colonic TET solves the limitations of the WMT input pathway to some extent [15]. It can not only complete multiple WMT treatments but also can be used for whole colonic administration of medication, avoiding intestinal injury and bleeding caused by repeated insertion of the enema tube or colonoscopy. And this is the only way which could be used for delivering medication while covering the whole colon, and there are no other methods which could be used for comparisons.

One of the major concerns of pediatricians about the use of TET techniques in children relates to their safety. Ding et al. reported that the FMT-related AEs associated with using colonic TET as the delivery method were lower when compared with the midgut [19]. Importantly, the latest systematic review showed that colonic TET was the pathway with the lowest incidence of delivery-related adverse events, compared with colonoscopy, enema, capsule, midgut tube, and gastroscopy [30]. In the present study, TET and WMT were successfully performed in all 47 cases (100%), and no severe TET-related complications occurred. The particles-caused tube obstruction was reported in another pilot study while delivering manually prepared fecal suspension [20]. However, there was no tube obstruction during WMT in the present study.

It should be emphasized that the TET tube does not affect the daily life of patients. Previous study in our center reported that 98.1% of adult patients were satisfied with WMT through TET [15]. In the present study, although some of the children's activities were restricted by TET, all parents were satisfied with TET. The preference for colonic TET became the first choice after the TET procedure,

showing that the parents experienced no difficulty in handling their children with a colonic TET tube. Although there is no single best universal delivery method that matches all patients, the choice made should be patient-specific. When considering the delivery route of WMT in children, disease condition, aesthetic factors, psychology, convenience, and pain should be considered much more carefully than adults during the entire workflow [22]. Though bowel preparation may be slightly difficult among children, than adults, but is comparatively less of a mental burden than the existing chronic disease that affects their daily life.

To the best of our knowledge, this is the first study to survey the safety and feasibility of colonic TET in children. This study does, however, have some limitations. First, the sample size of this pilot study was too small for comparison of the retention time of colonic TET among different diseases, but a larger prospective study based on these preliminary results is ongoing. In addition, this study did not evaluate clinical responses to whole-colon administration compared with other traditional treatments that will be a part of our future studies.

6. Conclusions

In conclusion, this article, for the first time, reports the use of colonic TET tube in 3-7 years old children. The results demonstrate that the novel concept of colonic TET is a feasible, practical, and safe technique for multiple WMTs or frequent colonic medication administration, with a high degree of parents' satisfaction. The results highlight the significance of colonic TET as a technique for colon-targeted medication delivery in pediatric patients. The use of colonic TET is opening a new era of whole colonic administration with reducing the stress of physicians, patients, and their families.

Data Availability

The questionnaire data used to support the findings of this study are included within the supplementary file.

Conflicts of Interest

Faming Zhang conceived the concept of GenFMTer and transendoscopic enteral tubing and related devices. Other authors declare that they have no competing interest.

Authors' Contributions

MZ and HB designed the study, included the patients, analyzed the data, and drafted the manuscript. QW, CL, and BC joined the clinical management. FZ designed the study and revised this article. Min Zhong and Heena Buch contributed equally to this study.

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Supplementary Materials

A supplementary file is submitted separately, containing the questionnaire results. *(Supplementary Materials)*

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Review Article

Fecal Microbiota Transplantation: A New Therapeutic Attempt from the Gut to the Brain

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Gut dysbacteriosis is closely related to various intestinal and extraintestinal diseases. Fecal microbiota transplantation (FMT) is a biological therapy that entails transferring the gut microbiota from healthy individuals to patients in order to reconstruct the intestinal microflora in the latter. It has been proved to be an effective treatment for recurrent *Clostridium difficile* infection. Studies show that the gut microbiota plays an important role in the pathophysiology of neurological and psychiatric disorders through the microbiota-gut-brain axis. Therefore, reconstruction of the healthy gut microbiota is a promising new strategy for treating cerebral diseases. We have reviewed the latest research on the role of gut microbiota in different nervous system diseases as well as FMT in the context of its application in neurological, psychiatric, and other nervous system-related diseases (Parkinson's disease, Alzheimer's disease, multiple sclerosis, epilepsy, autism spectrum disorder, bipolar disorder, hepatic encephalopathy, neuropathic pain, etc.).

1. Introduction

The gut microbiota is often considered an "invisible organ" that significantly affects human health and disease. More than 100 trillion microorganisms have been found in the human gastrointestinal (GI) tract, which encodes close to 3,000,0000 genes compared to the 23,000 genes within the human genome [1, 2], and are crucial for maintaining the balance between different physiological activities. The cross-talk between the GI tract and the central nervous system, commonly known as the gut-brain axis, plays an important role in the pathophysiology of neurological diseases. Studies increasingly show that dysbacteriosis can lead to or exacerbate various neurological and psychiatric disorders, such as Parkinson's disease [3, 4], Alzheimer's disease [5–7], autism spectrum disorder [8], multiple sclerosis [9], and epilepsy [10]. In addition, patients with neurological dysfunction often present GI symptoms [11], which underscores the causative role of the gut in neuropathological progression and provides a solid rationale for therapeutically targeting the gut microbiota in these diseases (Table 1). The current therapies targeting the intestinal microbiota include the use of antibiotics, probiotics, prebiotics, synbiotics, and fecal microbiota transplantation (FMT) that entails transplanting functional microbiota from healthy individuals into the GI tracts of patients. FMT can reconstruct the healthy gut microecology and improve clinical symptoms. Apart from its direct therapeutic effect in GI diseases, FMT has also been shown to improve neurological and psychological symptoms by modulating the gut-brain axis (Figure 1) [12]. In this review, we have summarized the gut microbiota in different nervous system diseases as well as the current applications of FMT in various neurological and psychiatric diseases and discussed the potential mechanisms and future directions.

2. Neurological Diseases

Studies [46–48] show that the GI tract and resident microbiota are susceptible to the neurological dysfunction associated with Parkinson's disease, Alzheimer's disease, multiple

Table 1: Characteristics, consequences, and application level of FMT in neuropsychological diseases.

Disease types	Alterations of gut microbiota	Altered substances caused by microbial dysbiosis	Application level of FMT	References
Neurological diseases				
Parkinson's disease	Increase in *Verrucomicrobiaceae*, *Ruminococcaceae*, *Proteobacteria*, *Clostridiaceae*, *Enterobacteriaceae*, *Bifidobacteriaceae*, *Lactobacillaceae*, *Pasteurellaceae*, *Christensenellaceae*, *Lactobacilli*, *Akkermansia*, *Ralstonia* Decrease in *Firmicutes*, *Prevotellaceae*, *Coprococcus*, *Bacteroides fragilis*, *Blauti*, *Roseburia*, *Faecalibacterium*	α-Synuclein, LPS, SCFAs, hydrogen production	Patient & animal	[4, 13, 14]
Alzheimer's disease	Increase in *Escherichia*, *Shigella*, *Chlamydia pneumoniae*, *Borrelia burgdorferi*, *Treponema pallidum*, *Burkholderiaceae*, *Staphylococcaceae*, *Porphyromonas gingivalis*, *Propionibacterium acnes* Decrease in *Eubacterium rectale*, *Bacteroides fragilis*	Inflammatory cytokines (IL-6, CXCL2, NLRP3, IL-1β, IL-10), Aβ, GABA, BDNF, DHA	Patient & animal	[5, 6, 13, 15]
Multiple sclerosis	Increase in *Firmicutes*, *Clostridium*, *Escherichia Shigella* Decrease in *Bacteroides*, *Faecalibacterium*, Eubacterium rectale, *Corynebacterium*, *Fusobacteria*	Proinflammatory cytokines, butyrate, lipid 654	Patient & animal	[13]
Epilepsy	Increase in *Firmicutes*, *Proteobacteria*, *Clostridium*, *Cronobacter*, *Akkermansia*, *Ruminococcus*, *Coprobacillus*, *Clostridium XVIII*, *Atopobium*, *Holdemania*, *Dorea*, *Saccharibacteria*, *Delftia*, *Paraprevotella*, *Gemmiger*, *Neisseria*, *Coprococcus*, *Fusobacterium*, *Methanobrevibacter*, *Phascolarctobacterium*, *Roseburia* Decrease in *Bacteroidetes*, *Actinobacteria*, *Prevotella*, *Bifidobacterium*	Proinflammatory cytokines (TNFα, IL-6, IL-1β), dopamine receptors D1 and D2	Patient & animal	[16, 17]
Tourette Syndrome	Increase in *Bacteroidetes*; in particular, *Bacteroides*, *Odoribacter*, and *Oscillospira* were identified as potential microbial biomarkers	SCFAs, D-alanine, tyrosine, dopamine	Patient & animal	[18]
Myalgic encephalomyelitis/chronic fatigue syndrome	Increase in *Roseburia*, *Holdemania*, *Enterococcus*, *Streptococcus spp.* Decrease in most *Bacteroidetes* genera.	Lactic acid, LPS, LPS-binding protein, soluble CD14, oxidative stress	Patient & animal	[19–21]
Guillain-Barré Syndrome	*Campylobacter jejuni* infection is associated with GBS while *Enterococcus faecalis* as a potential protective role	LPS, peripheral nerve gangliosides	Animal	[22, 23]
Stroke	Decreased neuronal injury and improved cognitive performance were observed in diabetic mice with bilateral common carotid arteries occlusion after receiving *Clostridium butyricum*	Trimethylamine N-oxide	Animal	[24, 25]
Amyotrophic lateral sclerosis	Increase in *Dorea* Decrease in *Butyrivibrio fibrisolvens*, *Firmicutes*, *Peptostreptococcus*, *Escherichia coli*, *Oscillibacter*, *Anaerostipes*, *Lachnospira*	Butyrate	Animal	[26–28]
Huntington's disease	Increase in *Bacteroidetes* Decrease in *Firmicutes*, *Lachnospiraceae*, *Akkermansiaceae*	Methionine, glycine	Animal	[29, 30]

Table 1: Continued.

