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Changes in microbiome composition after fecal microbiota transplantation via oral gavage and magnetic navigation technology-assisted proximal colon/cecum enema in antibiotic knock-down rats: a comparative experimental study

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Abstract

Background Fecal microbiota transplantation (FMT) transfers fecal matter from a donor into the gastrointestinal tract of a recipient to induce changes to the gut microbiota for therapeutic benefit; however, differences in the composition of gut microbiota after FMT via different donor material delivery routes are poorly understood. In this study, we first developed a novel technique for FMT, magnetic navigation technology(MAT)-assisted proximal colon enemas, in healthy Sprague–Dawley rats. Besides, the difference in fecal microbiota composition after FMT via oral gavage and proximal colon/cecum enemas was determined in antibiotic knock-down rats, in addition to the impact on intestinal barrier function.

Methods A device consisting of an external magnet and a magnet-tipped 6 Fr tube was used in the MAT group ($n=6$), and the control group ($n=6$) where fecal matter was delivered without magnetic navigation. The feasibility and safety of this method were assessed by angiography and histology. Next, the fecal microbiota of donor rats was transplanted into antibiotic knock-down rats via oral gavage ($n=6$) and MAT-assisted proximal colon/cecum enema ($n=6$) for a week. Analysis of fecal 16 S rRNA was conducted to determine differences in the composition of gut microbiota between different groups. The rat intestinal barrier integrity were evaulated by H&E and ZO-1/MUC2 immunofluorescence staining.

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Results The end of the fecal tube could be placed in the cecum or proximal colon of rats in MAT group; however, this was rarely achieved in the control group. No colon perforation or bleeding was detected in either group. After fecal microbiota transplantation, the microbiota α-diversity and β-diversity were comparable among the different delivery routes.At the family level, the relative abundances of *Muribaculaceae*, *Oscillospiraceae*, and *Erysipelotrichaceae* were higher in the gavage treatment group, whereas *Lactobacillaceae* and *Saccharimonadaceae* were higher in the enema treatment group (all $p < 0.05$). FMT by enema was superior to gavage in maintaining the integrity of the rat intestinal barrier, as assessed by an elevation in the density of goblet cells and increased expression of mucin-2.

Conclusions Fecal microbiota tube placement using magnetic navigation was safe and feasible in rats.Different delivery route for FMT affects the gut microbiota composition and the integrity of the rat intestinal barrier. Future experimental designs should consider the colonization outcomes of critical microbial taxa to determine the optimal FMT delivery routes in scientific research as well as clinical practise.

Keywords Fecal microbiota transplantation, Delivery route, Gut microbiota composition, Antibiotic knock-down rat

Introduction

Fecal microbiota transplantation (FMT), a procedure involving the transplantation of healthy donor stool into a recipient's gastrointestinal tract to directly alter gut microbiota and reshape its composition, has been widely recognized as both a method to determine the microbiome's causal role in gut dysbiosis-related diseases models and a novel disease-modifying therapy with demonstrated clinical benefits [1–4].

Multiple routes of FMT delivery with different locations of donor material infusion are available in clinical practice, such as colonoscopy of the proximal colon or terminal ileum and/or cecum, feeding tubes for the stomach or duodenum and/or proximal jejunum, and capsules for different locations, depending on the material of the capsule being used [5, 6]. A systematic study concluded that for the treatment of recurrent *Clostridioides difficile* infection, the cure rates of FMT differed depending on the location of the donor material infusion [6]. Thus, the location of the donor material infusion may influence the degree of donor microbial engraftment. However, there has been no research on the effect of donor material infusion location on the recipient microbiome composition after FMT.

Rodents, such as rats and mice, are among the most popular laboratory animals used in life sciences. Unfortunately, FMT delivery in rats or mice is mostly performed via oral gavage because performing FMT via colonoscopy and capsules is complex and difficult [7]. Recently, we have develop a novel method for for enteral tube placement using magnetic navigation technique and validated its effectiveness and safety in swine [8]. Based on this, a novel method for cecum/proximal colon enema using magnetic navigation technology (MAT) was developed, and its safety and effectiveness were verified in rats. Furthermore, differences in the microbiome composition after FMT via gavage and proximal colon enema and its effect on intestinal barrier integrity were also explored in antibiotic knock-down rats.

Methods

Animals

The rats used in this study were provided by the Experimental Animal Center of Xi'an Jiaotong University, which is a certified management system for the breeding and delivery of rodents devoted to life science research. All rats were handled by specially trained staff and maintained in specialized isolators. All surgical procedures were performed in a disinfected laminar-flow microbiological safety cabinet. All animal procedures were approved by the Animal Experiment Ethics Committee of Xi'an Jiaotong University and were performed in accordance with the ARRIVE guidelines and the Guide for the Care and Use of Laboratory Animals (8th edition, 2011).

Experimental design

Feasibility and safety of MAT-assisted FMT tube placement in the cecum/proximal colon of rats

Twelve male Sprague–Dawley rats were randomly assigned to one of two groups: (1) the MAT group ($n = 6$), in which the tube for FMT was placed with MAT assistance, and (2) the no MAT group ($n = 6$), in which the tube for FMT was placed without MAT. The magnetic device used in this study consisted of an external magnet and a tube with an inner magnet. The external and inner magnets were made of sintered neodymium-ferrum-boron materials (NdFeB N45). The parameters of the external magnet were a length of 3 cm, width of 1.5 cm, and thickness of 1 cm, and the parameters of the inner magnet were an inner diameter of 1.5 mm, outer diameter of 3 mm, and thickness of 2 mm. A standard 6 Fr, 30 cm flexible tube was modified by inserting an inner magnet into the distal tip (Fig. 1a and b), which was used as an FMT tube. This device is designed to be reused in multiple experiments.

Before the experiment, the rats were kept under abrosia for 8 h but had access to drinking water. The rats were individually transferred to the procedure room and placed in an anesthetic induction chamber. Anesthesia

was induced using 5% isoflurane in oxygen (2.4 L/min) for 2 min. Anesthesia was maintained using 2% isoflurane in oxygen (2.4 L/min) during the experiment. All rats in both groups were fixed on an operating plate after anesthesia. In the MAT group, the tube with the inner magnet was inserted from the rat anus, and the hand-held external magnet was placed over the lower abdomen to capture the internal magnet and then moved slowly along the path of the colon to the start of the cecum (Fig. 1c). In the no MAT group, the FMT tube was inserted through the anus without any guidance. Two milliliters of 0.9% sodium chloride solution were injected into the rat intestine through the tube to simulate the FMT procedure. The tube was removed after intubation depth was recorded in both groups. After the procedure, the devices were cleaned and used for the next procedure. Each rat in the two groups underwent daily procedures for three consecutive days. After the last catheterization, the position of the catheter end was determined through catheter angiography (Fig. 1d).

After awakening, all rats returned to a normal diet and were observed for blood in the stool. All rats were euthanized by cardiac injection of potassium chloride 24 h after the procedure to observe whether there was any colon injury or perforation (Fig. 1e). Colon and cecum tissues, especially suspected areas of injury in both groups, were sampled for histopathological analysis.

