Gut Pathogens

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Fecal microbiota transplantation alleviates immunosuppressant-associated diarrhea and recurrent urinary tract infection in kidney transplant recipients: a retrospective analysis

Jianmin Hu[1†], Ding Liu[1†], Guorong Liao[1], Ying Guo[1], Min Li[1], Jun Liao[1], Hua Chen[1], Song Zhou[1], Siqiang Yang[1], Shichao Li[1], Yongguang Liu[1*] and Ming Zhao[1*]

Abstract

Background Immunosuppressant administration subsequent to organ transplantation exerts a substantial influence on gut microbiota composition, thereby affecting patients' prognosis and quality of life.

Methods and results We conducted a retrospective analysis involving 18 patients who experienced severe diarrhea or recurrent urinary tract infection (rUTI) due to prolonged immunosuppressant usage after kidney transplantation. Following episodes of severe diarrhea or rUTI, these individuals underwent fecal microbiota transplantation (FMT), resulting in notable alleviation of clinical symptoms. No unexpected adverse or serious adverse events were reported. In comparison to the pre-FMT period, the α-diversity of the intestinal microbiota in patients did not exhibit a significant difference following FMT; however, there was a notable distinction in the β-diversity and analysis of similarity (ANOSIM). In addition, our findings indicated a significant decline in the relative abundance of the bacterial genera *Veillonella*, *Enterococcus*, and *Oribacterium*, whereas a marked elevation was observed in the relative abundance of *Faecalibacterium*, *Roseburia*, *Sutterella*, *Parasutterella*, and *Ruminiclostridium 5* after FMT in patients. Furthermore, there was a notable alteration in the metabolic pathway of gut microbiota in patients following FMT, with a significant enrichment observed in pathways such as Flavone and flavonol biosynthesis, Cytoskeleton proteins, Chromosome-related processes, NOD-like receptor signaling pathway, Progesterone-mediated oocyte maturation, and Antigen processing and presentation.

Conclusion FMT exhibited an effective approach for managing rUTI and diarrhea arising from postoperative immunosuppressant exposure in kidney transplant recipients.

†Jianmin Hu and Ding Liu contributed equally to this work.

*Correspondence:
Yongguang Liu
liuyg168@smu.edu.cn
Ming Zhao
zhaoming02@hotmail.com

Full list of author information is available at the end of the article

Keywords Fecal microbiota transplantation, Kidney transplantation, Immunosuppressant, Side effects, Retrospective study

Background

Kidney transplantation is a critical therapeutic intervention for patients with end-stage renal disease, significantly improving recipient survival rates [1, 2]. Post-transplantation care aims at preserving graft function and securing long-term patient survival [3, 4]. However, the prognosis of kidney transplant recipients varies considerably due to multiple influencing factors, including infections, injuries, glucocorticoids, cytokines, growth factors, and environmental conditions [5, 6]. Despite these insights, current research has limitations, and the identified factors may not fully explain the observed variability in transplantation outcomes.

The human body harbors a diverse microbiota that colonizes various regions, including the skin, gastrointestinal tract, nasal cavity, oral cavity, and reproductive tract [7]. Studies have shown significant alterations in the gut microbiota composition of kidney transplant recipients before and after surgery. These changes are closely associated with complications such as infections, rejection, and diarrhea, ultimately impacting patient prognosis [8, 9]. Potential underlying mechanisms include immune modulation, metabolic changes, and microbial dysbiosis.

Accumulating evidence highlights a correlation between gut microbiota and postoperative complications in renal allograft recipients [10, 11]. First, long-term immunosuppressant use in kidney transplant recipients compromises immune function [12, 13], leading to gut microbiota dysbiosis [9, 14]. Second, microbiota dysbiosis may trigger intestinal complications such as *Clostridioides difficile* (*C. difficile*) infection (CDI), inflammation, and diarrhea [15, 16]. These complications exacerbate physiological dysfunction, creating a detrimental feedback loop between immunosuppression and microbiota dysregulation, ultimately contributing to poor clinical outcomes [17, 18].

Fecal microbiota transplantation (FMT), a therapeutic procedure involving the transfer of processed donor feces to recipients, aims to restore gut microbiota homeostasis and alleviate intestinal symptoms [19, 20]. FMT has proven effective for treating CDI and pseudomembranous colitis, leading to its endorsement in clinical guidelines by agencies such as the US FDA [21]. Emerging evidence also supports its role in managing immune checkpoint inhibitor (ICI)-associated colitis [22, 23], and randomized controlled trials have validated its efficacy in inflammatory bowel disease (IBD) [20, 24]. Although limited case reports suggest potential benefits of FMT in post-kidney transplantation complications [25–27], their small sample sizes and observational designs restrict definitive conclusions. Thus, rigorous clinical trials are urgently needed to evaluate FMT's efficacy in this context.