Disease types	Alterations of gut microbiota	Altered substances caused by microbial dysbiosis	Application level of FMT	References
Psychiatric diseases				
Autism spectrum disorder	Increase in *Bacteroides*, *Barnesiella*, *Clostridium*, *Roseburia* Decrease in *Bifidobacterium*, *Coprococcus*, *Dialister*, *Faecalibacterium*, *Prevotella*, *Streptococcus*	Butyrate, lactate	Patient & animal	[31–33]
Bipolar disorder	Increase in *Bacteroidetes*, *Actinobacteria*, *Coriobacteria*, *Lachnospira*, *Enterobacteriaceae*, *Flavonifractor* Decrease in *Firmicutes*, *Ruminococcaceae*, *Roseburia*, *Faecalibacterium*, *Coprococcus*	Butyrate	Patient & animal	[34–37]
Depression	Increase in *Enterobacteriaceae*, *Prevotella*, *Klebsiella*, *Alistipes* Decrease in *Lachnospiraceae*, *Faecalibacterium*, *Coprococcus*, *Dialister*, *Ruminococcus*, *Lactobacillus*, *Bifidobacterium*	Butyrate, inflammatory cytokines	Patient & animal	[36, 38, 39]
Anxiety	Increase in *Fusobacterium*, *Ruminococcus*, *Escherichia Shigella* Decrease in *Faecalibacterium*, *Eubacterium*, *Sutterella*		Animal	[40, 41]
Other system-related neurological diseases				
Hepatic encephalopathy	Increases in *Enterobacteriaceae*, *Streptococcaceae*, *Porphyromonadaceae*, *Staphylococcaceae*, *Enterococcaceae* Decrease in *Lachnospiraceae*, *Ruminococcaceae*, *Rikenellaceae*, *Clostridium XIV*, *Phascolarctobacterium*	Ammonia, urease, SCFAs, aromatic amino acids	Patient & Animal	[42, 43]
Neuropathic pain	Associated: *Lactobacillus fermentum KBL374* & *KBL375*, *Bacteroides fragilis*, *Escherichia coli*, *Lactobacillus*, *Streptococcus* spp., *Enterococcus* spp., *Corynebacterium glutamicum*, *Peptostreptococcus*, *Clostridium sporogenes*	LPS, bacterial flagellin, indole, SCFAs, PUFAs, BAs	Patient & animal	[44]
Sepsis-associated encephalopathy	Associated: absence of anaerobes, including *Staphylococcus species* and *Escherichia coli*, with CDI, high relative abundance of pathogenic gram negatives, and *Enterococci*	LPS, SCFAs, BAs	Patient & animal	[45]

LPS: lipopolysaccharide; SCFAs: short-chain fatty acids; IL-6: interleukin-6; CXCL2: C-X-C motif chemokine ligand 2; NLRP3: recombinant NLR family, pyrin domain containing protein 3; IL-1β: interleukin-1β; IL-10: interleukin-10; Aβ: amyloid β-protein; GABA: γ-aminobutyric acid; BDNF: brain-derived neurotrophic factor; DHA: docosahexaenoic acid; TNFα: tumor necrosis factor-α; PUFAs: polyunsaturated fatty acid; Bas: bile acids.

sclerosis, epilepsy, and stroke. The gut-brain axis is adversely affected by the destruction of intestinal epithelial barrier, loss of intestinal neurons, and overproduction of proinflammatory cytokines. In addition, gut microbial abundance and diversity undergo significant changes during neurological disorders, especially that of bacteria producing anti-inflammatory factors. FMT can significantly adjust the richness of intestinal species and restore the proportion of anti-inflammatory bacteria and is therefore increasingly being considered for treating diseases of the nervous system (Table 2).

2.1. Parkinson's Disease. Parkinson's disease (PD) is a progressive neurodegenerative disease characterized by accumulation of Lewy bodies [69]. PD patients often present with GI symptoms such as constipation [70]. According to the theory of intestinal origin of PD, a prion-like neurotrophic protein is misfolded into α-synuclein (α-syn) and transported from the GI tract to the central nervous system (CNS) [71]. Studies on the mouse model of PD have confirmed that α-syn can indeed be transferred from the gut to the brain by crossing the blood-brain barrier [72]. Consistent with this, several studies [3, 4, 73, 74] have reported considerable differences between the gut microbial composition and metabolites of healthy individuals and PD patients. Scheperjans et al. [73] compared the fecal microbiome of PD patients with that of 72 healthy controls and detected 77.6% lower prevalence of *Prevotellaceae* in the former. In addition, PD patients' postural instability and gait difficulty were positively associated with the higher abundance of *Enterobacteriaceae*, suggesting a causative association between the microbiota-gut-brain axis

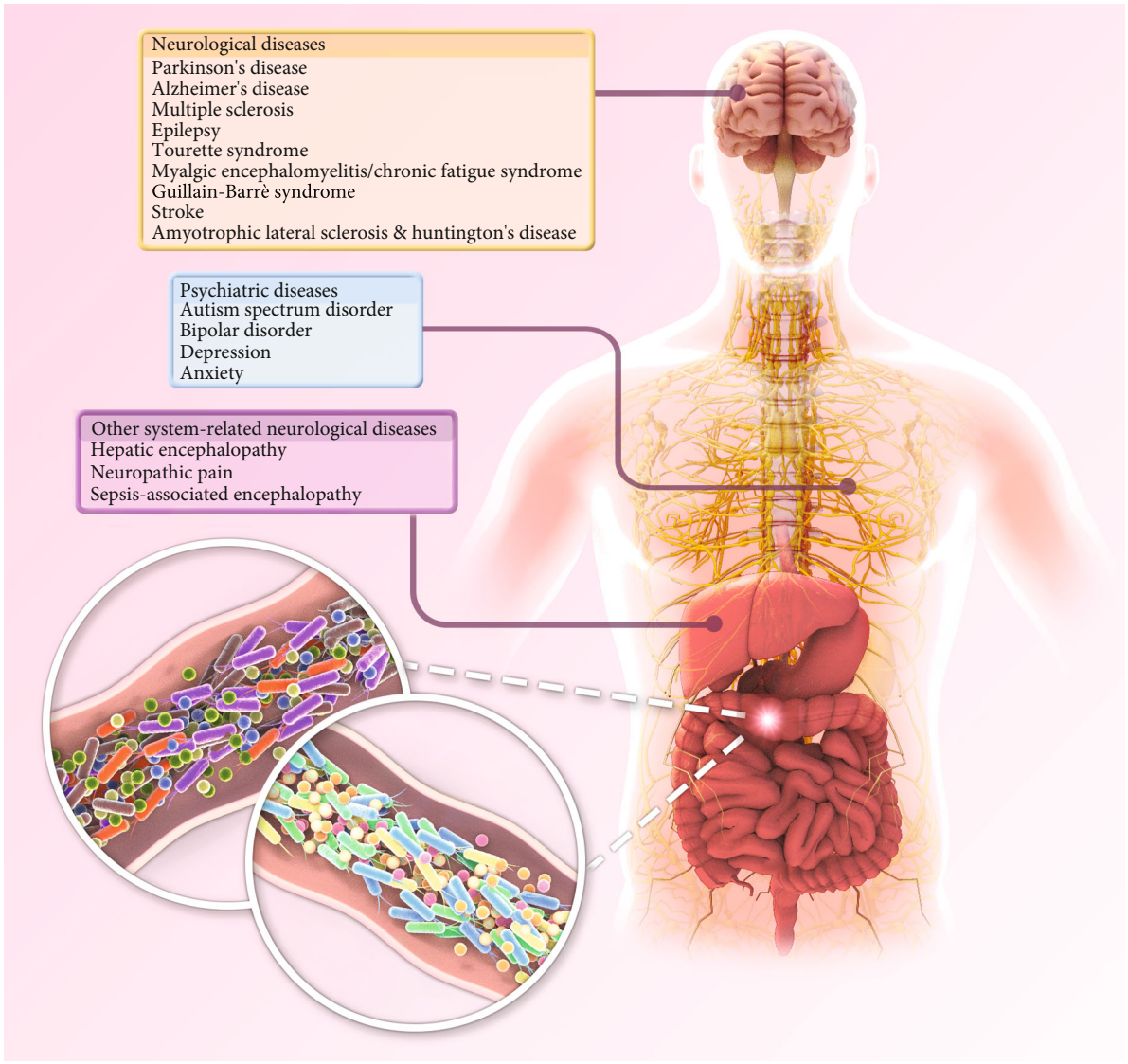

Figure 1: Current applications of FMT in various neurological and psychiatric diseases. Normal gut microbiota plays an important role in maintaining the functional stability of the gut-brain axis. Excessive reproduction of pathogenic bacteria or reduction of probiotics can lead to gut microbiota disorder and mediate a variety of neurological and psychological diseases. As an important therapeutic method to reconstruct gut microbiota, FMT has been tried to be applied to a variety of diseases related to gut-brain axis.

and progression of disease. Furthermore, Keshavarzian et al. [4] observed a greater proportion of LPS-producing proinflammatory bacteria (e.g., *Ralstonia*) and fewer bacteria producing the anti-inflammatory short-chain fatty acids (SCFAs) (e.g., *Blautia*, *Coprococcus*, *Roseburia*, and *Faecalibacterium*) in the gut of PD patients. Studies [75, 76] have shown that L-dopa can be metabolized into dopamine by gut microbial tyrosine decarboxylase, which is not easily affected by aromatic amino acid decarboxylase inhibitors such as carbidopa. In addition, the germ-free α-syn overexpressing (ASO) mice exhibited less severe motor and digestive symptoms (constipation), as well as lower microglia activation compared to their SPF counterparts [77], indicating that the gut microbiota is directly involved in PD's development.

Consistent with the above, Sun et al. [78] showed that FMT from healthy mice significantly improved the motor function in PD mice by mitigating intestinal inflammation and neuroinflammation and increasing the levels of dopamine and 5-hydroxytryptamine. The anti-inflammatory effects were mediated via TLR4/bk1/NF-κB/TNF-α pathway blockade, reduced activity of microglia and astrocytes, and increased producing SCFAs. In contrast, FMT from PD mice had a pathological effect on healthy recipients. Huang et al. [49] recently reported that three rounds of FMT over a period one week improved constipation and motor symptoms such as leg tremors in a PD patient. However, the tremors recurred 2 months after FMT, whereas constipation was relieved even after 3 months.

2.2. Alzheimer's Disease. Alzheimer's disease (AD) is a neurodegenerative disease characterized by cognitive decline due to the loss of neurons and synapses following deposition of neurofibrillary tangles (NFT) and misfolded amyloid β (Aβ) protein plaques [79]. Several studies [5, 6] have shown

Table 2: Clinical application for FMT based on the gut-brain axis.

Disease type	Studies	Study type	*N*	Location	Age	Sex	Complication	Administration route	FMT frequency	Donor	Clinical outcome
Neurological diseases											
Parkinson's disease	Huang et al. [49]	Case report	1	China	71	M	PD, constipation	TET tube (colon)	3 times	Healthy volunteer	Constipation cured, PD symptoms relieved for 2 months
Alzheimer's disease	Hazan [50]	Case report	1	USA	82	M	AD, rCDI	Colonoscopy	Once	His wife	MMSE score increased from 20 to 29
Multiple sclerosis	Borody et al. [51]	Case series	3	Australia	30/29/80	M/M/F	MS, constipation, vertigo, impaired concentration/MS, constipation/MS, constipation, proctalgia fugax, difficulty in walking	NA	5 FMTs/10 FMTs/5 FMTs	NA	Constipation resoluted, MS improved, 15 years post-FMT without relapse/constipation resolved, neurological symptoms improved, 3 years maintained normal motor/bowel symptoms resoluted, neurological improved
	Makkawi et al. [52]	Case report	1	Canada	61	F	MS, rCDI	Enema	Once	Her partner	rCDI resolved, prevented MS progression for over 10 years
Epilepsy	He et al. [53]	Case report	1	China	22	F	Epilepsy, CD	TET tube (colon)	3 times	Healthy volunteer	Seizure-free without antiepileptic drugs, decreasing CDAI to 104 points after 12 months and maintained until the end of 20-month follow-up
Tourette Syndrome	Zhao et al. [54]	Case report	1	China	9	M	TS	Gastroscope & colonoscopy	Once	Healthy volunteer	YGTSS-total tic score decreased from 31 to 5, motor severity score fell from 16 to 5, vocal severity score fell from 15 to 0, shifting from severe to mild
	Ding et al. [55]	Open-label clinical trial	11	China	19.2 ± 7.4	M	TS	TET tube (nasojejunal)	3 times	Healthy volunteer	45.5% (5/11), 45.5% (5/11) and 36.4% (4/11) of patients achieved improvement (≥30% reduction in YGTSS-total tic score) at week 1, week 4, and week 8 post-FMT, respectively. GTS-QoL score decreased at week 8 post-FMT
Myalgic encephalomyelitis/chronic fatigue syndrome	Borody et al. [56]	Larger cohort study	60	Australia	55.0 ± 11.5	36 F, 24 M	CFS (52 with IBS, 4 with constipation)	Single TC infusion ($n = 5$), two-day infusion (TC and enema, $n = 52$), three-day infusion (TC, 2-day enema, $n = 3$)	Once/twice/3 times	13 nonpathogenic enteric bacteria from healthy individual	35/60 patients responded after single FMT while7 patients responded after secondary FMT, giving a total of 42/60 improved patients

Table 2: Continued.