Assessment of gut microbiome composition difference after FMT via oral gavage and cecum/proximal colon enema in antibiotic knock-down rats

Eighteen male Sprague-Dawley rats were administered 2.5 mL of an antibiotic cocktail (ceftriaxone sodium [350 mg/kg], gentamicin [126 mg/kg], and metronidazole [0.5 g/L]) via oral gavage twice daily for 1 weeks to construct an acute antibiotic knock-down rat model; these rats were subsequently referred to as ABx rats. They were randomly assigned to one of three groups: (1) Gavage group ($n=6$): the ABx rats in this group underwent FMT once a day for a week; the donor fecal material was administered to the stomach through the upper gastrointestinal tract; (2) Enema group ($n=6$): the ABx rats in this group were underwent FMT once a day for a week; the donor fecal material was administered to the cecum or proximal colon of rats through the anus after MAT-assisted FMT tube placement; (3) ABx group ($n=6$): these rats did not undergo FMT after antibiotic treatment. Six rats of the same strain and batch were selected as fecal material donors for normal feeding. At the end of the experiment, the characteristics of the fecal pellet microbiota from rats in the three groups (gavage, enema, and ABx) were collected and analyzed using 16 S rRNA gene sequence analysis. Finally, all rats in the three groups (gavage, enema, and ABx) were euthanized by cardiac injection of potassium chloride and colon tissue samples were collected for intestinal barrier evaluation. The experimental design is illustrated in Fig. 2. A detailed description of these methods is provided in the Supplementary Material.

Hematoxylin and Eosin (H&E) staining

Colonic segments from each animal were perfused with ice-cold phosphate-buffered saline three times and fixed with 4% paraformaldehyde for 24 h, embedded in paraffin, and cut into 4 μm-thick sections. After staining with H&E as per standard protocols, the tissue sections were microscopically scanned using PRECICE 500B (UNIC TECHNOLOGIES, Beijing, China). The colon tissues were histologically scored based on the extent of immune cell infiltration in the mucosa and submucosa, distorted crypt structure, epithelial damage, dysplasia, and edema of the serosal and muscular layers.

Immunofluorescence staining

The colon tissue sections were subjected to antigen-retrieval in Tris-EDTA buffer (10 mM Tris base, 1 mM EDTA solution, 0.05% Tween 20, pH 8.0) at 65 °C overnight. After permeabilization with 3% hydrogen peroxide solution and 3% bovine serum albumin, slides were incubated with the primary antibodies Zo-1 at 4 °C overnight. The appropriate HRP-labeled secondary antibody Muc-2 was applied prior to counter-staining of the nucleus with CY3-TSA. Slides were photographed using a confocal microscope (Olympus FV1200, 400× magnification), and arbitrary fluorescence intensity units were measured using Imaging J software.

Statistical analysis

Statistical analysis was performed using SPSS version 20.0 software (SPSS Inc., Chicago, USA). Normally distributed continuous data are presented as mean ± SEM and analyzed using an unpaired, two-tailed t-test. Continuous data with non-normal distribution are presented as median and analyzed using the Wilcoxon rank sum test. Changes in gut microbiota were analyzed using LEfSe. The Mann – Whitney U test was used to compare the taxa of the fecal microbiota in different groups. A significance level of $P<0.05$ was considered statistically significant.

Results

MAT-assisted FMT tube location in all rats

In the MAT group, upon completion of FMT tube delivery and placement, five rats had FMT tubes located in the cecum and one rat in the proximal colon. In the control group, two rats had FMT tubes located in the proximal colon, and four rats had FMT tubes located in the distal colon (Table 1; Fig. 1d). The depth of intubation was

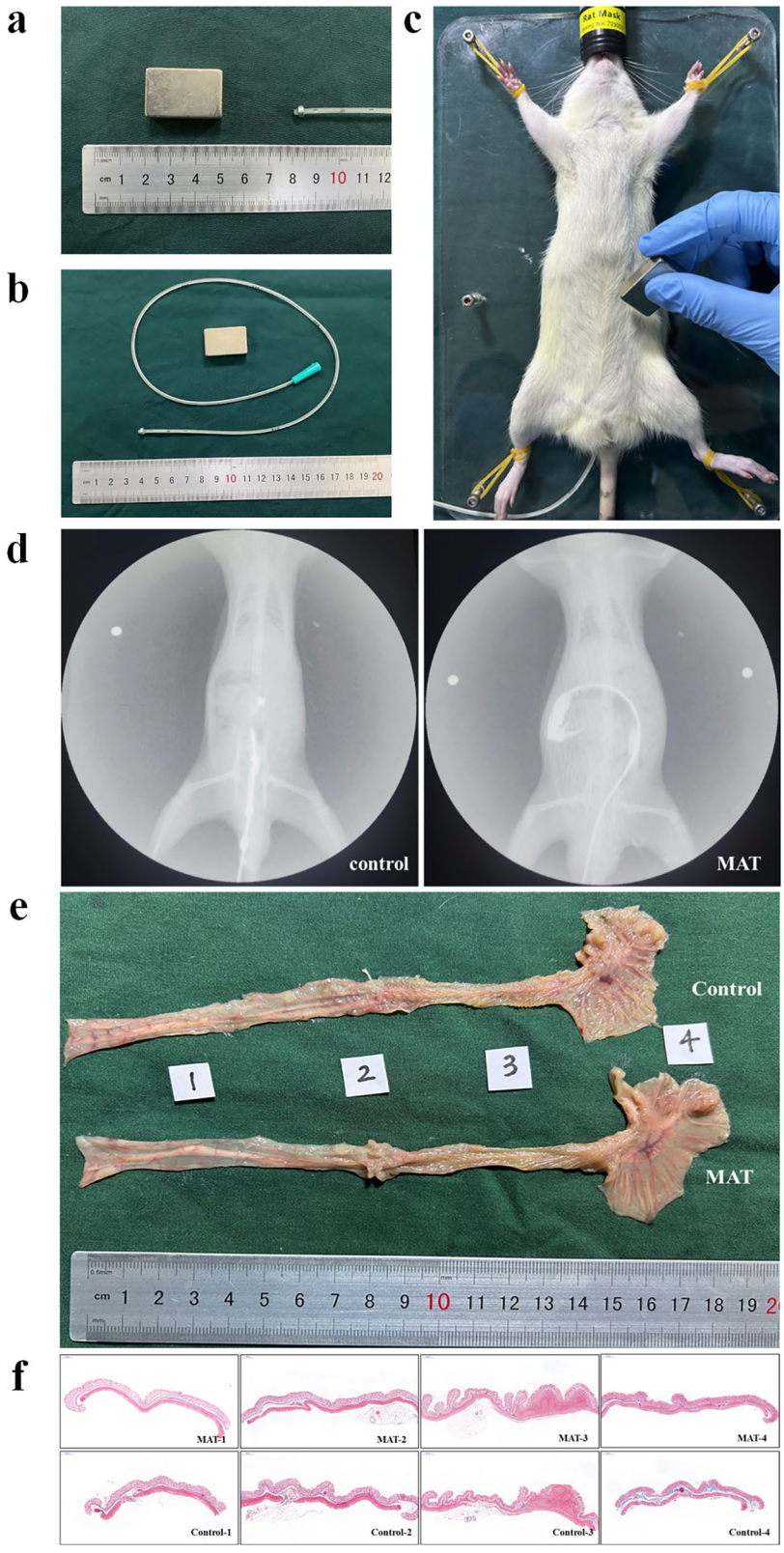

Fig. 1 The effectiveness and safety of MAT-assisted FMT tube placement in rats. (**a**) Magnetic device and operation process. (**b**) External magnets and fecal microbiota transplantation tube with internal magnets at the ends. (**c**) Fecal microbiota transplantation tube placement using a magnetic navigation technique in rats. (**d**) Positioning the FMT tube end using radiography. (**e**) Gross appearances of the colon and cecum after FMT tube placement. (**f**) Histology of different parts of the colon and cecum after FMT tube placement