Immunosuppressant administration following organ transplantation profoundly alters gut microbiota composition, potentially compromising patients' clinical outcomes and quality of life [28, 29]. In this retrospective study, we analyzed gut microbiota changes before and after FMT in 18 kidney transplant recipients who developed severe diarrhea or recurrent urinary tract infection (rUTI) due to prolonged immunosuppressant use. Our objectives were to evaluate FMT's therapeutic efficacy and elucidate its mechanisms through microbiota structural, compositional, and metabolic pathway analyses. Post-FMT, significant symptom alleviation was observed in both diarrhea and rUTI. Mechanistically, FMT modulated gut microbiota structure, composition, and metabolic pathways, particularly enriching pathways linked to flavone biosynthesis and xenobiotic degradation. These findings suggest that FMT may optimize gut microbiota to reduce immunosuppressant-associated complications, thereby improving prognosis and quality of life in transplant recipients.

Materials and methods

Study cohort

The study was approved by the Ethics Commitment of Zhujiang Hospital, Southern Medical University (approval number 2023-KY-049-02) and conducted with adherence to the Declaration of Helsinki. All participants provided written consent to partake in the research.

A retrospective analysis was conducted at Zhujiang Hospital, Southern Medical University, to explore the medical histories of patients who developed diarrhea or rUTI after undergoing immunosuppressive therapy following kidney transplantation. These patients had previously tried adjusting immunosuppressants, specifically CellCept and Myfortic—both analogues of mycophenolate mofetil (MMF). Despite clinicians' efforts to reduce MMF dosage or replace it with Mizoribine, the patients' conditions remained unimproved. For symptomatic management, montmorillonite powder and loperamide were employed as antidiarrheal agents, accompanied by oral or intravenous rehydration to correct electrolyte imbalances, such as hypokalemia. Although these interventions provided short-term relief, they were ineffective in preventing recurrence. Concurrently, colonoscopy was conducted to exclude organic pathologies, including IBD or neoplasms, while PCR testing was performed to rule out infectious etiologies such as *C. difficile* and

Campylobacter. All enrolled patients met the criteria for chronic diarrhea (defined as lasting more than two months) or recurrent diarrhea (characterized by two or more episodes within a six-month period) and demonstrated refractoriness to the aforementioned treatments. Based on the interaction mechanism between gut dysbiosis and immunosuppressive therapy [30], FMT was introduced as a novel therapeutic strategy aimed to enhance prognosis by restoring the intestinal microecological balance. Prior to obtaining consent from patients to undergo FMT and participate in associated research studies, it was imperative for clinicians to engage in comprehensive communication and dialogue with them. This process encompassed a detailed explanation of the current state of FMT research, the potential therapeutic benefits, and the inherent uncertainties, thereby enabling patients to make well-informed decisions.

To eliminate the possibility of other gastrointestinal disorders, a thorough physical examination, blood examination, gastroscopy, and colonoscopy were conducted on the patients. FMT was carefully administered by a skilled gastroenterologist via endoscopic delivery of fecal microbiota suspension. To guarantee optimal efficiency and patient comfort, the transplant plan outlined a schedule of once every two days, ultimately culminating in a cumulative total of three successful transplants, all completed efficiently within a span of five days. The inclusion criteria for the study on diarrhea are as follows: (1) Patients aged between 18 and 85 years; (2) Patients who were undergoing immunosuppressive regimens following kidney transplantation; (3) Patients experiencing diarrhea with a frequency of three or more times per day, characterized by watery or mushy stools; (4) fecal samples from patients were analyzed for *C. difficile*, a prevalent bacterium known to induce diarrhea, using reverse transcription polymerase chain reaction (RT-PCR). Toxin genes (specifically tcdA and tcdB, targeted in the PCR assay) were analyzed for *C. difficile* in this study; all tested samples returned negative results.The exclusion criteria for diarrhea in this study included: (1) the presence of systemic diseases and immune deficiencies, pregnancy, planning to become pregnant or breastfeeding, severe mental illness, or alcohol or drug abuse; (2) patients who have received treatment with probiotics or antibiotics within 8 weeks prior to entering the study, or those who consumed a low fermentable oligosaccharides, disaccharides, monosaccharides, and polyols (FODMAP) diet; and (3) patients with other causes of diarrhea, such as nervous diarrhea or infectious diarrhea.

In this study, rUTI was characterized by the occurrence of three or more symptomatic episodes of UTI within a 12-month timeframe, or two or more episodes within a 6-month period. The clinical presentation typically encompassed lower urinary tract symptoms, including dysuria, urgency, and increased urinary frequency. The diagnostic criteria were as follows: Urinalysis indicated pyuria, defined as the presence of 10 or more leukocytes per microliter, with or without hematuria and nitrite positivity. Urine culture demonstrated significant growth of uropathogen, with a concentration of $\geq 10^5$ colony-forming units per milliliter in midstream urine. Patients with UTI were commonly administered cefoperazone sodium and sulbactam sodium (CSSS) via injection. Inclusion criteria for rUTI are as follows: (1) patients aged between 18 and 85 years; (2) patients who were undergoing immunosuppressive regimens subsequent to kidney transplantation; (3) patients with a positive urine culture indicating the presence of bacteria or positive urine white blood cells.