Disease type	Studies	Study type	*N*	Location	Age	Sex	Complication	Administration route	FMT frequency	Donor	Clinical outcome
Psychiatric diseases											
Autism spectrum disorder	Ward et al. [57]	Case series	9	Canada	7.7 ± 5.4	NA	ASD	Capsules & enema	Twice	Healthy volunteer	ASD symptoms were not changed in the 21-year-old subject, while markedly improving in 1 of two 8-year-old subjects
	Zhao et al. [58]	Open-label, randomized waitlist-controlled trial	48	China	NA	NA	ASD	Gastroscope & colonoscopy	Twice	Healthy volunteer	CARS score in the FMT group showed a statistically 10.8% decrease compared to a 0.8% decrease in the waitlist group after the first FMT and remained marginally reduced after the second FMT
	Kang et al. [32]	Open-label clinical trial	18	USA	7 to 16 years	NA	ASD	Oral vs. enema	For 7–8 weeks	Healthy volunteer	80% reduction of GI symptoms post-FMT, ASD symptoms improved significantly and remained improved 8 weeks post-FMT
	Kang et al. [32]	Follow-up of a clinical trial	18	USA	7 to 16 years	NA	ASD	Oral vs. enema	For 7–8 weeks	Healthy volunteer	Two years post-FMT, most GI symptom improvements continued, and autism-related symptoms improved even more
Bipolar disorder	Hinton [59]	Case report	1	Australia	33	F	BD	NA	9 FMTs over a period of 2 months	Her husband	Symptom-free from depression
Depression	Cai et al. [60]	Case report	1	China	79	F	MDD	Gastroscope	Once	Her grandson	PHQ-9 scores improved
Other system-related neurological diseases											
Hepatic encephalopathy	Kao et al. [61]	Case report	1	Canada	57	M	Liver cirrhosis, HE	Colonoscopy & enema	5 FMTs	Healthy volunteer	Stoop test, serum ammonia, and quality of life all significantly improved; appetite, alertness and overall well-being improved
	Bajaj et al. [62]	Open-label, randomized clinical trial	20	USA	64.5 ± 5.1 (FMT) vs. 62.9 ± 9.8 (SOC)	M	Liver cirrhosis, HE	Enema	Once	Healthy volunteer	Significantly improved in PHES total score and EncephalApp Stroop in the FMT group
	Bajaj et al. [63]	A phase 1, randomized, placebo-controlled trial	20	USA	63.3 ± 4.2 (FMT) vs. 64.2 ± 6.2 (SOC)	16 M, 4 F	Liver cirrhosis, HE	Capsules	15 capsules of FMT/placebo	Healthy volunteer	EncephalApp improved

Table 2: Continued.

Disease type	Studies	Study type	*N*	Location	Age	Sex	Complication	Administration route	FMT frequency	Donor	Clinical outcome
Neuropathic pain	Cai et al. [64]	Case report	1	China	46	F	Diabetic neuropathy	Colonoscopy	Twice	Healthy volunteer	The glycemic control improved, with a remarkable relief of the symptoms of painful DN
Sepsis	Li et al. [65]	Case report	1	China	29	F	Bacteremia, shock	Nasoduodenal tube	Once	Healthy volunteer	Fever went down, and the stool output had a marked reduction
	Li et al. [66]	Case report	1	China	44	F	Shock, respiratory failure, AKI	Nasoduodenal tube	Once	Healthy volunteer	Patient's septic symptoms and severe diarrhea were successfully controlled
	Wei et al. [67]	Case report	2	China	65/84	M	Shock, respiratory failure, bacteremia, AKI	Nasoduodenal tube	Once	Healthy volunteer	MODS and severe diarrhea were alleviated in both patients
	Gopalsamy et al. [68]	Case report	1	Georgia	57	M	MDRO infection, respiratory failure	PEG tube	Once	NA	Death (not due to FMT)

FMT: fecal microbiota transplantation; M: male; F: female; PD: Parkinson's disease; TET: transendoscopic enteral tubing; MMSE score: minimental state examination score; AD: Alzheimer's disease; rCDI: recurrent clostridium difficile infection; CDAI: Crohn's disease activity index; MS: multiple sclerosis; CD: Crohn's disease; YGTSS-total tic score: Yale Global Tic Severity Scale-total tic score; TS: Tourette Syndrome; GTS-QoL score: Gilles de la Tourette Syndrome-quality of life score; CFS: chronic fatigue syndrome; IBS: Irritable bowel syndrome; TC: transcolonoscopic; ASD: autism spectrum disorder; CARS score: childhood autism rating scale score; BD: bipolar disorder; MDD: major depressive disorder; PHQ-9 scores: Patient Health Questionnaire-9 scores; HE: hepatic encephalopathy; PHES total score: psychometric hepatic encephalopathy score total score; DN: diabetic neuropathy; AKI: acute kidney injury; MODS: multiple organ dysfunction syndrome; MDRO infection: multidrug-resistant organism infection; PEG tube: polyethylene glycol tube.

that the gut microbiota composition in AD patients differs considerably from that of healthy elderly individuals. For instance, the AD patients have a higher relative abundance of LPS-producing bacteria such as *Burkholderiaceae*, *Staphylococcaceae*, *Porphyromonas gingivalis*, and *Propionibacterium acnes*, as well as fungi in their intestine compared to healthy controls. In addition, patients with cerebral amyloidosis (Amy+) and cognitive impairment have more proinflammatory bacteria in their feces and higher levels of circulating inflammatory cytokines (IL-6, IL-1β, etc.) compared to healthy individuals and Amy- patients [80–84]. Likewise, Cattaneo et al. [85] also detected higher circulating levels of IL-6, IL-1β, and other inflammation-related factors like CXCL2 and NLRP3, along with reduced levels of the anti-inflammatory IL-10 in Amy+ patients relative to that in controls and Amy- patients. Furthermore, the Amy+ patients showed lower abundance of *Eubacterium rectale* and a higher abundance of *Escherichia*/*Shigella* compared to both healthy controls and Amy- patients. A significantly positive correlation was observed between the levels of proinflammatory factors and the abundance of *Escherichia*/*Shigella*. Several bacterial species are known to secrete neurotransmitters and alter the expression of synaptic plasticity, which may play a role in the pathogenesis of AD [86]. In addition to these direct effects, some changes in gut microbiota may indirectly promote AD by triggering neuroinflammation [87]. Consistent with this hypothesis, there are reports that probiotics can improve cognitive function in not only animal models but also AD patients or adults with cognitive impairment [88–90]. Furthermore, the age-related decline in cognitive ability may also be related to the concomitant decrease in the number of anti-inflammatory bacteria in the human gut [91, 92].

Recent studies [93, 94] have shown that antibiotic-mediated depletion of the gut microbiota alleviated Aβ-pathology and neuroinflammation in a mouse model of AD, and the therapeutic effect of antibiotics was partially reversed following FMT from AD mice. In addition, germ-free mice receiving feces from healthy old mice had worse cognitive function compared to the recipients of feces from younger mice due to lower fecal levels of nervous system-related metabolites (such as GABA) in the former [95]. Kim et al. [96] transplanted the fecal microbiota from health control mice into the recently developed AD-like pathology with amyloid and neurofibrillary tangle (ADLPAPT) transgenic mouse model and observed a significant reduction in cerebral amyloid plaques, NFTs and reactive gliosis, which correlated to improve cognitive and memory function. Hazan [50] reported the case of an 82-year-old AD patient who showed remission of *Clostridium difficile* infection (CDI) symptoms after receiving a single FMT from his 85-year-old wife and a negative stool test 2 months later. Interestingly, the minimental state examination (MMSE) score of the patient increased from 20 (mild cognitive impairment) to 26 (normal cognitive function) 2 months after FMT, and he reported memory retention and significant improvement in mood (MMSE score 29) after 4 and 6 months, respectively.

2.3. Multiple Sclerosis. Multiple sclerosis (MS) is a demyelinating disease of the CNS with uncertain etiology, although genetics, infection, and environmental factors have been implicated as key pathological factors [97]. The gut microbiota regulates the production of myelin sheath in the prefrontal cortex of mice [98, 99] and maintains the integrity of the blood-brain barrier [100] by producing SCFAs [101]. This is suggestive of a dysregulated gut microbiome in MS since the loss of blood-brain barrier integrity is also a cardinal sign of this disorder. In addition to the direct role in demyelination and blood-brain barrier disruption, the gut microbiota and its metabolites also regulate neuroinflammation [102–104], although the exact relationship between gut microorganisms and MS-related neuroinflammation needs a further study. The intestinal microbiota of MS patients have a lower relative abundance of Treg cell-inducing bacteria [9, 105], which may increase the proportion of peripheral Th1 and Th17 cells [98]. In addition, the risk of relapse in MS patients is associated with the depletion of Fusobacteria, expansion of the phylum Firmicutes, and presence of Archaea (Euryarchaeota) [106]. Oral gavage with *Prevotella histicola* not only reduced the severity of symptoms in a mouse model of MS but also decreased the number of Th1 and Th17 cells, while increasing that of Treg cells [107]. A randomized control trial (RCT) on 40 MS patients showed that probiotic (*Lactobacillus acidophilus*, *Lactobacillus casei*, *Bifidobacterium bifidum*, and *Lactobacillus fermentum*) supplementation for 12 weeks significantly increased the circulating levels of IL-8 and TNF-α and improved the expanded disability status scale (EDSS) scores [108].

The clinical and pathophysiological characteristics of MS are best simulated in the experimental autoimmune encephalomyelitis (EAE) mouse model [109]. Oral gavage of the fecal microbiota from MS patients exacerbated the symptoms in EAE mice and decreased the levels of the anti-inflammatory cytokine IL-10 [98, 110]. Li et al. similarly showed that FMT from healthy mice alleviated the symptoms in EAE mice by reducing activity of microglia and astrocytes and restoring the blood-brain barrier integrity and axonal myelination [111]. The therapeutic effects of FMT in MS have been reported in only two studies so far [51, 52]. In one patient with secondary progressive MS complicated with recurrent CDI, FMT mitigated the recurrent infection and prevented disease progression of MS. However, the EDSS score of the patient stabilized without any improvements in the symptoms. Therefore, although FMT has limited therapeutic effect; it has the potential to provide long-term benefits for MS patients [52]. Furthermore, 3 MS patients with severe constipation were able to defecate normally after FMT, and their exercising ability was also improved significantly [51].