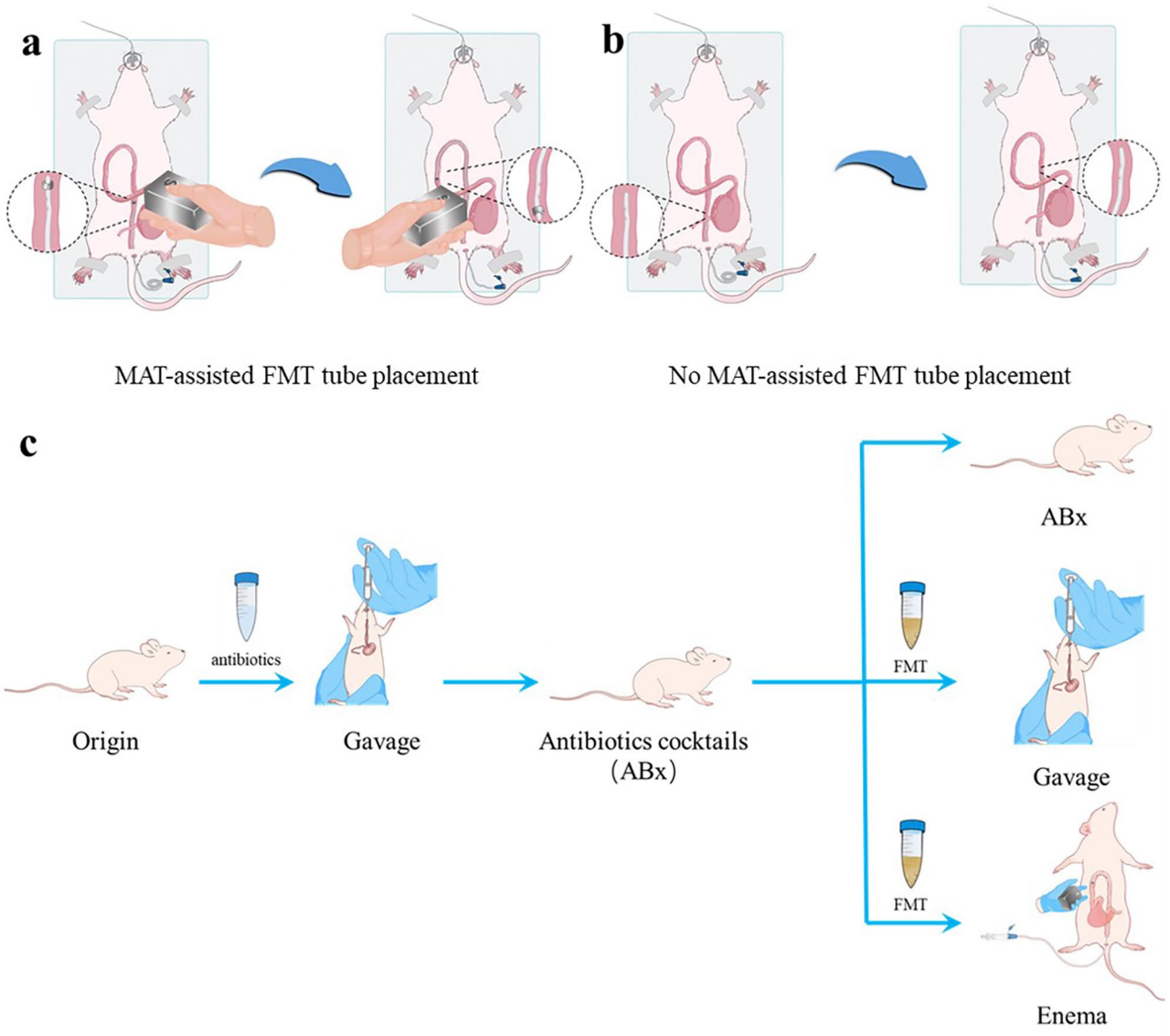

Fig. 2 Schema of the experimental design. (**a**) MAT-assisted FMT tube placement. (**b**) No MAT-assisted FMT tube placement. (**c**) Design scheme for studying the differences in the composition of gut microbiota after FMT via different donor material delivery routes

Table 1 Surgery data for the magnetic navigation technique (MAT) and control groups

	MAT ($n=6$)	Control ($n=6$)	*p*-value
Procedure time, min	5.3 [4.9–5.9]	5.1 [4.8–6.0]	0.40
Intubation depth, cm	14.6 [13.2–15.6]	7.7 [6.2–9.1]	< 0.05
Tube end position			< 0.05
Cecum	1	0	
Proximal colon	5	2	
Distal colon	0	4	

longer in the MAT group than in the control group (14.6 [13.2–15.6] vs. 7.7 [6.2–9.1], $p<0.05$). The procedure time was comparable between the two groups (5.3 [4.9–5.9] vs. 5.1 [4.8–6.0], $p=0.40$) (Table 1). No bleeding or perforation of the colon or cecum was observed in either group postoperatively (Fig. 1e). Tissue samples were obtained from four parts of the colon, and the structure of each layer of the colon was observed to be intact in both groups; no obvious mucosal damage was detected in either group (Fig. 1f).

Characteristics of the abx rats in different groups

Food intake, water intake, and weight during the FMT were compared among the three groups. No significant difference was detected in food intake among the three groups, whereas the ABx rats in the Gavage and ABx groups consumed more water than those in the Enema group ($p<0.05$, Supplementary Fig. 1a, 1b). Interestingly, rats in the Gavage group showed a slower increase in body weight than those in the Enema and ABx groups

($p < 0.05$; Supplementary Fig. 1c). Notably, four rats in the Gavage group developed diarrhea after 3 days of FMT treatment, while only one rat in the Enema group exhibited this symptom. In addition, no rats in the Abx group experienced diarrhea, except for changes in fecal color.

Diversity analysis of the gut microbiome model evaluation

A total of 1286,121 clean reads of 16 S rRNA reads were obtained from 18 samples. In total, 4,828 amplicon sequence variants (ASVs) were identified, of which 858, 1655, and 1454 were specific to the ABx, Gavage, and Enema groups, respectively (Fig. 3a). In addition, there were 77, 78, and 442 ASVs in the ABx + Gavage, ABx + Enema, and Gavage + Enema treatment groups, respectively (Fig. 3b). The α-diversity of the gut bacteria was evaluated using the Chao1, ACE, and Shannon indices. The α-diversity was significantly higher in the Gavage and Enema treatment groups than in the ABx treatment group based on the Chao1, ACE, and Shannon indices, indicating that the number of gut microbes was increased after FMT through either Enema or Gavage ($p < 0.05$, Fig. 3c and d, S2a). However, no significant difference was found in bacterial α-diversity between the Enema and Gavage treatment groups ($p > 0.05$, Fig. 3c and d, S2a). Next, the global microbial β-diversity was evaluated using QIIME 2, and the result of the principal coordinates analysis (PCoA) showed that the Enema treatment was completely separated from the ABx treatments when the PC1 contribution rate was 27.54% and PC2 contribution rate was 17.46%, while the Gavage treatment was completely separated from the ABx treatment when the PC1 contribution rate was 33.15% and PC2 contribution rate was 12.96% (Fig. 3e). In addition, the results of the principal component analysis with a PC1 contribution rate of 48.06% and a PC2 contribution rate of 14.45% showed that the Enema and Gavage groups were different (Figure S2b). The Anosim analysis showed that the global microbial β-diversity was significantly different between the three groups; however, no significant differences in the microbial community's structure were observed between the Enema treatment and Gavage treatment groups ($p < 0.05$, Fig. 3f, S2c).