The exclusion criteria for patients with rUTI are as follows: (1) those with systemic diseases and immune deficiencies, (2) pregnant women, those planning to become pregnant, or currently breastfeeding mothers, (3) individuals experiencing severe mental illness or engaging in alcohol or drug abuse, (4) patients undergoing treatment with probiotics or antibiotics within 8 weeks prior to study enrollment, (5) individuals following a FODMAP diet, and (6) patients with uncomplicated rUTI, which refer to rUTI occurring in the absence of significant complexity factors such as urinary tract obstruction, structural abnormalities, or immunocompromise.

Examination of clinical laboratory indicators

Blood samples were obtained from patients using vacuum blood collection tubes with anticoagulant reagents or without additives for biochemical index detection and routine blood work. Biochemical indicators were measured using Roche biochemistry instrument following the manufacturer's protocol. Flow cytometry was employed to determine levels of white blood cells, red blood cells, platelets, and hemoglobin. C-reactive protein levels were measured using enzyme linked immunosorbent assay (ELISA).

Stool donor screening procedures

The rigorous screening process for stool donors adhered to the guidelines outlined in the Chinese Fecal Microbiota Transplantation Donor Guidelines [31, 32] and performed by Xiamen Treatgut Biotechnology Co., Ltd, China. Thorough scrutiny of the donor's medical history and lifestyle practices was undertaken to preclude any potential exposure to infectious agents or engagement in substance abuse. Comprehensive evaluations, including physical examinations and blood analyses, were conducted to eliminate the presence of gastrointestinal, metabolic, or neurological disorders. Parameters assessed during the blood examination encompassed complete blood count, blood glucose levels, electrolyte

concentrations, and inflammatory markers. Additionally, liver function tests and thyroid function tests were administered. Serological screening tests were performed to ascertain the donor's status with respect to human immunodeficiency virus (HIV), syphilis, and hepatitis A, B, and C. Furthermore, meticulous examinations were conducted to test the presence of pathogenic bacteria such as *Shigella* spp., *Salmonella* spp., *Campylobacter* spp., *Yersinia* spp., and toxin-producing *C. difficile*[31]. Screening for rotavirus, fecal ova, and parasites was also conducted. The identified donor, four current college students (two females and two males), yielded negative results across all administered tests and examinations. The donor exhibited a non-smoking habit, maintained a state of good health, did not ingest any medications, and possessed a body mass index (BMI) within the range of 18.5–23.9 kg/m^2. Importantly, the donor exhibited no familial relationships to any participants enrolled in this study.

Extraction and preservation of intestinal microbiota

The methodology employed for the acquisition and preservation of intestinal microbiota entails a rigorous screening procedure to identify suitable donors, in accordance with the aforementioned criteria [33]. In this study, a total of four fecal samples were collected from four donors. Each sample was processed individually. Briefly, fresh stool specimens weighing more than 100 g were obtained, agitated, and diluted with physiological saline. The resultant samples were subsequently tightly sealed in anaerobic containers at a temperature of 4 °C, with a storage duration not exceeding 4 h. Next, the elimination of larger particulate matter, such as residual food remnants, was accomplished through the filter screens which have inner diameters ranging from 2000 μm to 75 μm, with specific sizes including 850, 355, 250, 180, 150, 125, and 90 μm. The residual suspension was subsequently centrifuged at 300×g for 6 min under ambient temperature conditions (26–28 °C) to precipitate intestinal microbiota, ultimately yielding a bacterial pellet.

To ensure the longevity and viability of the acquired gut microbiota, a preservation solution specifically designed for intestinal microbiota (Xiamen Treatgut Biotechnology Co., Ltd) was incorporated into the bacterial liquid, which was subsequently frozen at a -80 °C freezer. Rigorous quality control measures were implemented, encompassing assessments of specifications, visual attributes, quantity, and weight across all produced materials. During transportation, it is imperative to employ dry ice to maintain optimal conditions for the preservation of the microbiota.

Fecal microbiota transplantation process

The procedure of fecal microbiota transplantation involved administering an intestinal microbiota suspension via gastrointestinal endoscopy injection. FMT was administered using a specimen collected from an individual donor according to a matching strategy reported by Zhang, Bangzhou et al. [31]. Prior to the initial FMT, bowel preparation with polyethylene glycol electrolyte powder (PEG) was performed. Subsequent transplantations ($n = 2$) within the 5-day regimen omitted repeated bowel cleansing but required ≥ 4-hour fasting (including oral intake cessation) pre-procedure. All infusions utilized endoscopically delivered, 37 °C-preconditioned microbial solutions into the duodenal bulb under monitored anesthesia, with consistent protocol adherence across all administrations. The transplantation regimen involved a frequency of once every other day, resulting in a total of three transplantations. During each transplantation session, a 50 mL volume of the suspension was injected at five-minute intervals, resulting in a total volume of 200 mL per session.

Follow-up after FMT

Patients were subjected to post-transplantation surveillance within 30 min to assess immediate adverse reactions such as diarrhea and fever. Subsequently, ongoing monitoring occurred over the course of one week to ascertain the presence of any adverse events. For extended monitoring, patients underwent telephonic follow-ups on a weekly basis for a duration of 12 weeks post-discharge. Thereafter, monthly follow-ups continued, primarily oriented towards investigating the status of FMT recipients with regard to diarrhea resolution, improvement, or exacerbation, abdominal pain manifestations, stool frequency and consistency, weight fluctuations, alterations in medical history, and modifications in medication regimens.