2.4. Epilepsy. Epilepsy is a chronic disease characterized by the sudden abnormal discharge from cerebral neurons, which leads to transient brain dysfunction. The individual susceptibility to epilepsy is associated with genetic and environmental factors, although the exact etiology of most cases remains unclear [16]. Nevertheless, the composition and distribution of gut microbes in patients with intractable epilepsy are distinct from that in healthy controls [17, 112, 113]. Peng et al. [17] found that compared to drug-sensitive patients, the intestinal Firmicutes/Bacteroides ratio and α-diversity were significantly higher in the drug-resistant patients. Interestingly, the

α-diversity of the latter was similar to that of healthy controls, most likely due to an aberrant increase in the number of rare bacterial genera such as *Clostridium XVIII*, *Atopobium*, *Holdemania*, *Dorea*, *Saccharibacteria*, *Delftia*, *Coprobacillus*, *Paraprevotella*, *Ruminococcus*, *Gemmiger*, *Akkermansia*, *Neisseria*, *Coprococcus*, *Fusobacterium*, *Methanobrevibacter*, *Phascolarctobacterium*, and *Roseburia*. In addition, the increased abundance of *Bifidobacterium* and *Lactobacillus* was associated with fewer seizures per year, and a ketogenic diet reduced the frequency of seizures by modulating the gut microbiota [114]. Sewal et al. [115] further observed that intraperitoneal injection of LPS increased the frequency of epileptic symptoms, which was accompanied by an increase in the blood-brain barrier permeability and in the cerebral levels of proinflammatory cytokines. Antibiotics can protect against epileptic seizures by altering the bacterial population, although there is evidence that they may even induce epilepsy [16]. In addition, probiotic strains such as *Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Lactobacillus casei*, *Lactobacillus helveticus*, *Lactobacillus brevis*, *Bifidobacterium lactis*, *Streptococcus salivarius subsp.*, and *Thermophilus* have also shown a positive effect in epilepsy patients [116, 117]. Olson et al. [118] observed that transplantation of ketogenic microbiota decreased the number of seizures in mice at a higher threshold. He et al. [53] reported a case of epilepsy complicated with Crohn's disease in a 17-year-old patient who showed improvements in neurological and intestinal symptoms following three rounds of FMT. Antiepileptic therapy with sodium valproate was discontinued after 20 months, and no epileptic seizures were observed.

2.5. Tourette Syndrome. Tourette Syndrome (TS) is a neurodevelopmental disorder characterized by motor and speech tics in childhood [119]. Liao et al. [120] found that probiotic supplementation improved tic-like behavior in mice, which coincided with an increased level of dopamine and norepinephrine. A study on 30 pediatric acute-onset neuropsychiatric syndrome and pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections syndrome patients revealed a significantly different gut microbial composition compared to that of healthy controls [18]. Another study found that [121] antibiotics that effectively reduce streptococcal infections can also mitigate the associated tic disorders. Zhao et al. [54] reported that FMT eliminated involuntary articulation, reduced involuntary shrugging, and increased attention span in a pediatric case of TS over a period of 8 weeks. In an open label clinical trial [55], 11 TS patients experienced a transient decrease in seizure severity following three rounds of FMT.

2.6. Myalgic Encephalomyelitis/Chronic Fatigue Syndrome. Myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) is characterized by unexplained persistent fatigue, disturbed sleep, cognitive impairment, fever, postural intolerance, lymphadenopathy, and irritable bowel syndrome. The gut microbiota is significantly altered in patients with ME/CFS [19], and the extent of microbial dysbiosis affects disease severity [122]. Sheedy et al. [123] observed increased relative abundance of gram-positive lactic acid-producing bacteria in the gut of ME/CFS patients, which may lower the mucosal pH and increase permeability. Moreover, the transfer of lactic acid from intestine to the blood may be one of the reasons for the increase of lactate level in cerebrospinal fluid of ME/CFS patients [124–126]. Selective transplantation of 13 nonpathogenic enteric bacteria through colonoscopy [56] significantly improved intestinal and other symptoms in 42/60 ME/CFS patients. In addition, 7/12 patients who were followed up for 15 to 20 years showed complete remission, indicating FMT is a promising treatment for ME/CFS.

2.7. Guillain-Barré Syndrome. Guillain-Barré Syndrome (GBS) is a paralytic autoimmune neuropathy caused by infection, especially *Campylobacter jejuni* infection in the GI tract, or other immune stimulation [127]. The innate immune response to campylobacteriosis is characterized by the accumulation of neutrophils and macrophages, inflammatory damage to the mucosa, gut barrier defects, and malabsorption, which eventually lead to bloody diarrhea [23]. Mice inoculated with *Campylobacter jejuni* from GBS patients showed increased levels of autoantibodies and peripheral nerve injury [128, 129], indicating a close association between gut dysbiosis and GBS pathogenesis. In fact, the cross-reaction between LPS produced by *Campylobacter jejuni*, and the peripheral gangliosides is one of the causative factors of GBS [130]. The combination of antibiotics and FMT significantly expedited *Campylobacter jejuni* clearance from the infected mice [131]. In addition, Brooks et al. [132] observed that human FMT increased the Th2 and autoimmune response in mice infected with *Campylobacter jejuni*. Finally, the outer core LPS of *Campylobacter jejuni* can directly initiate the peripheral neuropathy of GBS by inducing production of neurotoxic antiganglioside autoantibodies [133].

2.8. Stroke. Stroke is an acute cerebrovascular accident characterized by muscular and sensory weakness. Studies show that the composition of gut microbiota of stroke patients differs considerably from that in healthy controls [134, 135], although there are some reports indicating transient or no change [136]. Furthermore, the possible role of gut dysbiosis in stroke is ambiguous [137]. One study showed that a stroke episode decreased intestinal motility and α-diversity and led to bacterial overgrowth, intestinal barrier damage, and increased infiltration of inflammatory immune cells in the gut-associated lymphoid tissue and brain, eventually increasing the infarct volume [138]. In addition, the translocation of gut microbiota and their metabolites may also be involved in the pathogenesis of stroke [139]. For instance, trimethylamine-N-oxide produced by gut microbiota may be associated with a higher risk of atherosclerosis-mediated cardiovascular events, including stroke [140, 141]. Prebiotic treatment exacerbated the functional damage and inflammation in a mouse model of stroke, which increased the infarct volume [138, 142]. However, transplantation of healthy microbiota reduced infarct volume [138], indicating that FMT can be considered for treating stroke patients.

2.9. Amyotrophic Lateral Sclerosis and Huntington's Disease. Amyotrophic lateral sclerosis (ALS), also known as motor neuron disease (MND), is a neurodegenerative disorder

characterized by progressive atrophy of the limb, trunk, chest, and abdomen muscles following upper and lower motor neuron injury [143]. The mouse model of ALS shows an altered gut microbiota structure compared to healthy mice, such as a lower relative abundance of butyrate-producing bacteria [144]. Although a definitive pathological role of the gut microbiota in ALS has not been reported in humans [26], the clinical potential of FMT is still being explored [145]. Huntington's disease is caused by an autosomal dominant mutation in the huntingtin gene and is inherited in most cases. Nevertheless, several studies have implicated nongenetic factors in the development of Huntington's disease, such as the gut microbiota. Metabonomics analysis of the sera of preonset and early onset Huntington's disease patients and healthy controls showed significant differences in the gut microbiota metabolites across all groups [146], indicating that changes in the microflora determine disease course. The role of gut dysbiosis in the pathogenesis of Huntington's disease has also been confirmed in a murine transgenic model [29]. However, further studies are needed to fully understand the causative role of the gut microbiota and its metabolites in the genesis, progression, and severity of Huntington's disease.

3. Psychiatric Diseases

There is growing evidence that gut dysbiosis also contributes to mental health and psychiatric disorders, such as autism spectrum disorder, bipolar disorder, depression, anxiety, obsessive-compulsive disorder, posttraumatic stress disorder, schizophrenia, and dementia through the gut-brain axis [147, 148]. FMT has gained attention as a viable therapeutic option for these conditions (Table 2).

3.1. Autism Spectrum Disorder. Autism spectrum disorder (ASD) is a group of neurodevelopmental disorders characterized by changes in social interaction and repetitive, stereotypical behavior [149]. Recent studies show that gut microbial community and metabolites of ASD patients are distinct from that of healthy individuals [8, 150]. Although a putative relationship between gut dysbiosis and ASD behavior has been established in rodent models and human subjects, studies have not been sufficient to confirm the causal relationship between gut microbiota and ASD symptoms. The predominant phyla of the healthy adult human gut are Bacteroidetes (e.g., *Bacteroides* and *Prevotella*), Firmicutes (e.g., *Clostridium*, *Lactobacillus*, and *Ruminococcus*), Proteobacteria (e.g., *Enterobacter*), and Actinobacteria (e.g., *Bifidobacterium*) and constitute more than 90% of the gut microbiota [151, 152]. Since germ-free mice are socially dysfunctional compared to wild-type mice, the gut microbiota likely play an important role in normal behavior [153]. Regular administration of probiotics including *Lactobacillus reuteri*, *Lactobacillus plantarum*, *Lactobacillus acidophilus*, *Lactobacillus rhamnosus*, and *Bifidobacterium longum* over a period of 3 weeks to 6 months improved autistic symptoms significantly in ASD children [154–156]. *Lactobacillus rhamnosus* and a placebo were, respectively, administered to 40 and 35 infants for the first 6 months of life in an RCT, and all subjects were followed over 13 years. Six infants of the placebo group were diagnosed with Asperger's syndrome or attention deficit hyperactivity disorder during the follow-up period whereas none in the probiotic group exhibited any signs of autism, indicating that early administration of probiotics can potentially reduce the risk of developing ASD [155]. A recent study has shown that there is a causal relationship between maternal diet, changes in gut microbiota, and social behavior. Among female neonatal rats fed with a high-fat diet, female rats born on a high-fat diet and for more than 4 weeks *Lactobacillus reuteri* restored gut microbial diversity and significantly improved their social behavior [157].

Sharon et al. [158] found that germ-free mice transplanted with the feces from children with ASD exhibited similar symptoms. In addition, the offspring of these FMT recipients also experienced these symptoms and showed alternative splicing of ASD-related genes in the brain. Likewise, FMT from healthy hamsters alleviated the ASD-like symptoms in the autism hamster model [159] by alleviating the brain oxidative stress response. In an open clinical trial on 18 children with ASD, FMT for 7 to 8 weeks could significantly improve digestive symptoms (abdominal pain, constipation, diarrhea, and indigestion) and the behavioral symptoms [32]. Furthermore, FMT also improved the bacterial diversity by significantly increasing the abundance of *Bifidobacterium*, *Desulfovibrio*, and *Prevotella*. The therapeutic effects persisted for 8 weeks after ceasing treatment. In another study, the ASD symptoms improved in 8/9 recipients of FMT and antibiotic treatment [57]. Zhao et al. [58] conducted an open label RCT on 24 autistic and 24 normal children that were treated with FMT for 2 months. Although FMT improved the behavioral and GI symptoms, the effects were transient.

3.2. Bipolar Disorder. Bipolar disorder (BD) is a type of mood disorder that clinical manifests as distinct episodes of depression, manic seizures, and their combination. Both the diversity and taxonomic composition of the gut microbiota in BD patients are significantly different from that of healthy individuals [36]. Painold et al. [160] further showed that the phylum Actinobacteria and class *Coriobacteria* were significantly more abundant in the gut of BD patients, whereas *Ruminococcaceae* and *Faecalibacterium* were more abundant in the healthy controls as per 16S rRNA gene sequencing and LEfSE analysis. They also observed a negative correlation between microbial α-diversity and duration of BD and identified bacterial clades associated with inflammatory status, serum lipids, depressive symptoms, oxidative stress, anthropometrics, and metabolic syndrome in the BD patients. Hu et al. [35] analyzed the gut microflora of 52 BD patients and 45 controls and found that the α-diversity of untreated BD patients was lower than that of the control group, and the predominant phyla were Bacteroidetes and Firmicutes, respectively. In addition, butyrate-producing bacteria were less abundant in the untreated patients, which was restored following quetiapine treatment. Furthermore, probiotics supplementation for a period of 3 months improved the cognitive and executive functions of 20 BD patients [161].