Effects of donor material infusion location on the composition of the gut microbiome after FMT in ABx rats

Five phyla (*Bacillota*, *Bacteroidota*, *Pseudomonadota*, *Patescibacteria*, and *Actinobacteriota*) together accounted for more than 98% of the relative abundance in all groups (Fig. 4a). The top five bacteria in terms of relative abundance at the phylum level were *Bacillota*, *Bacteroidota*, *Pseudomonadota*, *Patescibacteria*, and *Actinobacteriota* in the three groups. Among these phyla, only the relative abundance of *Patescibacteria* was significantly different between the Gavage and Abx rats, whereas it was comparable between the Enema and Abx rats (Fig. 4a–c). Interestingly, the relative abundance of *Bacillota* in rats in the Enema group was higher than that in the Gavage group; however, the trend of *Patescibacteria* was the opposite (Fig. 4c). The changes in the gut microbiota composition at the class and order levels are shown in Figure S3a–f. At the family level, the top ten bacteria were *Muribaculaceae*, *Lachnospiraceae*, *Lactobacillaceae*, *Prevotellaceae*, *Erysipelotrichaceae*, *Oscillospiraceae*, *unclassified Clostridia UCG 014*, *Ruminococcaceae*, *Bacillaceae*, and *Peptostreptococcaceae* among the three groups (Fig. 4d-f). The relative abundances of *Oscillospiraceae* and *Erysipelotrichaceae* between the ABx and Enema groups, and *Muribaculaceae*, *Lactobacillaceae*, *Erysipelotrichaceae*, and *Oscillospiraceae* between the ABx and Gavage groups were significantly different (Fig. 4f). In the Gavage and Enema groups, *Muribaculaceae*, *Lachnospiraceae*, *Lactobacillaceae*, *Prevotellaceae*, *unclassified Clostridia UCG 014*, *Oscillospiraceae*, *Ruminococcaceae*, *Peptostreptococcaceae*, *Saccharimonadaceae*, and *Erysipelotrichaceae* were the most abundant. The relative abundances of *Muribaculaceae*, *Oscillospiraceae*, and *Erysipelotrichaceae* were higher in the Gavage group, whereas *Lactobacillaceae* and *Saccharimonadaceae* showed higher relative abundances in the Enema group (Fig. 4e–f).

A biomarker that statistically differed between the groups was also identified using linear discriminant analysis effect size, which indicated significant differences at some taxonomic levels among the three groups with a threshold score of linear discriminant analysis > 3.0 (Fig. 5a). The Cladogram assay also revealed microbiome differences among the three groups at various phylogenetic levels (Fig. 5b). At the family level, *Bacillaceae*, *Lactobacillaceae*, and *Erysipelotrichaceae* contributed to the difference in the ABx group; *Monoglobaceae* and *Peptostreptococcaceae* were responsible for the difference in the Enema group; and *Muribaculaceae*, *Oscillospiraceae*, and *Saccharimonadaceae* contributed to the difference in the Gavage group (Figure S4a–h).

Effects of donor material infusion location on the gut microbiota phenotype

Nine potential phenotypes, including aerobic and anaerobic, containing mobile elements, facultatively anaerobic, forming biofilms, Gram-negative, Gram-positive, potentially pathogenic, and stress-tolerant bacteria, among the three groups were detected via BugBase potential prediction (Fig. 6a–i, S5-7). The relative abundance of aerobic bacteria containing mobile elements, gram-positive bacteria, and stress-tolerant bacteria in the Enema group increased significantly compared with that in the Gavage group, which might be related to the increase in *Lactobacillaceae.* Furthermore, the phenotype containing

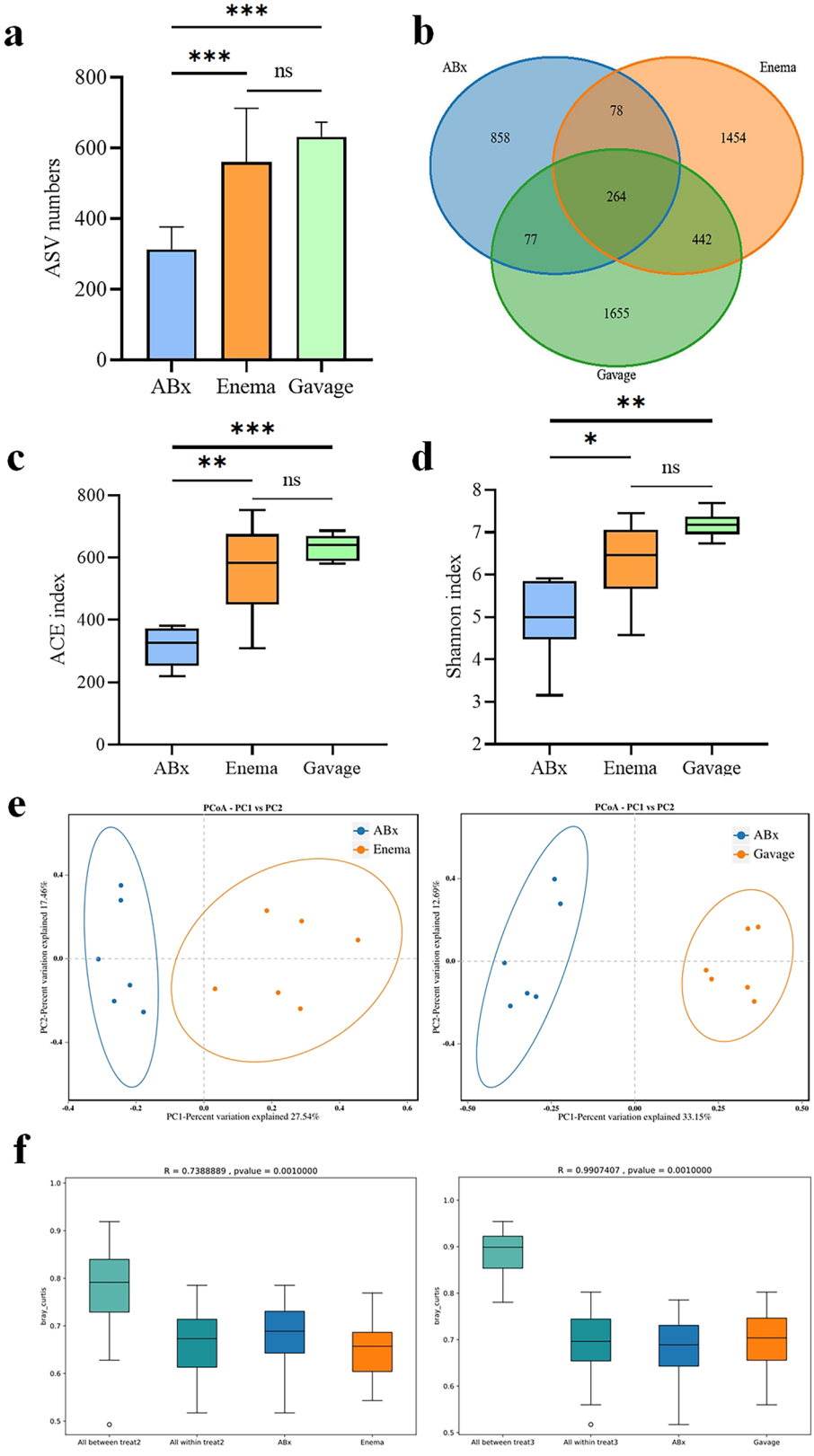

Fig. 3 Diversity analysis of the gut microbiome. (**a**) ASV numbers. (**b**) Venn graph. (**c**) ACE index of α diversities analysis. (**d**) Shannon index of α diversities analysis. (**e**) PCoA analyses. (**f**) Anosim analysis. Data are expressed as means ± SEM ($n = 6$)