Stool sample collection, storage and nucleic acid extraction

Within the cohort of 18 participants under investigation, 11 subjects underwent comprehensive evaluations of their gut microbiota, both before and after FMT. These evaluations encompassed 5 cases with diarrhea (P1, P4, P5, P6, and P9) and 6 cases with rUTI (P11, P12, P13, P15, P16, and P18). The remaining seven patients underwent exclusive pre-transplantation testing. A stool sample was obtained from each patient one week prior to FMT, with a subsequent sample collected one-month post-FMT. Stool specimens were acquired from individuals, preserved in a designated solution (Xiamen Treatgut Biotechnology Co., Ltd) at room temperature, and expeditiously dispatched to the laboratory for nucleic acid extraction, library construction, and quality assessment. DNA was

extracted from approximately 0.25 g of fecal samples using the QIAamp Fast DNA Stool Mini Kit (Qiagen, CA, USA) according to the manufacturer's instructions. The concentrations and purity of the isolated DNAs were assessed using spectrophotometry (MultiskanTM GO, ThermoFisher Scientific, USA). The DNA extracts were evaluated for quality by agarose (1.5%) gel electrophoresis in 1× Tris-Acetate-EDTA buffer. Samples were stored at -20 ℃ before being used as templates for next-generation sequencing library preparation.

16S rRNA gene sequencing

The primers used were synthesized by identifying the V4 variable regions of the bacterial 16S rRNA gene. The forward primer sequence, 5'-GTGYCAGCMGCCGCGGTAA-3', and the reverse primer sequence, 5'-GGACTACNVGGGTWTCTAAT-3', were specifically designed in accordance with the 16 S Illumina Amplicon Protocol of the Earth Microbiome Project (https://earthmicrobiome.org/protocols-and-standards/16s/). 16 S rRNA genes were amplified using the above primers with the barcode. All PCR reactions were carried out in 20 μL reaction volumes with 10 μL of KAPA HiFi HotStart ReadyMix (KAPA Biosystems, USA), 0.2 μM of forward and reverse primers, and about 10 ng template DNA. Thermal cycling consisted of initial denaturation at 95℃ for 3 min, followed by 30 cycles of denaturation at 95℃ for 20 s, annealing at 60℃ for 30 s, and elongation at 72℃ for 30s. Finally, incubation at 72℃ for 10 min was performed. The DNA extracts were also evaluated for quality by agarose (1.5%) gel electrophoresis in 1× Tris-Acetate-EDTA buffer. Samples were stored at -20 ℃ before being used as templates for next-generation sequencing library preparation. We mixed the same volume of 1× loading buffer (containing SYBR green) with PCR products and performed electrophoresis on 2% agarose gel for detection. Samples with a bright main strip between 400 and 450 bp were chosen for further experiments. PCR products were mixed in equidensity ratios. Then, the mixture of PCR products was purified with the GeneJET Gel Extraction Kit (ThermoFisher Scientific). Sequencing libraries were generated using the TruSeq® DNA PCR-Free Sample Preparation Kit (Illumina) following the manufacturer's recommendations, and index codes were added. The library quality was assessed on the Qubit@ 2.0 Fluorometer (ThermoFisher Scientific) and Agilent Bioanalyzer 2100 system. Finally, the library was sequenced on an Illumina MiniSeq, and 150 bp paired-end reads were generated by Xiamen Treatgut Biotechnology Co., Ltd.

Bioinformatics analyses

Among the 18 participants enrolled in the study, 11 individuals—consisting of five patients with diarrhea (P1, P4, P5, P6, and P9) and six patients with rUTI (P11, P12, P13, P15, P16, and P18)—underwent comprehensive gut microbiota assessments both before and after fecal microbiota transplantation. In contrast, seven other patients received only pre-transplantation testing and did not undergo any subsequent evaluation of their intestinal microbiota following the transplantation. Consequently, our analysis primarily focused on the sequencing data obtained exclusively from these 11 patients who had undergone both pre- and post-FMT assessments.

Flash software [34] (version 1.2.11, https://ccb.jhu.edu/software/FLASH/index.shtml) was employed for the pur pose of splicing and implementing quality control on the off-machine paired-end data, resulting in the generation of high-quality clean reads (Table S1). Subsequently, chimeras were systematically filtered. The USEARCH software (http://www.drive5.com/usearch/) was applied to cluster valid sequences from all samples into operational taxonomic units (OTUs) at a 97% similarity threshold. Species annotation of representative sequences was conducted based on the SILVA database release 138, facilitating the elucidation of community structure at each taxonomic level. The data underwent rarefication processing, performed according to the sequence number of the smallest sample. Species with an average relative abundance less than one in ten thousand were removed. The analysis of microbial communities encompassed α-diversity analysis for assessing bacterial relative abundance and diversity using the R package microbiome v1.22.0, while β-diversity analysis was utilized to discern differences between the two groups of microbial communities using the R package vegan v2.6.4. Principal Coordinate Analysis (PCoA) based on BrayCurtis distances between OTU abundance profiles was employed to visually represent the variability in community structure between these two groups. Microbial diversity analysis, executed through R software, was employed to compare variations in the relative abundance of bacterial taxa at the phylum, class, order, family, and genus levels.