Hinton [59] reported a case of a 29-year-old female patient diagnosed with type I DSM-IV BD who had been

treated with various drugs, including lithium, lamotrigine, valproate, quetiapine, olanzapine, and various benzodiazepines, that led to significant weight gain and poor quality of life. Nine rounds of FMT in 11 months not only alleviated depression and mania but also helped her lose the excess weight and remain asymptomatic without using other drugs.

3.3. Depression. Depression is a common mental disease typically characterized by persistent feelings of sadness and loss of interest in daily activities. It results from a combination of both genetic and environmental factors, and a major cause is stress [162]. Studies increasingly show that the gut microbiota can shape cognition through the microbiota gut-brain axis, and mice with altered microbiota usually exhibit depression-related behaviors [163]. Kelly and Borre [164] analyzed the intestinal flora of 34 patients with depression and 33 matched healthy subjects and found that the microbial abundance and biodiversity were decreased in the patient group. FMT from these patients into germ-free rats induced depression-like behavior such as lack of pleasure and anxiety in the latter, along with increased levels of tryptophan. A meta-analysis of 71 studies published between 2003 and 2019 [165] further revealed that probiotics and prebiotics can significantly improve symptoms of anxiety and depression compared to untreated or placebo-treated controls and provide additional benefits to patients with other diseases such as irritable bowel syndrome.

Zhang et al. [163] found that FMT from depressed patients into germ-free mice led to depressive behavior in the latter. Similar results were observed after antibiotic treatment as well. Furthermore, FMT from the NLRP3-knockout mice significantly improved the behavioral symptoms in a mouse model of depression. Likewise, Xie [166] also found that the fecal microbiota of healthy mice alleviated depressive symptoms. In a recent case report [60] of an older woman diagnosed with depression, a single FMT improved sleep cycle, appetite, and general mood within 4 days of treatment. The patient was able to live independently after 2 weeks and showed an increase in weight. Six months later, her weight had returned to normal, constipation symptoms had improved, and the Patient Health Questionnaire-9 score decreased from 21 to 4.

3.4. Anxiety. Anxiety is one of the most common types of neurosis and is characterized by feelings of tension/worry without a clear objective, restlessness, and autonomic nerve dysfunction. Clinically, it is classified into chronic/generalized anxiety and acute anxiety or panic attack [167]. A large case-control study [168] showed that the use of antibiotics increased the risk of anxiety and depression, and the risk increased with the frequency of usage, suggesting a causative or ancillary role of the gut microbiota. Furthermore, there is evidence that depression can lead to secondary changes in the composition of the gut microbiota, resulting in a regulatory feedback loop between depression and dysbacteriosis [169]. Compared to the SPF mice, sterile mice showed significantly higher anxiety in the elevated maze test, and oral administration of the JB-1 probiotic strain effectively reduced the anxious behavior and improved performance. Furthermore, a systematic review of 21 studies including 1503 subjects with anxiety disorders concluded that microbiota-targeted therapies [41], including probiotics supplements, single probiotics, double probiotics, multiple probiotics, dietary fiber supplement, and low FODMAP diet, can alleviate symptoms of anxiety by regulating the gut microbiota.

De Palma et al. [170] transplanted fecal microbiota from healthy control and diarrhea-predominant irritable bowel syndrome (IBS) patients with (IBS-A) or without anxiety into germ-free mice and analyzed the changes in intestinal function and behavior. The gut microbiota of mice transplanted with the feces of IBS patients showed unique clustering characteristics compared to that of control fecal recipients. Anxiety-like behavior was determined with the light/dark preference test and platform jumping test, which showed that the IBS-A recipient mice had the least preference for light and showed the delay in jumping off a high platform, both of which are indicative of a higher degree of anxiety. These studies clearly indicate the involvement of gut dysbiosis in the severity of anxiety symptoms.

4. Other System-Related Neurological Diseases

Several neurological and psychological diseases are frequently complicated with digestive system symptoms. Likewise, some diseases predominantly affecting the nonnervous systems may also have a neurological component and are commonly manifested as encephalopathies. For instance, decompensated hepatic encephalopathy and peripheral neuropathy are severe complications of cirrhosis and diabetes, respectively, and sepsis patients often present delirium, coma, and other neurological symptoms. The role of the gut microbiota in these encephalopathies is increasingly being recognized, thereby indicating the therapeutic potential of FMT for these diseases (Table 2).

4.1. Hepatic Encephalopathy. Hepatic encephalopathy (HE) is a serious complication of cirrhosis and is caused by brain dysfunction. The increased content of hepatic ammonia in cirrhosis patients with mild HE indicates the pathological involvement of intestinal dysbiosis. For instance, the intestinal tract of cirrhotic patients with/without mild HE frequently harbors urease-positive *Streptococcus salivarius*, which is absent in healthy individuals [171]. Thus, *S. salivarius* is a promising therapeutic target in liver cirrhosis patients with mild HE. Sung et al. [43] confirmed that fecal microbiota can predict the clinical prognosis of patients with liver cirrhosis and HE, such as *Lactobacillus*, *Bacteroides*, *Clostridium_incertae_sedisof*, and *Clostridium XI*, which were associated with patients' mortality. Furthermore, Kawaguchi et al. [172] showed that rifaximin improved both liver and neuropsychological function in liver cirrhosis patients with HE by adjusting the gut microbial structure.

A promising case study of a 57-year-old patient with HE due to alcoholic and hepatitis C cirrhosis [61] showed that FMT in addition to lactulose objectively improved reaction time, serum ammonia, and quality of life scores. However, these improvements were transient and subsided to the baseline levels within 7 weeks of FMT cessation. Furthermore, Bajaj et al. [62] conducted an RCT on male cirrhotic patients

diagnosed with recurrent HE and found that FMT reduced hospitalization rate and improved cognitive ability in these patients during the 5-month follow-up. In another clinical trial conducted by Bajaj et al. [63], administration of FMT capsules to HE patients restored the gut microflora by significantly increasing the abundance of *Bifidobacterium* and *Ruminococcaceae* and decreasing that of pathogenic genera like *Streptococcus* and *Veillonella*. The FMT-induced changes in the gut microbiota led to an increase in duodenal E-cadherin and defensin-α5 expression and reduced serum levels of IL-6 and LBP.

4.2. Neuropathic Pain. Neuropathic pain is caused by peripheral or CNS injury (such as nerve injury or chemotherapy injury) or diabetes and is characterized by abnormal sensations or pain even after normal stimulations [173]. The composition and function of the gut microbiota in diabetic patients differ significantly from that of healthy controls [174]. FMT from conventionally reared mice increased the insulin resistance in germ-free mice [175], whereas subjects with metabolic syndrome showed increased insulin sensitivity following FMT [176]. Gut microbiota can also directly regulate the excitability of spinal dorsal root neurons or indirectly regulate inflammation in the peripheral and central nervous system [177]. Oxaliplatin can cause peripheral neuropathy and pain, but this phenomenon is not obvious in mice with antibiotic cleaning or in mice with complete loss of gut microbiota. Furthermore, if FMT was performed on the appellate mice, the pain would be restored, indicating that the gut microbiota has an effect on neuropathic pain [178]. Another study found that probiotics alleviated the characteristics of paclitaxel-induced neuropathic pain *in vitro* [179], although their efficacy is dependent on the type of neuropathic pain. For instance, *Lactobacillus Reuteri* or *Bifidobacterium* were not effective against the neuropathic pain induced by chronic compression injury in rats [180].

A case study [64] of a woman with type 2 diabetes mellitus and diabetic neuropathy showed that two rounds of FMT improved limb pain and paresthesia, which was manifested as decreased visual analogue pain score (VAS) and increased tibial nerve motor conduction velocity, without any significant improvement in EMG sensory dysfunction. In addition, the fasting blood glucose level also decreased and stabilized, and glycosylated hemoglobin content decreased post-FMT.

4.3. Sepsis-Associated Encephalopathy. Sepsis is an acute systemic infection caused by various pathogenic bacteria that invade the bloodstream and rapidly proliferate and produce life-threatening toxins. Sepsis-associated encephalopathy is a key neurological manifestation of sepsis, with symptoms ranging from delirium to coma. It occurs in almost 70% of the ICU patients and is associated with higher ICU and hospital mortality, as well as poor long-term outcomes (including cognitive and functional outcomes) [181, 182]. The toxins and other harmful antigens secreted by pathogenic bacteria or viruses can be neutralized by the antibodies produced by antigen-primed B cells. Intestinal microorganisms have been shown to induce the clonal expansion of specific B cell populations and increase production of antibodies to prevent the spread of infection [183]. Li et al. found that FMT effectively improved the spatial memory and EEG abnormalities in an LPS-induced rat model of sepsis combined with cervical vagotomy, and the therapeutic effect of FMT was likely mediated through the vagus nerve [184]. In addition, several case reports indicate that non-CDI sepsis patients with prolonged ICU stay and complications including bacteremia, MDR bacterial infection, respiratory failure, and organ dysfunction significantly benefitted from FMT. A total of 5 patients received FMT, of which 4 showed clinical improvement and 1 died from non-FMT-related causes [65–68].

5. Discussion

Nervous system diseases are highly complex and show cognitive, motor, and even systemic manifestations. Given that gut dysbiosis is a potential causative factor of neurological dysfunction, FMT-mediated restoration of the gut microbiota can stall the symptoms or progression of nervous system diseases through immune, endocrine, metabolic, and/or neural pathways. The metabolites and cytokines produced by gut bacteria determine intestinal and systemic inflammation and, therefore, the intestinal barrier function. However, there are several limitations of using FMT in treating neurological, mental, and psychological diseases: (1) for many diseases, the therapeutic effects of FMT are limited to animal models and isolated cases. Although transplantation of human feces to animal models has shown encouraging results, the GI and physiological differences between humans and animals preclude the extrapolation of the results to sick or healthy humans. (2) The fecal feeding behavior often observed in mice [185] may also affect the microbiota analysis and the efficacy of FMT. In addition, animals housed in the same cage may have a closer gut microbial structure, which can also affect the results. (3) The efficacy of FMT depends on the types of antibiotics, microbial composition, intervention procedure, and donors. The exact influence of these factors and the potential adverse effects of FMT are currently unknown due to lack of long-term follow-up and appropriate controls. Therefore, it is crucial to establish scientific standards in order to gauge the therapeutic efficacy of FMT [186]. (4) The role of the gut microbiota in the early development of nervous system also needs to be elucidated. For instance, a study on 39 infants showed that the α-diversity of gut microbiota was also associated with functional connectivity between the auxiliary motor area and the inferior parietal lobule, and this functional connectivity affects the cognitive level at 2 years of age [187]. (5) Many successful cases of FMT in the treatment of neurological diseases/psychiatric diseases often have obvious GI symptoms, and the improvement of neurological symptoms/mental symptoms is also related to the GI symptoms. For patients with neurological diseases/psychiatric diseases but without obvious GI symptoms, whether the curative effect of FMT will be reduced or unchanged is also worth our concern.

Despite the promising results, the rationale for the clinical application of FMT is currently based on animal models and a few case reports and clinical studies. Large-scale randomized double-blind controlled trials are still needed to clarify the role of FMT in neurological diseases. At present,

Table 3: Clinical trials of FMT involving in nervous and mental disease.