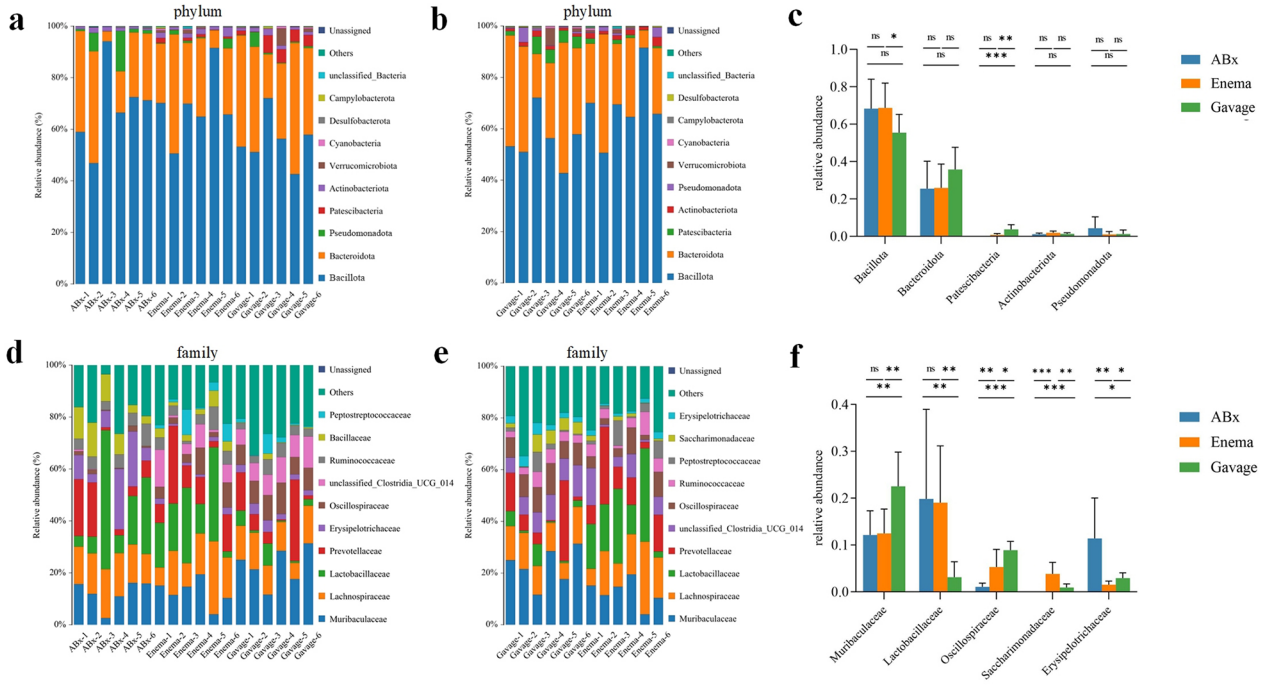

Fig. 4 The composition of the gut microbiome after FMT in rats among three groups. (**a**) Relative abundance distribution at the phylum level among three groups. (**b**) Relative abundance distribution in the Gavage and Enema group at the phylum level. (**c**) A bar graph of the phylum distribution with a TOP 5 abundance. (**d**) Relative abundance distribution at the family level among three groups. (**e**) Relative abundance distribution at family levels in the Gavage and Enema groups at the phylum level. (**f**) A bar graph of family distribution with statistical differences between the Gavage and Enema groups. Data are expressed as means ± SEM ($n = 6$)

Gram-negative and potentially pathogenic bacteria increased remarkably in the Gavage group compared to the Enema group, which might be related to the increase in *F16* and *S24-7* (*Muribaculaceae*).

Differences in intestinal barrier function among the three groups

Changes in the gut microbiota composition affect intestinal mucosal barrier function. Therefore, we tested the intestinal mucosal barrier function in rats from the three groups (Fig. 7a–h). The density of goblet cells and the expression and fluorescence intensity of the mucosal protein, mucin-2 (Muc-2), in the colon were significantly increased in the Enema group compared to those in the ABx and Gavage groups, as assessed by Alcian blue and immunofluorescence staining, respectively (Fig. 7a–c). In addition, the fluorescence intensity of zonula occludin-1 in the colon was higher in the Enema group than in the ABx group but comparable to that in the Gavage group. These results suggest that FMT treatment by enema is superior to gavage in maintaining the integrity of the rat intestinal barrier by elevating the density of goblet cells and promoting the expression of Muc-2.

Similarities in the microbial characteristics among donor, gavage and enema groups

In total, 10,678 amplicon sequence variants (ASVs) were identified, of which 5082, 2433, and 2192 were specific to the Donor, Gavage, and Enema groups, respectively (Fig. 8a). In addition, there were 124, 175, and 322 ASVs in the Donor + Gavage, Donor + Enema, and Gavage + Enema treatment groups, respectively (Fig. 8b). The α-diversity of the gut bacteria was evaluated using the ACE and Shannon indices. However, no significant difference was found in bacterial α-diversity based on the ACE and Shannon indices (Fig. 8c and d). Besides, the result of the principal coordinates analysis (PCoA) showed that the Enema treatment was completely separated from the donor when the PC1 contribution rate was 26.97% and PC2 contribution rate was 15.26%, while the Gavage treatment was also completely separated from the donor when the PC1 contribution rate was 27.07% and PC2 contribution rate was 12.96% (Fig. 8e). The Anosim analysis showed that the global microbial β-diversity was significantly different between the three groups ($p < 0.05$, Fig. 8f).