Prediction of metabolic functions using Kyoto encyclopedia of genes and genomes (KEGG) and clusters of orthologous groups (COG) analyses

To predict the functional attributes of microbial communities based on 16 S rRNA gene sequencing data, we employed the Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt2) approach (http://picrust.github.io/picrust/) [35]. This method utilized comprehensive 16 S rRNA sequences from bacteria with known genomes to infer the functional gene profiles (including homologous genes) of their last common ancestor. By constructing a phylogenetic tree based on species taxonomy, PICRUSt2 maps the composition of the sequenced microbiota to the

KEGG and COG databases, enabling the prediction of the metabolic potential of the microbiota.

Statistical analysis

Statistical analysis was performed using SPSS (version 21.0) and R (version 4.3.1).

Comparisons among the three groups were performed using the Kruskal-Wallis test, followed by paired Dunn's test for post-hoc analyses. Inter-group differences were assessed via the independent t-test or Mann-Whitney U test, depending on data distribution. Data are presented as mean ± standard deviation. The paired Wilcoxon test was used for the analysis of alpha diversity between baseline and post-FMT treatment. To identify differentially abundant bacterial taxa at the genus level, linear discriminant analysis effect size (LEfSe, http://huttenhower.sph.harvard.edu/lefse/) [36] was applied to OTUs. Permutational multivariate analysis of variance (PERMANOVA) [37] was employed as a non-parametric approach to evaluate significant differences in gut microbiota composition between groups. Statistical significance thresholds were defined as $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$. All P-values were adjusted for multiple comparisons using the Benjamini-Hochberg false discovery rate (FDR) correction.

Results

Clinical characteristics of patients who were exposed to immunosuppressants following kidney transplantation

This study encompassed a cohort of 18 patients who received kidney transplantation between 2020 and 2022. Subsequent to the kidney transplantation, all patients were administered substantial doses of immunosuppressants to mitigate the risk of rejection. Among them, 10 experienced severe diarrhea, while the remaining 8 patients endured persistent rUTI. From 2020 to 2022, these patients underwent FMT, with subsequent efficacy evaluations and monitoring extending through to 2023. Additionally, only 11 patients underwent 16 S rRNA sequencing of their gut microbiota before and after FMT (Fig. 1A).

Among the subset of patients who exhibited severe diarrhea, 3 were male and 7 were female. The age range of these patients spanned from 25 to 63 years, with a median age of 43 years. Additionally, the patients' BMI ranged from 14.5 to 25.8, with a median BMI of 19.84. Notably, five of the patients had a medical history involving surgical procedures or prior conditions such as hypertension, thyroidectomy, tuberculous pleurisy, and colectomy (Table 1).

rUTI were observed in a cohort of 8 additional patients, consisting of 1 male and 7 females. The age range of these patients was 31 to 65 years, with a median age of 48 years. The patients had a BMI ranging from 15.4 to 25.6, with a median BMI of 20.19. Among these patients, two had a history of surgery or previous medical conditions, including hypertension and uterine polyps. Furthermore, four patients showed a lack of resistance to beta-lactam antibiotics, while four patients demonstrated resistance to beta-lactam antibiotics. In addition, 1 patient tested positive for *Klebsiella pneumoniae*, and 7 patients tested positive for *Escherichia coli* (Table 2).

FMT significantly improves immunosuppressant exposure-related complications in patients who received kidney transplantation

To assess the effectiveness of FMT, the frequency of diarrhea and the average urinary white blood cell (WBC) count were recorded for patients pre- and post-FMT. Data revealed that patients with pre-transplantation diarrhea experienced a frequency of diarrhea ranging from 3 to 10 times, with an average of 6.9 times. One month after transplantation, the frequency of diarrhea decreased to 0–2 times, with an average of 0.6 times. Six months after transplantation, the frequency of diarrhea ranged from 0 to 3 times, with an average of 0.6 times. After 12 months, the frequency of diarrhea ranged from 0 to 3 times, with an average of 1 time. These results indicate a significant reduction in the frequency of diarrhea among patients following fecal microbiota transplantation (Fig. 1B). The average urinary leukocyte score of patients diagnosed with rUTI prior to transplantation ranged from 1.00 to 2.00, with a mean score of 1.40. Following transplantation, the average urinary leukocyte score ranged from 0.00 to 1.30, with a mean score of 0.67, demonstrating a statistically significant reduction in score (Fig. 1C). Additionally, no unexpected adverse or serious adverse events were reported.