NCT number	Conditions	FMT route	Phases	Status	Locations
NCT02255617	Hepatic encephalopathy	Colonoscopy & enema	Phase 1, phase 2	Completed	Canada
NCT02636647	Hepatic encephalopathy	Enema	Phase 1	Completed	United States
NCT03420482	Hepatic encephalopathy	Capsules	Phase 2	Recruiting	United States
NCT03152188	Hepatic encephalopathy	Capsules	Phase 1	Completed	United States
NCT03439982	Hepatic encephalopathy	Colonoscopy & enema	Phase 1, phase 2	Recruiting	Canada
NCT03796598	Hepatic encephalopathy	Capsules & enema	Phase 1, phase 2	Recruiting	United States
NCT03408886	Autism spectrum disorder	Pill (no detail)	Phase 2	Recruiting	United States
NCT03426826	Autism spectrum disorder	Gastroscope	Phase 1	Recruiting	United States
NCT03829878	Autism spectrum disorder	Capsules	Phase 2	Not yet recruiting	United States
NCT04182633	Autism spectrum disorder	Oral administration of FM (no detail)	Phase 2	Recruiting	United States
NCT04246398	Children with autism	Capsules	Not applicable	Not yet recruiting	Israel
NCT03026231	Parkinson's disease	Capsules	Phase 1, phase 2	Withdrawn	United States
NCT03671785	Parkinson disease	Capsules	Phase 1	Recruiting	United States
NCT03808389	Parkinson disease	Nasojejunal	Not applicable	Recruiting	Belgium
NCT03876327	Parkinson disease	Not applicable	Phase 2, phase 3	Completed	Israel
NCT03183869	Multiple sclerosis	Enema	Phase 2	Terminated	Canada
NCT03594487	Multiple sclerosis	Colonoscopy	Phase 1	Recruiting	United States
NCT03975413	Multiple sclerosis	Not applicable	Not applicable	Active, not recruiting	United States
NCT04203017	Multiple sclerosis	Capsules	Phase 1	Recruiting	Russian Federation
NCT03691987	Chronic fatigue syndrome/myalgic encephalomyelitis	Enema	Phase 2	Recruiting	Norway
NCT04158427	Chronic fatigue syndrome/myalgic encephalomyelitis	Colonoscopy	Not applicable	Enrolling by invitation	Finland
NCT03233100	Depressive symptoms, anxiety symptoms, gut-brain disorders	Not applicable	Not applicable	Unknown status*	China
NCT03281044	Major depressive disorder	Capsules	Phase 2	Terminated	Switzerland
NCT04001439	Depression in schizophrenia	Capsules	Not applicable	Not yet recruiting	France
NCT03998423	Alzheimer disease	Capsules	Phase 1	Terminated	United States
NCT02889627	Epilepsy	Microbiota suspension infused into midgut or lower gut (no detail)	Phase 2, phase 3	Recruiting	China
NCT03279224	Bipolar depression	Colonoscopy	Phase 2, phase 3	Recruiting	Canada

Table 3: Continued.

NCT number	Conditions	FMT route	Phases	Status	Locations
NCT03766321	Amyotrophic lateral sclerosis	Nasojejunal	Not applicable	Recruiting	Italy
NCT04132427	Pitt-Hopkins syndrome	Oral (no detail)	Phase 2	Recruiting	United States
NCT03416751	Alcohol abuse	Enema	Phase 1	Completed	United States
NCT03928808	Anorexia nervosa	Nasogastric tube	Early phase 1	Suspended	United States
NCT02336789	Disorientation as to people, time and place	Colonoscopy	Not applicable	Unknown status*	Israel
NCT04014413	Hepatic encephalopathy, multiple sclerosis, autism, alcohol dependence	Not applicable	Not applicable	Recruiting	China

*Study has passed its completion date, and status has not been verified in more than two years. Date from https://clinicaltrials.gov/.

33 clinical trials are ongoing on the potential therapeutic effects of FMT on mental and nervous system diseases (Table 3). Furthermore, the modes of delivering fecal microbiota also need to be improved. While a capsular form is more comfortable for the patients, fecal bacterial liquid in the form of washed/selective microbiota transplantation [188, 189] may be more effective in reducing the potential side effects.

Data Availability

Data from the review are available upon request from the corresponding authors (Y.Q.N. and Y.J.Z.).

Conflicts of Interest

The authors declare no competing financial interests.

Authors' Contributions

H.M.X., H.L.H., and Y.L.Z. are involved in the design of the study and drafting of the article. H.L.Z. and J.X. are involved in the statistical analysis and interpretation of the data. D.W.S. and Y.D.L. are involved in the design of the figure and tables. Y.J.Z. and Y.Q.N. planned and directed the project and interpreted the results. All authors read and approved the final manuscript. H.M.X., H.L.H., and Y.L.Z. contributed equally to this article.

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Research Article

The Efficacy of Washed Microbiota Transplantation on *Helicobacter pylori* Eradication: A Pilot Study

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Aim. The fecal microbiota transplantation by washed preparation was recently coined as washed microbiota transplantation (WMT). This pilot study is aimed at exploring the feasibility and efficacy of WMT on *Helicobacter pylori* eradication. *Methods*. Consecutive patients who had been treated with WMT for various indications and who were positive for *H. pylori* infection before WMT treatment but had never received eradication therapy for *H. pylori* infection were invited to take a follow-up ^{13}C-urea breath test. The associations of demographic, clinical factors, and laboratory indicators for gastric function and intestinal barrier function with the therapeutic effect were determined. *Results*. A total of 32 eligible patients were included, and the overall *H. pylori* eradication rate was 40.6% (13/32). Patients with *H. pylori* eradication had a higher pepsinogen ratio (PGR) than those without (13.00 ± 6.97 *vs.* 8.31 ± 3.733; $P = 0.02$). Female patients had a higher, albeit not statistically significant, eradication rate than male patients (53.85% *vs.* 31.58%; $P = 0.208$). Compared with lower gastrointestinal tract delivery route, middle gastrointestinal tract delivery route seems to be a more suitable way for the treatment of *H. pylori* infection (58.33% vs 16.67%; $P = 0.152$). There was no significant difference in other demographic and clinical factors between patients with and without *H. pylori* eradication. *Conclusion*. *H. pylori* infection is eradicated in a proportion of patients who have received WMT. An increased pre-WMT PGR appears to be associated with the therapeutic effect. Further studies are required to confirm the efficacy of WMT, especially in combination with currently recommended regimens in randomized controlled trials.

1. Introduction

Helicobacter pylori is a type of microaerophilic, spiral-shaped, Gram-negative bacteria, which is colonized in the human stomach and easily resists the extreme environment of gastric acid [1]. It is a major pathogen of chronic gastritis, peptic ulcer, and gastric cancer and is also associated with irritable bowel syndrome [2, 3]. Early in 1994, the World Health Organization defined *H. pylori* as a class I carcinogen of gastric cancer, which accounted for 90% of noncardiac cancer cases [4]. It is estimated that about 50% of the population worldwide is infected with *H. pylori*, and the prevalence in developing countries is much higher than that in developed countries [5]. It is well known that *H. pylori* infection is difficult to eradicate naturally without drug intervention [6]. Triple therapy consisting of a bismuth salt or proton pump inhibitors and two antibiotics has shown good performance in the early battle with *H. pylori* infection. With the widespread application of antibiotics in clinical practice, *H. pylori* resistance to antibiotics has increased to different degrees worldwide. To solve this problem, the treatment plan for *H. pylori* infection has changed from initial triple therapy to quadruple therapy, and the treatment period has been gradually extended, which seriously affects patient compliance as well as the quality of life [5, 7]. However, the success rate of *H. pylori* eradication is still declining, and *H. pylori* eradication is now becoming a difficult challenge for clinical physicians [8]. Moreover, the recurrence of *H. pylori*

infection, including the recrudescence and reinfection of *H. pylori*, is also of concern; the recurrence rate is estimated to be 10.9% of patients after eradication treatment in developing countries, and quadruple therapy is required for most recurrent cases to reeradicate *H. pylori* infection [9–11].

Previous studies have shown that *H. pylori* infection causes gastrointestinal microbiota disorder, and this change is reversible after *H. pylori* eradication [12–14]. In addition, antibiotic-based treatments for *H. pylori* eradication have been shown to cause gut microbiota dysbiosis and lead to the increase of *erm (B)* gene (a gene encoding erythromycin-resistant methylase), which would compromise the efficacy of eradiation therapy regimens including a macrolide [12, 15, 16]. Therefore, it may be possible to reverse the colonization of *H. pylori* by restoring the gastrointestinal microbiota. It has been proven that supplementation of probiotics such as *Lactobacillus acidophilus* and *Saccharomyces boulardii* in traditional triple therapy can effectively improve the eradication rate of *H. pylori* infection and reduce the incidence of adverse events. However, there are still no probiotics that can be used alone to eradicate *H. pylori* infection [17, 18].

Fecal microbiota transplantation (FMT), in which the fecal microbiota of a healthy individual is transplanted into the patient's intestines, has been shown to effectively restore the gastrointestinal microbiota and treat gastrointestinal diseases. It has been demonstrated that FMT is efficacious for the treatment of a variety of gut microbiota-related diseases, including digestive system and nondigestive system diseases [19–22]. FMT on the basis of washed microbiota preparation, known as washed microbiota transplantation (WMT), has been proven to decrease adverse events caused by traditional fecal suspension preparation and greatly improve the efficacy [23, 24]. We speculate that WMT can also be used, alone or in combination with currently recommended regimens, to eradicate *H. pylori* infection, by restoring the gut microbiota. However, this hypothesis has not been tested.

Therefore, the aim of this pilot study was to explore the feasibility and efficacy of WMT on *H. pylori* eradication.

2. Materials and Methods

2.1. Patients. Consecutive patients, who had been treated with WMT for various indications at the First Affiliated Hospital of Guangdong Pharmaceutical University (Guangdong, China) and were positive for *H. pylori* infection within 1 year before WMT treatment but had not been treated with any eradication therapy for *H. pylori* infection during the period, were identified by reviewing the hospitalization data. Then, a telephone call was conducted, and those patients who were not receiving eradication therapy for *H. pylori* infection after WMT were invited to take a follow-up ^{13}C-urea breath test (UBT), which was performed at least 4 weeks after the completion of WMT and withdrawal of proton pump inhibitors and antibiotics. Only patients who took the follow-up examination were included in the final analysis.

Gastric function indicators including pepsinogen I (PGI), pepsinogen II (PGII), pepsinogen ratio (PGR), gastrin 17 (G-17), and intestinal barrier function indicators including diamine oxidase (DAO), D-lactate, and lipopolysaccharide (LPS) detected the week before and after WMT were analyzed.

The protocol of this study was approved by the Ethics Committee of the First Affiliated Hospital of Guangdong Pharmaceutical University, and all patients who took the follow-up ^{13}C-UBT provided written consent according to the Measures for Ethical Review of Biomedical Research Involving Human Beings (http://www.gov.cn/gongbao/content/2017/content_5227817.htm).

2.2. WMT

2.2.1. Stool Donors. The methods for donor screening and stool suspension preparation were consistent with the Nanjing Consensus on Methodology of Washed Microbiota Transplantation [24]. The donors' ages ranged between 18 and 25 years old, and their body mass indexes were between 18.5 and 23.9. All donors needed to pass a structured questionnaire firstly, and those who met the requirements were invited to participate in further interview and psychological and physical examinations. Donors with infectious diseases, digestive diseases, metabolic diseases, chronic fatigue syndrome, autoimmune diseases, allergic disease, and neuropsychiatric diseases were excluded. All qualified donors were required to receive a training about healthy diet prior to stool donation.