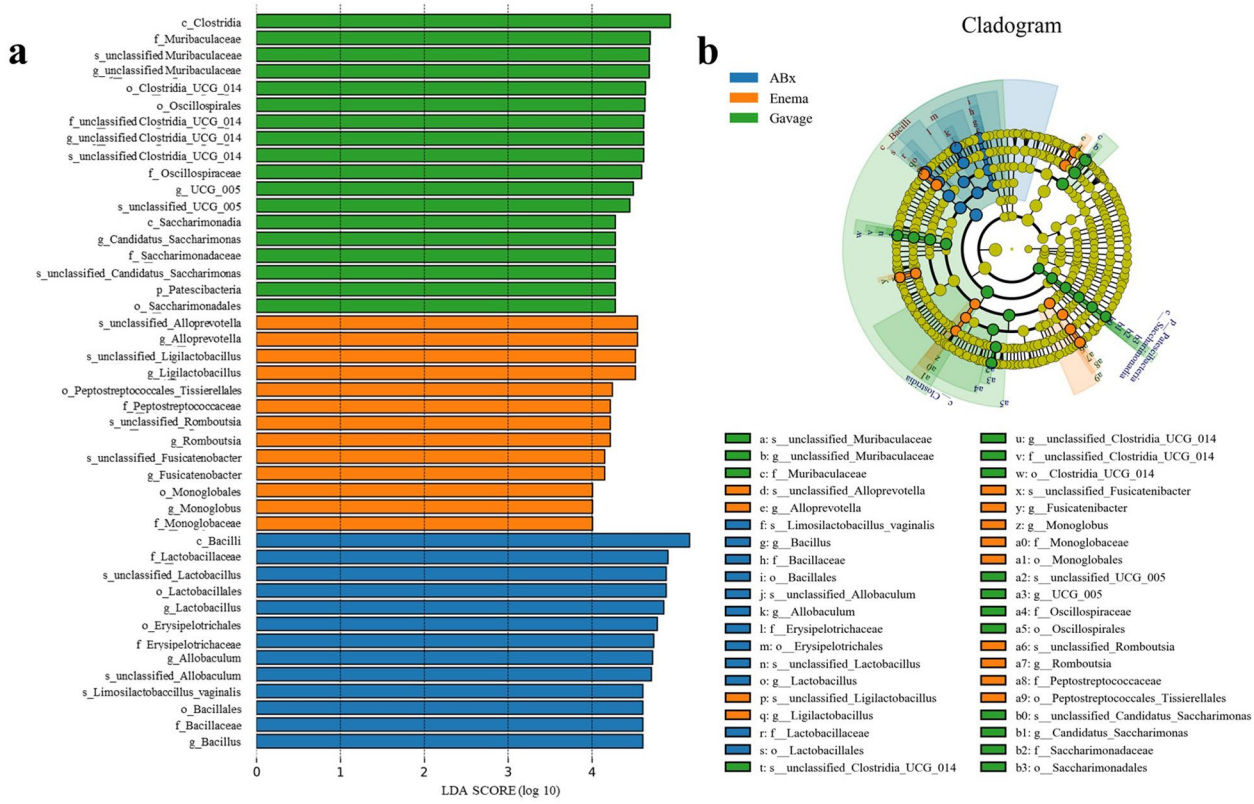

Fig. 5 Lefse branch plot. (**a**) Histogram of distribution of linear discriminant analysis values revealing the microbiome of different taxa among the three groups. (**b**) The different bacterial-rich taxa among the three groups. Green, orange, and blue show different bacterial taxa in the Gavage, Enema, and ABx groups, respectively, and yellow shows no significant differences between groups

Discussion

FMT has proven to be a successful therapy for some infectious and noncommunicable diseases [9–13]; however, these positive outcomes have rarely been replicated in other microbiome-related disorders. Initial evidence suggests that the degree of donor microbial engraftment is associated with FMT success [14, 15]. Several factors influence donor microbial engraftment, including those related to donors and recipients, as well as working protocols. Among these factors, the route of donor material delivery is considered a key determinant of clinical FMT success [16]. Different donor material delivery routes correspond to different donor material infusion locations. Currently, the common locations of donor material infusion in clinical settings include the stomach or duodenal and/or proximal jejunum via feeding tubes and the proximal colon, terminal ileum, and/or cecum via colonoscopy. Capsules can allow donor material to be released into the stomach, small bowel, and proximal colon, depending on the capsule material being used. In the present study, we attempted to detect differences in microbiome composition after FMT via different locations of donor material infusion in rats. However, in rats, the donor material can only be released in the stomach or distal colon instead of the proximal colon/cecum because performing FMT via colonoscopy or capsules is complex and difficult [7]. MAT has been used to achieve the transpyloric placement of enteral feeding tubes in the distal small bowel and colonoscopic enteral tube placement in colon [8, 17, 18]. Therefore, a novel method for placing rat FMT tubes in the proximal colon/cecum was developed in the present study. The results indicated that the FMT tube with a magnet at its end can move to the proximal colon or cecum under the guidance of another magnet, whereas it can only move to the distal colon without guidance. Moreover, no intestinal bleeding, perforation, or intestinal mucosal damage was observed after FMT tube placement using MAT. Thus, this novel method is safe and effective for placing FMT tubes in the proximal colon or cecum in rats.

Next, we continued to explore the effect of donor fecal delivery routes on receipent fecal microbiome composition in antibiotic knock-down rats. To our knowledge, this is the first animal study to focus on differences in donor microbial composition after FMT via different locations of donor material infusion. In the present study, the Chao1, ACE, and Shannon indices were applied to measure α-diversity among the three groups. The result showed that FMT via either gavage or proximal colon/cecum enema could improve gut microbiome α-diversity

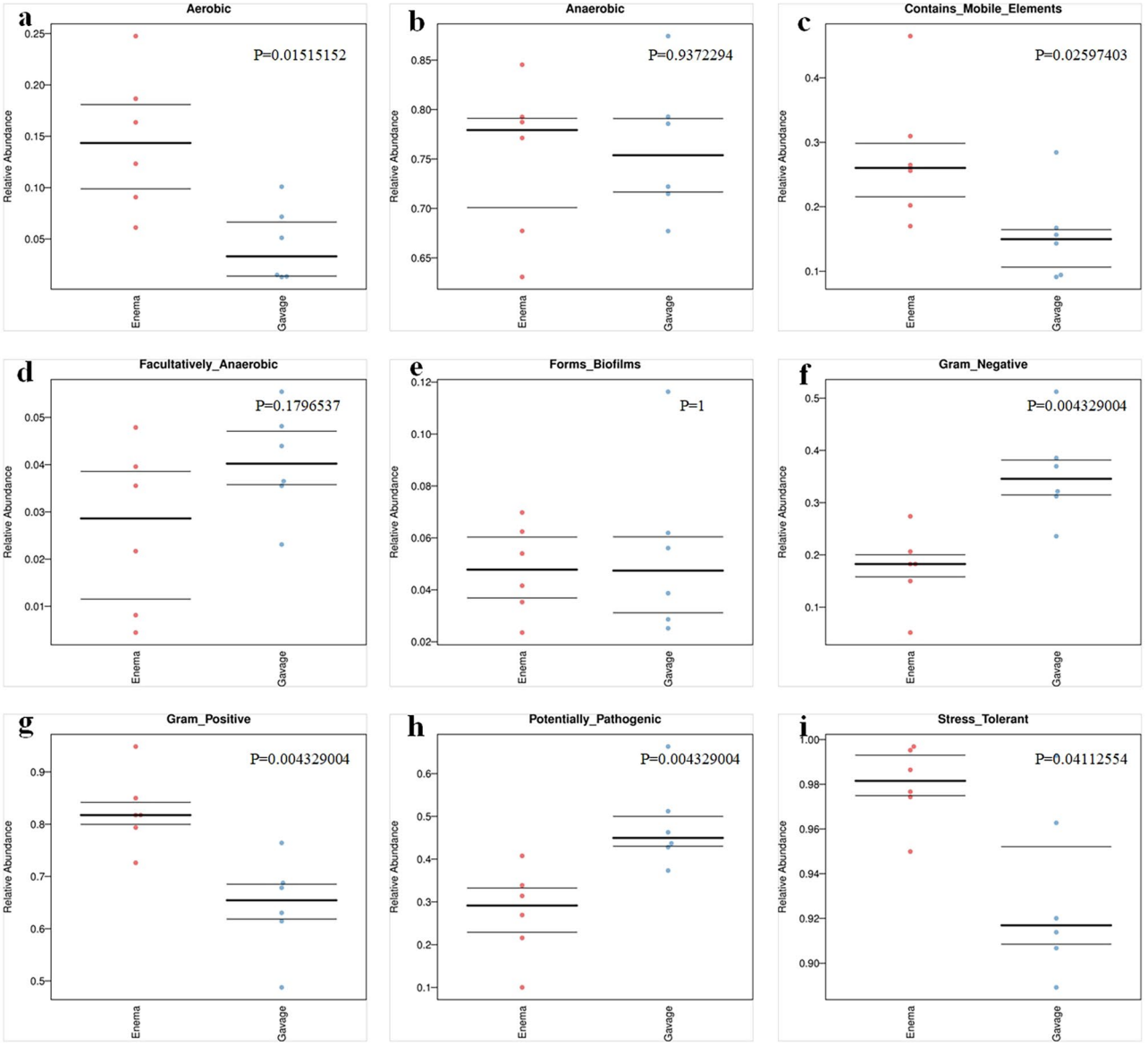

Fig. 6 Prediction of the BugBase phenotype in the Gavage and Enema groups. (**a**) Aerobic, (**b**) Anaerobic, (**c**) Containing mobile elements, (**d**) Facultatively anaerobic, (**e**) Forming biofilms, (**f**) Gram-negative, (**g**) Gram-positive, (**h**) Potentially pathogenic, (**i**) Stress-tolerant

in ABx rats. However, the diversity of the gut microbiome was comparable after FMT at different donor material infusion locations. In addition, the degree of similarity in species diversity among different samples was also evaluated by PCoA; the result showed that the gut microbiome β-diversity in ABx rats after FMT was definitely influenced by the location of donor material infusion.