Intestinal microbiota composition of patients before and after FMT

In this study, within the investigated cohort of 18 participants, 11 subjects underwent comprehensive assessments for gut microbiota both pre- and post-FMT, including 5 cases of diarrhea (P1, P4, P5, P6, and P9) and 6 cases of rUTI (P11, P12, P13, P15, P16, and P18). The remaining 7 patients exclusively underwent pre-transplantation testing. Next, using 16 S rRNA sequencing of these 11 patients, data revealed the presence of 98 distinct bacterial genera in the fecal samples of patients prior to FMT, while post-FMT samples exhibited 88 unique bacterial genera. Besides, 264 bacterial genera were shared both pre- and post-FMT (Fig. 2A). The relative abundance of intestinal microbiota pre- and post-FMT underwent scrutiny across various taxonomic levels. At the genus level, *Veillonella* predominated before FMT, whereas post-FMT, *Bacteroides* exhibited the highest relative abundance (Fig. 2B). At the phylum level, an

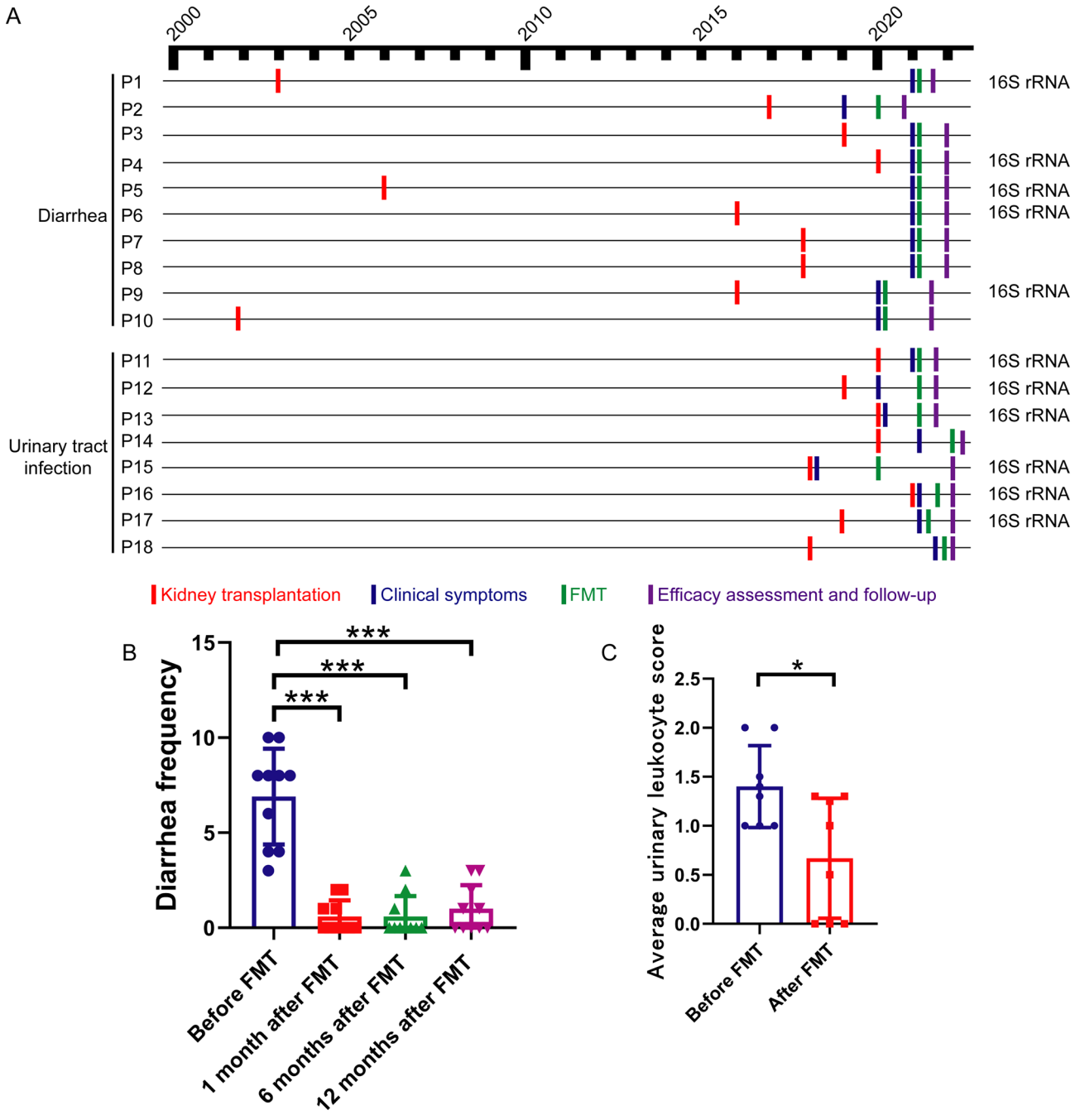


Fig. 1 FMT significantly improves immunosuppressant exposure-related complications in patients who have undergone kidney transplantation. (**A**) Diarrhea or rUTI in patients who have received kidney transplants. (**B**) Diarrhea frequency before and after FMT in kidney transplant patients with diarrhea. (**C**) The average urinary leukocyte score before and after FMT in kidney transplant patients with rUTI. Comparisons among the three groups were performed using the Kruskal-Wallis test, followed by paired Dunn's test for post-hoc analyses. *$P < 0.05$; ***$P < 0.001$. FMT, fecal microbiota transplantation; rUTI, recurrent urinary tract infection

augmentation in the relative abundance of *Bacteroidetes* was observed post-FMT. Furthermore, at the class level, a reduction in the relative abundance of *Negativicutes* was evident post-FMT, while at the order level, a decrease in *Selenomonadales* and at the family level, a decline in *Veillonellaceae* were observed after FMT (Fig. 2C).