2.2.2. Stool Suspension. The stool samples provided by the donor were collected, weighed, added with sterile saline according to the ratio of feces to saline (1 : 5), and then mixed evenly. The mixture was filtered through the intelligent microbial separation system (GenFMTer; FMT Medical, Nanjing, China), and five stages of filtration were carried out. The obtained suspension was then immediately centrifuged at a speed of 2500 rpm for 3 minutes and repeated three times. The final sediment was suspended again with sterile saline in accordance with the ratio of sediment to saline (1 : 1).

2.2.3. WMT Preoperative Preparation. Metoclopramide was injected intramuscularly 30 minutes before WMT, and a proton pump inhibitor (Omeprazole or Lansoprazole) was injected intravenously one hour before WMT.

2.2.4. WMT Procedure. Before WMT, an endoscopic administration tube (nasojejunal or transendoscopic enteral tube) was placed in the stomach (or upper) or jejunum (or middle) along the upper gastrointestinal tract or in the right hemicolon (or lower) along the lower gastrointestinal tract and fixed with titanium clips with the assistance of a gastroscope or enteroscope, and then, the endoscopic administration tube was flushed with normal saline to confirm the patency [25]. The gastrointestinal tract delivery route was dependent on the patient's wish or tolerance. The stool suspension was infused according to the standard of 200 mL per person. Finally, the patient was asked to stay in the lying position for 30 min, with restriction of strenuous exercise. During the course of treatment, the frequency of WMT was once a day for three consecutive days, and the actual course patients received was adjusted according to the patient's condition.

2.3. Detection of H. pylori Infection. ^{13}C-UBT was used for the *H. pylori* detection before and during WMT for some patients and at least 4 weeks after the completion of WMT for all included patients. During the test, the patient's first breath was collected after fasting for 2 h, followed by oral administration of ^{13}C-urea (Beijing Boran Pharmaceutical Co., Ltd., Beijing, China), and the second breath was collected 30 min later. The values of CO_2 at baseline and 30 min later were measured by an isotope ratio mass spectrometer (Beijing Richen-Force Science & Technology Co., Ltd., Beijing, China). Positivity was defined when the $^{12}C/^{13}C$ ratio (δ value) was greater than 4 in the breath sample before and after administration of the ^{13}C-urea [26].

A commercial enzyme-linked immunosorbent assay kit (Beijing Wantai DRD Co., Ltd., Beijing, China) was used to detect the *H. pylori* antibody, and the *H. pylori* infection status was diagnosed according to the manufacturer's instructions [19]. For patients who underwent upper endoscopy, gastric specimens obtained by endoscopy were embedded in paraffin and sectioned, followed by hematoxylin and eosin staining for histological examination and the rapid urease test [27]. Patients with dark blue arcs or S-shaped bacteria in the section under the light microscope and/or with color change in the reagent (Fujian Sanqiang Biochemical Co., Ltd., Fujian, China) were considered *H. pylori* positive.

In this study, *H. pylori* status before or during WMT was defined as positive when any one of the four tests (*i.e.*^{13}C-UBT, serology test for *H. pylori*-IgG antibody, rapid urease test, pathological examination) was positive. To further strengthen the quality of the study, further analysis for patients who tested positive for *H. pylori* infection by at least two tests or at least twice by a single test within 1 year before WMT was performed. *H. pylori* eradication was defined as a negative *H. pylori* status in the follow-up ^{13}C-UBT at least 4 weeks after the completion of WMT.

2.4. Data Analysis. Categorical data are expressed as the frequency and percentage and numerical data as the mean ± standard deviation. The enumeration data were tested by the chi-square test and Fisher's exact test; the odds ratio (OR) and 95% confidence interval were calculated. If a normal distribution was obeyed, Student's *t*-test was used for the comparison between the groups; otherwise, the Kruskal-Wallis *H* test was used for replacement. In addition, the variables with $P < 0.20$ were analyzed in multivariate logistic regression analysis. SPSS software version 19.0 (IBM, Armonk, NY, USA) was used to analyze the data. The difference was defined as statistically significant when $P < 0.05$.

3. Results

3.1. Effect of WMT on H. pylori Infection. A total of 352 hospitalized patients who received WMT were identified, of whom 19 did not have a history of *H. pylori* detection and 248 were *H. pylori*-negative. Thus, 85 H. *pylori*-positive patients before and during WMT were further reviewed. Of the 85 *H. pylori*-infected patients, only 32 had a ^{13}C-UBT after WMT and did not receive *H. pylori* eradication therapy (Figure 1). Finally, 32 patients, including 19 (59.4%) males and 13 (40.6%) females, were included in the analysis. Among these patients, 13, 5, 5, and 2 patients were diagnosed with *H. pylori* infection by a single *H. pylori*-serology test alone, ^{13}C-UBT alone, rapid urease test alone, and pathological examination alone, respectively, and the remaining seven patients were diagnosed with *H. pylori* infection by at least two tests ($n = 6$) or at least twice by a single test ($n = 1$). The average age of these patients was 57.22 ± 18.29, ranging from 9 to 86 years. Irritable bowel syndrome (IBS) was the most common indication for WMT, accounting for 59.38% ($n = 19$), followed by nonalcoholic fatty liver disease (NAFLD), hepatic encephalopathy (HE), gastroesophageal reflux disease (GERD), gouty arthritis (GA), alcoholic hepatitis (AH, all 6.25%, $n = 2$), hepatic cirrhosis (HC), functional dyspepsia (FD), and attention deficit hyperactivity disorder (ADHD, all 3.13%; $n = 1$). Of the 32 patients, 13 (40.6%) became negative, while the other 19 (59.4%) remained positive for *H. pylori* infection in the ^{13}C-UBT after WMT. The 13 patients became *H. pylori* negative, one patient used moxifloxacin for urinary tract infection for 1 week, five had never used any antibiotics, and seven were unsure or could not remember whether they used antibiotics during the one year prior to WMT. In addition, of the seven patients who were positive for *H. pylori* infection by at least two tests or at least twice by a single test within 1 year before WMT, two (28.57%) became negative at the follow-up visit.

3.2. Associations of WMT with Therapeutic Effects. There was no significant difference in the eradication rate between male and female patients (31.58% *vs.* 53.85%, OR = 0.396, 95% CI: 0.92–1.699, $\chi^2 = 1.587$, $P = 0.208$), and among those with different indications (26.3%, 100%, 50%, 100%, 0%, 100%, 100%, 0%, and 0% for IBS, NAFLD, HE, GERD, GA, AH, HC, FD, and ADHD, respectively; $P = 0.120$) (Table 1). The rate was not significantly different among patients who received WMT via upper, middle, and/or lower gastrointestinal tract delivery route; however, the rate with middle gastrointestinal tract delivery route only appeared to be higher than that with lower gastrointestinal tract delivery route only (58.33% *vs* 16.67%, OR = 7, 95% CI: 0.613–79.871; $P = 0.152$). There was no significant difference in the age (60.38 ± 14.25 *vs.* 55.00 ± 20.67 years; $P = 0.422$), course times of WMT (2.46 ± 1.13 *vs* .2.74 ± 1.66 times; $P = 0.607$), frequencies of WMT (7.15 ± 3.44 *vs.* 8.32 ± 4.97 times; $P = 0.471$), and duration from the completion WMT to last ^{13}C-UBT (428.23 ± 262.17 *vs.* 600.89 ± 424.31 days; $P = 0.202$) between patients with and without *H. pylori* eradication (Table 1). Data were available on intestinal barrier function for all patients and on gastric function for 31 patients (one patient who remained positive for *H. pylori* infection did not undergo the test). There were no significant differences in the values of DAO (6.03 ± 6.00 U/L *vs.* 4.92 ± 2.97 U/L), D-lactate (13.06 ± 6.913 U/L *vs.* 13.91 ± 8.43 U/L), LPS (7.06 ± 8.65 U/L *vs.* 9.82 ± 7.18 U/L), PG I (126.33 ± 56.73 μg/L *vs.* 138.44 ± 88.39 μg/L), PG II (12.10 ± 7.27 μg/L *vs.* 19.66 ± 15.51 μg/L), and G-17 (5.65 ± 11.36 pmol/L *vs.* 8.02 ± 11.45 pmol/L) between patients with and without *H. pylori* eradication.

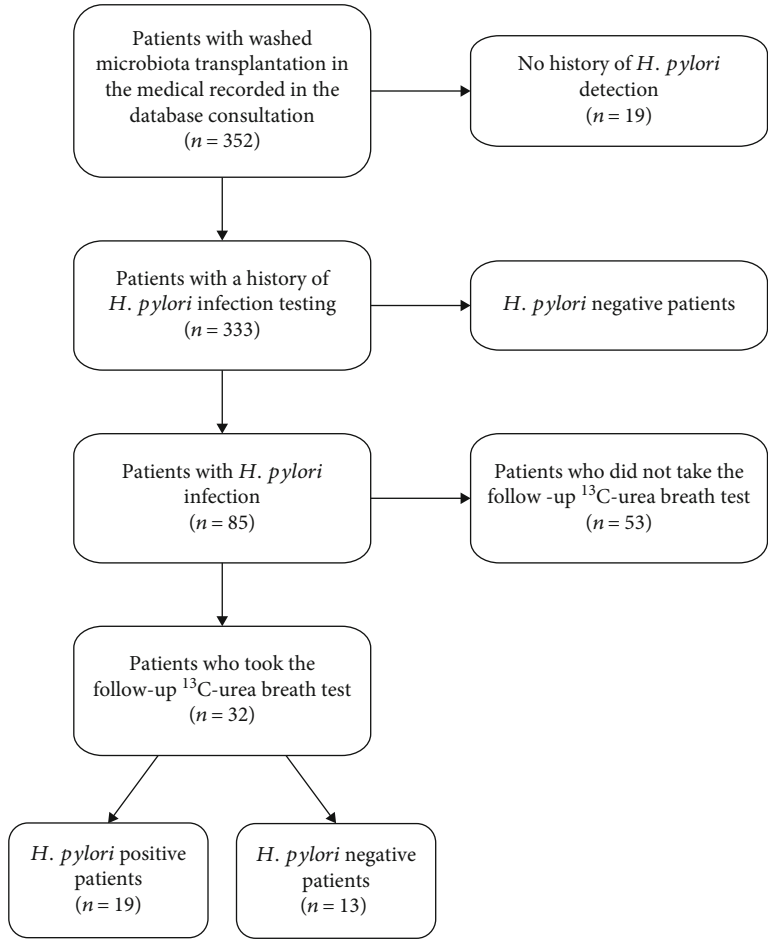

Figure 1: Flowchart for the inclusion of patients.

The PGR was significantly higher in patients with *H. pylori* eradication than those in whom *H. pylori* infection was persistent (13.00 ± 6.97 *vs.* 8.31 ± 3.73; $P = 0.022$). However, the subsequent multivariate logistic regression analysis did not show any association between pre-WMT PGR and the efficacy of WMT (OR = 1.152, 95%: 0.959-1.384, $P = 0.130$).

4. Discussion

In this study, we found that 40.6% of patients who received WMT had *H. pylori* infection eradicated after the treatment, which was significantly associated with an increased pre-WMT PGR but not with patient age, gender, indications, delivery route, course and frequency of WMT, and the intestinal function.