As for microbial species composition analysis, *Bacillota* and *Bacteroidetes* comprised 90% of the gut microbiome [19]. *Bacillota*, including *Lactobacillaceae*, play an important role in preserving the intestinal barrier and modulating inflammation [20, 21]. In the present study, the relative abundance of *Lactobacillaceae* was higher in rats after FMT via enema than by gavage, and the intestinal mucosal barrier integrity of the rats in the Enema group seemed to be better than that in the Gavage group. In addition, the relative abundance of *Muribaculaceae*, formerly known as the S24-7 family, was higher in the Gavage group than in the Enema group. A previous study indicated that an increased abundance of *Muribaculaceae* is associated with diarrhea in weaning pigs [22]. More rats in the gavage group showed symptoms of diarrhea than those in the enema group (66.67% vs. 25%). In addition, the relative abundances of *Oscillospiraceae*, *Saccharimonadaceae*, and *Erysipelotrichaceae* differed after FMT via gavage and enema. The main reason for

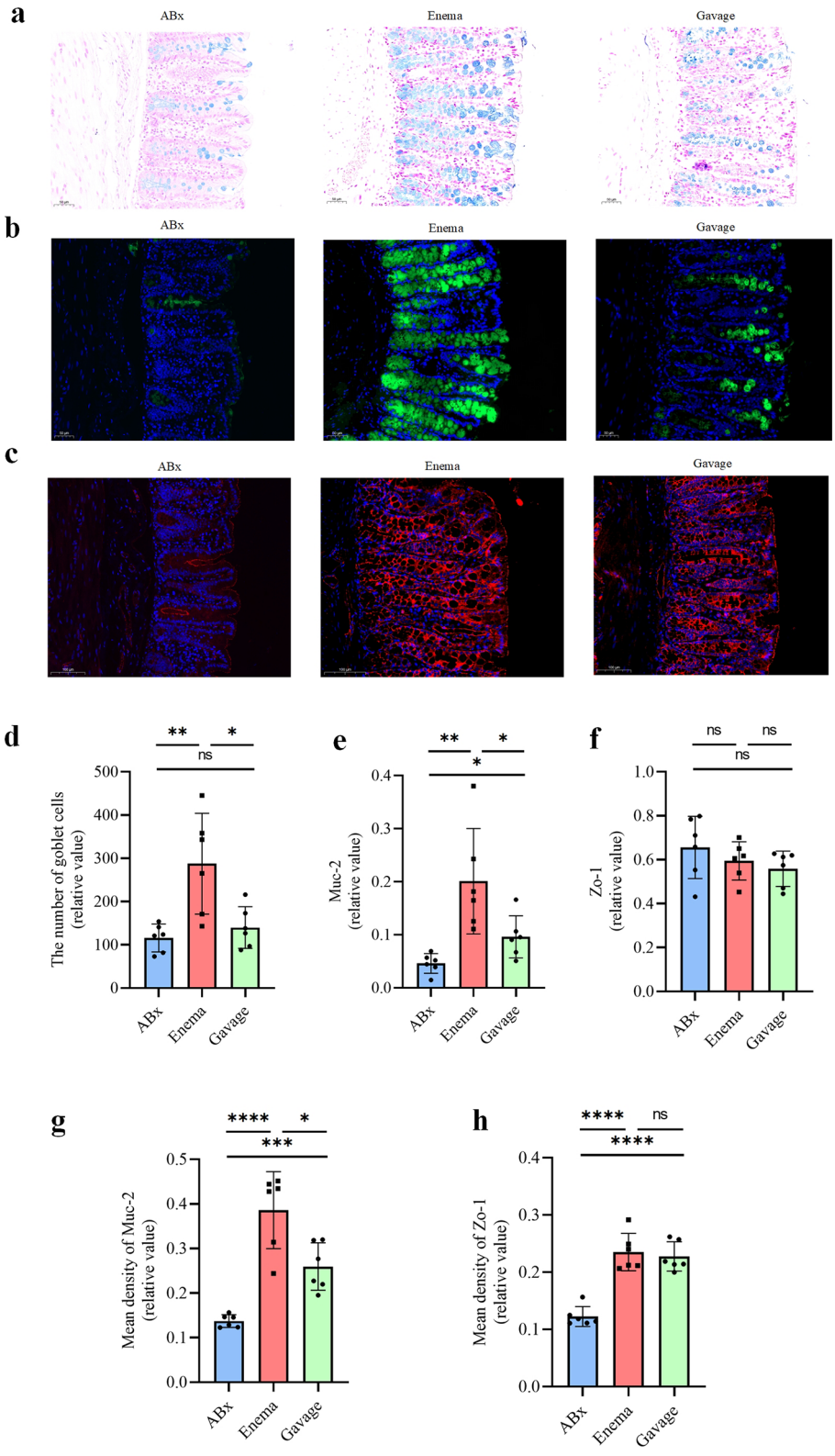

Fig. 7 Comparison of intestinal barriers in different groups. (**a**) Alcian blue staining of the colon (scale bar, 50 μm), (**b**) Representative immuno-fluorescence images of mucin-2 (Muc-2) in the colon (scale bar, 50 μm), (**c**) Representative immuno-fluorescence images of zonula occluden-1 (Zo-1) in the colon (scale bar, 100 μm), (**d**) The number of goblet cells in the colon ($n=6$), (**e**) Quantification of Muc-2 protein levels ($n=6$), (**f**) Quantification of Zo-1protein levels ($n=6$), (**g**) Quantification of Muc-2 fluorescence intensity ($n=6$), (**h**) Quantification of Zo-1 fluorescence intensity ($n=6$)