Diversity of intestinal microbiota in patients before and after FMT

Next, analysis of data from the aforementioned 11 subjects demonstrated a notable trend towards heightened α-diversity post-FMT, albeit this increase failed to achieve statistical significance (Fig. 3A). However,

Table 1 Clinical characteristics of patients with diarrhea

Gender		*N*
	male	3
	female	7
Age (y) /median (range)		43 (25–63)
Race		
	Han nationality	10
	minority nationality	0
BMI /median (range)		19.84 (14.5–25.8)
Surgical history and past medical history except for kidney transplantation		
	yes	5
	no	5
Course of disease (month) /median (range)		5.5 (1–24)
Stool consistency based on (Bristol Stool Form Scale)		
	Type 6: Fluffy pieces with ragged edges, a mushy stool	3
	Type 7: Watery, no solid pieces (entirely liquid)	7

Table 2 Clinical characteristics of patients with recurrent urinary tract infections

Gender		*N*
	male	1
	female	7
Age (y) /median (range)		48 (31–65)
Race		
	Han nationality	8
	minority nationality	0
BMI /median (range)		20.19 (15.4–25.6)
Surgical history and past medical history except for kidney transplantation		
	yes	2
	no	6
Course of disease (month) /median (range)		5 (2–11)
Frequency of urinary tract infections / median (range)		3.5 (2–5)
Urine culture		
	beta-lactam resistant (-)	4
	beta-lactam resistant (+)	4
	Klebsiella pneumoniae (+)	1
	Escherichia coli (+)	7

a significant difference was observed in the ANOSIM index, a measure of group similarity, with an R-value of 0.148 and a P-value of 0.0273 (Fig. 3B). Additionally, both Principal Component Analysis (PCA) and PCoA, which reflect β-diversity in the gut microbiota, showed distinct differences between patients before and after FMT (Fig. 3C). These results indicated shifts in the intestinal microbiota composition among patients subsequent to FMT. It was worth noting that FMT has been shown to improve gut microbiota composition of patients. However, it should be emphasized that, although there were considerable improvements, the gut microbiota of these patients has not yet fully attained the level of that of healthy donors (Fig. S1 and Fig. S2).

Microbiota characterization at the genus level in patients before and after FMT

To explore distinctive genera pre- and post-FMT, LEfSe was configured with a threshold of 3. The data showed a significant decrease in the relative abundance of the bacterial genera *Veillonella*, *Enterococcus*, and *Oribacterium*, while there was a notable increase in the relative abundance of *Faecalibacterium*, *Roseburia*, *Sutterella*, *Parasutterella*, and *Ruminiclostridium 5* following FMT in patients. (Fig. 4A, C). Besides, the evolutionary relationship diagram illustrated the phylogenetic connections and taxonomic hierarchies among various bacterial families. Notably, within the pre-FMT samples, there was a significant divergence observed between *Enterococcaceae* and *Enterobacteriaceae*. Conversely, in the post-FMT samples, it was the *Burkholderiaceae* that exhibited a notable difference, demonstrating how microbial communities shift and evolve in response to FMT intervention (Fig. 4B). Moreover, differences in bacterial relative abundance before and after FMT were observed at the order and family levels (Fig. S3).

Metabolic pathways of intestinal microbiota in patients before and after FMT

After acquiring the 16 S rRNA sequencing data of the gut microbiota, we employed the KEGG database to predict metabolic functions. KEGG analysis unveiled the top 20 metabolic pathways exhibiting significant differences, with notable decreases observed in pathways such as Amino acid metabolism, Valine, leucine and isoleucine degradation, Lysine degradation, Ascorbate and aldarate metabolism, Selenocompound metabolism, Tryptophan metabolism, Caprolactam degradation, Pertussis, C5-Branched dibasic acid metabolism, Geraniol degradation, and Biosynthesis of siderophore group nonribosomal peptides following FMT. Conversely, pathways that demonstrated significant increases included Flavone and flavonol biosynthesis, Cytoskeleton proteins, Chromosome-related processes, NOD-like receptor signaling