In the present study, we found that WMT, a microbial therapy, had a certain efficacy on *H. pylori* infection. If confirmed, it is undoubtedly a breakthrough for the traditional eradication therapy that relies significantly on antibiotics, as WMT can be used as a direct or indirect means for *H. pylori* infection. Although *H. pylori* infection can be eradicated by triple or quadruple antibiotic-based therapy in over 80% of patients, the problem of *H. pylori* resistance has gradually emerged with the extensive use of antibiotics [5, 7]. The multidrug resistance rate of *H. pylori* varies from 10% to 40%, and even sextuple resistance has been detected in some countries [28]. The increased antibiotic resistance in *H. pylori* will further reduce patients' quality of life and increase the cost-effectiveness of antibiotic-based therapy [8]. In addition, prolonged eradication therapy for *H. pylori* infection also leads to dysbiosis of the intestinal microbiota and increases the expression of resistance genes, which may further induce various diseases [12, 15, 16, 29]. However, with the emergence of WMT and fecal suspension capsules, the safety and convenience of WMT have been significantly improved [23, 30]. It is notable that WMT may be used for refractory *H. pylori* infection (defined as those who have failed the first eradication treatment or with recurrence of *H. pylori* infection). Chen et al. found that *Lactobacillus rhamnosus* and *Lactobacillus acidophilus* had effective antimicrobial activity against multidrug-resistant *H. pylori* by inhibiting *H. pylori*-induced inflammation and promoting the growth of probiotics [31]. WMT, which is also a microbial therapy, may affect the colonization of *H. pylori* by increasing the abundance of the above probiotics and reducing the inflammation caused by refractory *H. pylori* infection. Previous studies have shown that WMT has better cost-effectiveness in treating some recurrent diseases such as *Clostridium Difficile* infection and inflammatory bowel disease, compared with conventional

Table 1: Associations of age, gender, indications, and procedures of WMT with *H. pylori* eradication by WMT.

Variable	*H. pylori* eradicated	*H. pylori* persistent	*P* value
Age	60.38 ± 14.25	55.00 ± 20.67	0.422
Gender			
Male ($n = 19$)	6 (31.58)	13 (68.42)	0.208
Female ($n = 13$)	7 (53.85)	6 (46.15)	
Indications			
IBS ($n = 19$)	5 (26.32)	14 (73.68)	
NAFLD ($n = 2$)	2 (100.00)	0 (0.00)	
HE ($n = 2$)	1 (50.00)	1 (50.00)	
GERD ($n = 2$)	2 (100.00)	0 (0.00)	
GA ($n = 2$)	0 (0.00)	2 (100.00)	0.120
AH ($n = 2$)	2 (100.00)	0 (0.00)	
HC ($n = 1$)	1 (100.00)	0 (0.00)	
FD ($n = 1$)	0 (0.00)	1 (100.00)	
ADHD ($n = 1$)	0 (0.00)	1 (100.00)	
Delivery route*			
Middle gastrointestinal tract only ($n = 12$)	7 (58.33)	5 (41.67)	
Lower gastrointestinal tract only ($n = 6$)	1 (16.67)	5 (83.33)	
Upper gastrointestinal tract and middle gastrointestinal tract ($n = 4$)	2 (50.00)	2 (50.00)	0.152
Middle and lower gastrointestinal tract ($n = 8$)	3 (37.50)	5 (62.50)	
Upper, middle and lower gastrointestinal tract ($n = 2$)	0 (0.00)	2 (100.00)	
WMT procedures			
Course times	2.46 ± 1.13	2.74 ± 1.66	0.607
Frequency	7.15 ± 3.44	8.32 ± 4.97	0.471
Duration (day)	428.23 ± 262.17	600.89 ± 424.31	0.202
Intestinal barrier function ($n = 32$)			
Diamine oxidase (U/L)	6.03 ± 6.00	4.92 ± 2.97	0.545
D-lactate (U/L)	13.06 ± 6.913	13.91 ± 8.43	0.768
Lipopolysaccharide (U/L)	7.06 ± 8.65	9.82 ± 7.18	0.332
Gastric function ($n = 31$)			
PG I (μg/L)	126.33 ± 56.73	138.44 ± 88.39	0.669
PG II (μg/L)	12.10 ± 7.27	19.66 ± 15.51	0.114
PG ratio (PG I/PG II)	13.00 ± 6.97	8.31 ± 3.733	0.022
Gastrin-17 (μg/L)	5.65 ± 11.36	8.02 ± 11.45	0.573

Data are expressed as the mean with standard deviation or number (%), where appropriate. *During WMT, an endoscopic administration tube was placed in the stomach (or upper) or jejunum (or middle) through the upper gastrointestinal tract or right hemicolon (or lower) through the lower gastrointestinal tract. $P = 0.152$, compared between patients receiving WMT *via* middle gastrointestinal tract delivery route alone and those with lower gastrointestinal tract delivery route alone. IBS: irritable bowel syndrome; NAFLD: nonalcoholic fatty liver disease; HE: hepatic encephalopathy; GERD: gastroesophageal reflux disease; GA: gouty arthritis; AH: alcoholic hepatitis; HC: hepatic cirrhosis; FD: functional dyspepsia; ADHD: attention deficit hyperactivity disorder; PG: pepsinogen.

therapies [32, 33]. Therefore, WMT may also be a good alternative to antibiotics for the eradication of refractory *H. pylori* infection. Due to the limitation of clinical data, we were unable to explore the role of WMT for refractory *H. pylori* infection in this pilot study. However, it is worthy of further in-depth investigation.

We observed that the success of WMT in eradicating *H. pylori* infection appeared to be associated with an increased PGR detected within 1 week prior to WMT. It has been demonstrated that a low PGR is a biomarker of precancerous lesions, such as atrophic gastritis, and thus indicates a high risk of developing gastric cancer [34–36]. Our observation suggests that WMT may have a therapeutic effect on *H. pylori* infection in patients with low risk of gastric cancer and those with high risk of gastric cancer may not benefit from WMT. Therefore, PGR may be used as one of the evaluation indicators for microbial intervention in the treatment of *H. pylori* infection. Further investigation is required to elucidate the mechanism for the favorite therapeutic effect of WMT in patients with a high PGR.

It is noticeable that female patients appeared to be more likely to have a higher eradication rate of *H. pylori* infection than male patients after WMT, which may be related to the differences in hormone levels. Hosoda et al. [37] found that some steroid hormones, including estradiol, androstenedione and progesterone, were effective in the inhibition of *H. pylori* infection, suggesting that high levels of estradiol, androstenedione, and progesterone in female patients may enhance the therapeutic effect of WMT in eradicating *H. pylori* infection. However, further investigation is required to determine whether the efficacy of WMT in eradicating *H. pylori* infection is better in females than in males and the underlying mechanism.

Four patients underwent WMT *via* the upper gastrointestinal tract (gastric) delivery route in the present study, but they had also received WMT *via* the middle gastrointestinal tract (small intestine) delivery route at the same time. Thus, we were unable to compare the difference in the efficacy between the three delivery routes. However, patients who received WMT *via* middle gastrointestinal tract delivery route alone appeared to have a higher eradication rate than those with a lower gastrointestinal tract delivery route although the difference was not statistically different, most likely due to the small number of cases in this preliminary study. The ability of the translated fecal microbiota to spread and colonize into the stomach may contribute to the difference in the eradication rate. *H. pylori* specifically colonizes in the stomach, where the low pH is hostile to the growth of other microbiota [3, 38]. Among the three delivery routes, the distance between the lower route (*i.e.*, the right hemicolon) and the stomach is the longest, and the pH of the upper route (*i.e.*, the stomach *per se*) is the lowest; thus, the fecal microbiota translated through these two routes may be difficult either to reach the stomach or to hardly survive in the low-pH environment of the stomach. Therefore, the middle route (*i.e.*, the jejunum) may be the most favorable location for the translated fecal microbiota to adapt, spread, and colonize the stomach, whereby exhibiting anti-*H. pylori* effects. However, many patients in the present study received several courses of WMT through more than one sites since the original purpose of WMT was for indications other than *H. pylori* infection; we could not determine the optimal delivery route. Therefore, well-designed studies with a large number of patients are needed to observe the optimal delivery route for the treatment of *H. pylori* infection with WMT.

It should be mentioned that although all patients enrolled in this study had never received *H. pylori* eradication therapy, some patients had received antibiotics for various conditions within one year prior to WMT. However, they did not take two or more antibiotics simultaneously and the duration of antibiotic use did not last for more than a week. It is generally accepted that successful eradication of *H. pylori* infection can only be achieved by a combined administration of at least two antibiotics in combination with a proton pump inhibitor with or without a bismuth salt, for 7–14 days [39]. It is rare, if any, that a single antibiotic can successfully eradicate *H. pylori* infection. To further confirm the efficacy of WMT for *H. pylori* infection, we analyzed the therapeutic effect of WMT in the patients who were positive for *H. pylori* infection by at least two tests or at least twice by a single test before WMT and observed that the eradication rate remained 28.57%. A serology test for IgG anti-*H. pylori* antibody was used as one of the diagnostic methods in the present study due to its high accuracy in the diagnosis of *H. pylori* infection [40]. It has been demonstrated that the existence of IgG anti-*H. pylori* antibody in patients represents current active *H. pylori* infection if the patients have never received any eradication therapy, which is the case in the present study, as spontaneous elimination of *H. pylori* infection in adults is extremely rare [41]. There might be a possibility that false-negative post-WMT UBT results were obtained in some patients. However, ^{13}C-UBT has been used in clinical practice as a standard method to determine *H. pylori* status after *H. pylori* eradication therapy, due to its high sensitivity and specificity [42–44]. Thus, it is unlikely that the false-negative results ^{13}C-UBT occurred in nearly 30% of cases after WMT. Therefore, we believe that our observation was not opportunistic. It should be acknowledged that the sample size of the present study was relatively small, due to the stringent inclusion criteria of this study, which may affect the accuracy of the study results to a certain extent. However, the present study, for the first time, demonstrates the therapeutic efficacy of WMT for *H. pylori* infection and thus provides a novel direction for searching regimens with promising efficacy in the eradication of *H. pylori* infection. In the future, more attention should be paid to optimization of WMT, confirmation of the efficacy, as well as safety, of WMT, especially in combination with currently recommended regimens for *H. pylori* infection in randomized controlled trials, determination of the influencing factors, and elucidation of the underlying potential mechanisms.

5. Conclusion

H. pylori infection was eradicated in a proportion of patients who received WMT. An increased pre-WMT PGR appeared to be associated with the therapeutic effect of WMT. Further clinical studies are required to confirm the efficacy, as well as safety, of WMT, especially in combination with currently recommended regimens in randomized controlled trials.

Data Availability

The [DATA TYPE] data used to support the findings of this study are available from the corresponding author upon request.

Disclosure

The funders have no role in study design, data collection and management, interpretation of data, writing of the report, and the decision to submit the report for publication.

Conflicts of Interest

All authors have no conflict of interest in this study.

Authors' Contributions

ZNY and HHXX jointly analyzed the data and wrote this article. RZ, LL, LHW, and XJL collected and analyzed the clinical data. WRX collected and analyzed the data on the detection of intestinal barrier function and gastric function. XXH designed the study and revised this article. All authors read and approved the manuscript. Zhi-Ning Ye and Harry Hua-Xiang Xia contributed equally to this work.

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