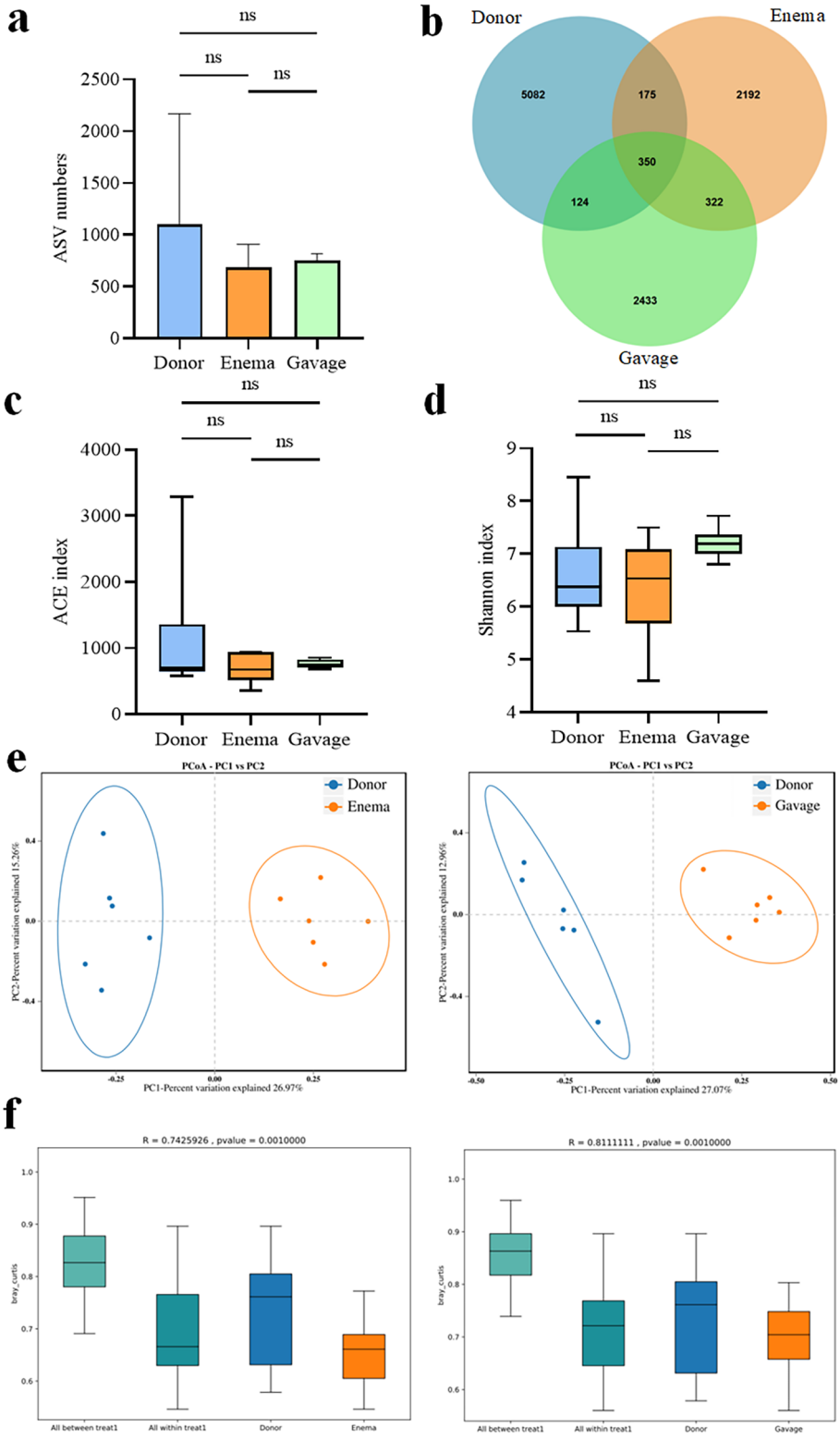

Fig. 8 Diversity analysis of the gut microbiome. (**a**) ASV numbers. (**b**) Venn graph. (**c**) ACE index of α diversities analysis. (**d**) Shannon index of α diversities analysis. (**e**) PCoA analyses. (**f**) Anosim analysis. Data are expressed as means ± SEM ($n = 6$)

these differences in recipient microbial composition may be the effects of digestive fluids (gastric acid, bile acids, and pancreatic juice) on the donor material. Interestingly, *Lactobacillaceae* are thought to survive harsh conditions [23]. However, the relative abundance of *Lactobacillaceae* was lower in rats after FMT via gavage than after FMT via enema; the reason for this phenomenon remains unclear. Notably, the relative abundances of most bacteria at the family level in ABx rats after FMT were comparable, although the location of the donor material infusion was different. In fact, there are still several factors other than the route of delivery that may influence the degree of donor microbial engraftment, including diversity and specific composition of the gut microbiome, immune system, host genetics, fecal amount and number of infusions, some adjuvant treatments, and the virome and mycobiome [16, 24].

Based on this, the location of the donor material infusion should not be ignored when FMT is used in both clinical applications and scientific research. In fact, clinical studies on fecal microbiota transplantation have found that different delivery methods may lead to different therapeutic effects. A small non-randomized cohort study found differences in multidrug-resistant organism decolonization after FMT via different delivery routes [25]. A systematic review also indicated evidence of a difference between delivery methods with respect to the response to FMT [26]. Therefore, microbiome phenotype prediction after FMT using different deliver routes was performed in the present study, and the relative abundance of aerobic bacteria containing mobile elements, Gram-positive, and stress-tolerant types in the Enema group seemed to be higher than that in the Gavage group, while the phenotype containing Gram-negative and potentially pathogenic bacteria increased remarkably in the Gavage group. These findings could contribute to the refinement of experimental designs and intervention strategies in future studies.

This study had some limitations. First, the internal magnet used in our study is made of sintered neodymium iron boron material (NdFeB N45) and coated with nickel, and its biocompatibility and effect on gut microbiome composition are concerning. However, the internal magnet only briefly remains in the body; therefore, we did not investigate these issues in the present study. Second, only two locations (stomach and proximal colon/cecum) of the donor material infusion were investigated in the present study. In fact, capsules, one of the donor material delivery routes used in clinical practice, can allow donor material to be released at different locations of the digestive tract depending on the material of the capsule being used. Unfortunately, performing FMT via capsule is difficult in rats. Clinical studies have found that capsules can achieve the same effect as colonoscopy after normalization of the gut microbiome [27]. However, this effect was partially delayed. This delay was due in part to the location of the capsular release of the FMT material. Thus, future clinical research is warranted to detect the effect of different locations of donor material infusion on the gut microbiome following FMT. Besides, in microbial diversity analysis, phenotypic predictions rely on reference databases dominated by culturable bacteria, which may introduce bias due to: (i) Strain-level variability in phenotypic traits (e.g., virulence genes absent in reference genomes), (ii) Potential misannotation of uncultured or novel taxa.

Conclusion

Compared to previous studies, we designed a simple and practical method to transplant the rat gut microbiota and successfully demonstrated its viability and feasibility in rats. In addition, the microbiome composition differed after FMT via oral gavage and proximal colon enema in antibiotic knock-down rats.

Abbreviations

FMT	Fecal microbiota transplantation
MAT	Magnetic navigation technology
ASV	Amplicon sequence variants
PCoA	Principal coordinates analysis

Supplementary Information

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Supplementary Material 1

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None.

Author contributions

Qiang Lu and Yi Lyu designed the study; Xian-Jie Bai, Yu-Chen Mei, Jia-Tong Zhao, Zhi-Ren Chen, Chen-Xi Yang, Xiao-Juan Dong, Jia-Wei Yu, Lin-Biao Xiang, Er-Zheng Zhou and Yong Chen performed the research and acquired the data; Jia-Yi Hao, Zhi-Jie Zhang and Xian-Jie Bai analyzed the data; Yu-Xuan Liuyang and Chen-Xi Yang drew schematic diagram. Xian-Jie Bai, Lu Ren, Ying-Min Yao drafted the manuscript. Qiang Lu, Lei Zhang, and Yi Lyu contributed significantly to the revision of the manuscript. All the authors have read and approved the final version of the manuscript.

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Data availability

Data is provided within the manuscript or supplementary information files.

Declarations

Ethics approval and consent to participate

All animal procedures were approved by the Animal Experiment Ethics Committee of Xi'an Jiaotong University and were performed in accordance with the ARRIVE guidelines and the Guide for the Care and Use of Laboratory Animals (8th edition, 2011).

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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