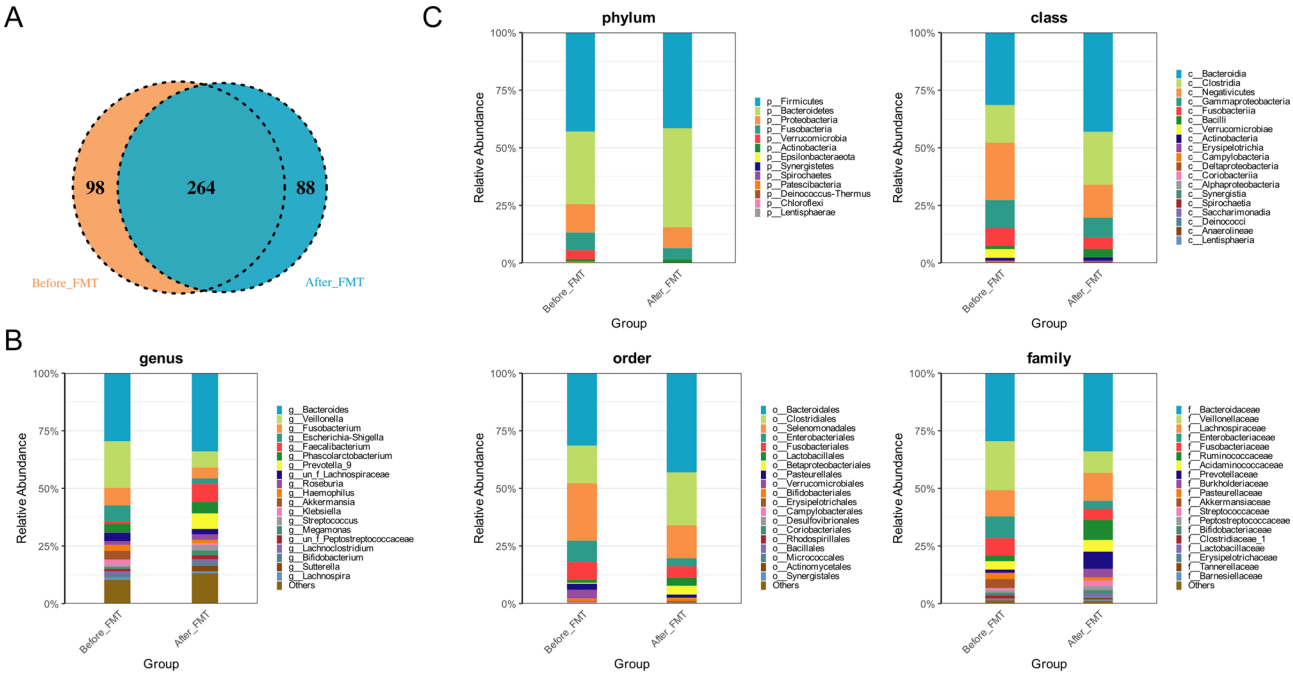


Fig. 2 Intestinal microbiota structure of patients before and after fecal microbiota transplantation. (**A**) Venn diagram depicting OTU distribution of gut microbiota before and after FMT in patients. (**B**) Histogram showing intestinal microbiota composition at the genus level before and after FMT in patients. (**C**) Histogram illustrating intestinal microbiota composition at the phylum, class, order and family levels before and after FMT in patients. FMT, fecal microbiota transplantation

pathway, Progesterone-mediated oocyte maturation, and Antigen processing and presentation (Fig. 5A). Subsequent analysis utilizing the COG database to assess the metabolic pathways, focusing on the top 20 in terms of relative abundance, revealed that several pathways exhibited a significant increase in abundance after FMT, including Uncharacterized conserved protein related to MYG1 family (COG4286), Predicted hydrolase of the alpha/beta superfamily (COG2819), Phosphoserine aminotransferase (COG1932), Predicted N6-adenine-specific DNA methylase (COG0116), Predicted DNA alkylation repair enzyme (COG4912), Plasmid maintenance system antidote protein (COG3093), Na+/phosphate symporter (COG1283), ABC-type Na + efflux pump, permease component (COG1668), Type IIA topoisomerase (DNA gyrase/topo II, topoisomerase IV), B subunit (COG0187), DNA or RNA helicases of superfamily II (COG1061), Predicted dinucleotide-utilizing enzyme (COG1712), Predicted nucleic acid-binding protein, contains PIN domain (COG1569), GTPases (COG2262), Predicted transcriptional regulator (COG3311), Prophage repressor (COG3617), Mu-like prophage tail protein gpP (COG4379). In contrast, Lipid A core-O-antigen ligase and related enzymes (COG3307) exhibited significantly inhibition post-FMT (Fig. 5B).

Correlations among intestinal bacterial taxa in patients before and after FMT

Conducting correlation analyses on the intestinal microbiota of subjects pre- and post-FMT revealed noteworthy associations between the abundances of numerous bacterial taxa. At the family level, *Lachnospiraceae* exhibited a significantly positive association with *Peptostreptococcaceae*, *Clostridiaceae*, and *Erysipelotrichaceae*. Additionally, positive associations were observed between *Fusobacteriaceae* and *Campylobacteraceae*, *Streptococcaceae* and *Lactobacillaceae*, *Clostridiaceae* and *Erysipelotrichacea*e, as well as *Tannerellaceae* and *Barnesiellaceae*. However, a negative relationship was indicated between *Bacteroidaceae* and *Veillonellaceae* (Fig. 6A). At the genus level, positive correlations were identified between *Fusobacterium* and *Lachnospira*, *Streptococcus* and *Sutterella*, *Streptococcus* and *Lactobacillus*, as well as *Sutterella* and *Lactobacillus*. Conversely, *Bacteroides* demonstrated a significant negative correlation with *Veillonella* (Fig. 6B).

Discussion

In this retrospective study, 18 patients who developed severe diarrhea or rUTI following kidney transplantation due to long-term immunosuppressant use underwent FMT, resulting in significant clinical symptom alleviation. Furthermore, we employed 16 S rRNA gene sequencing to assess the structure and composition

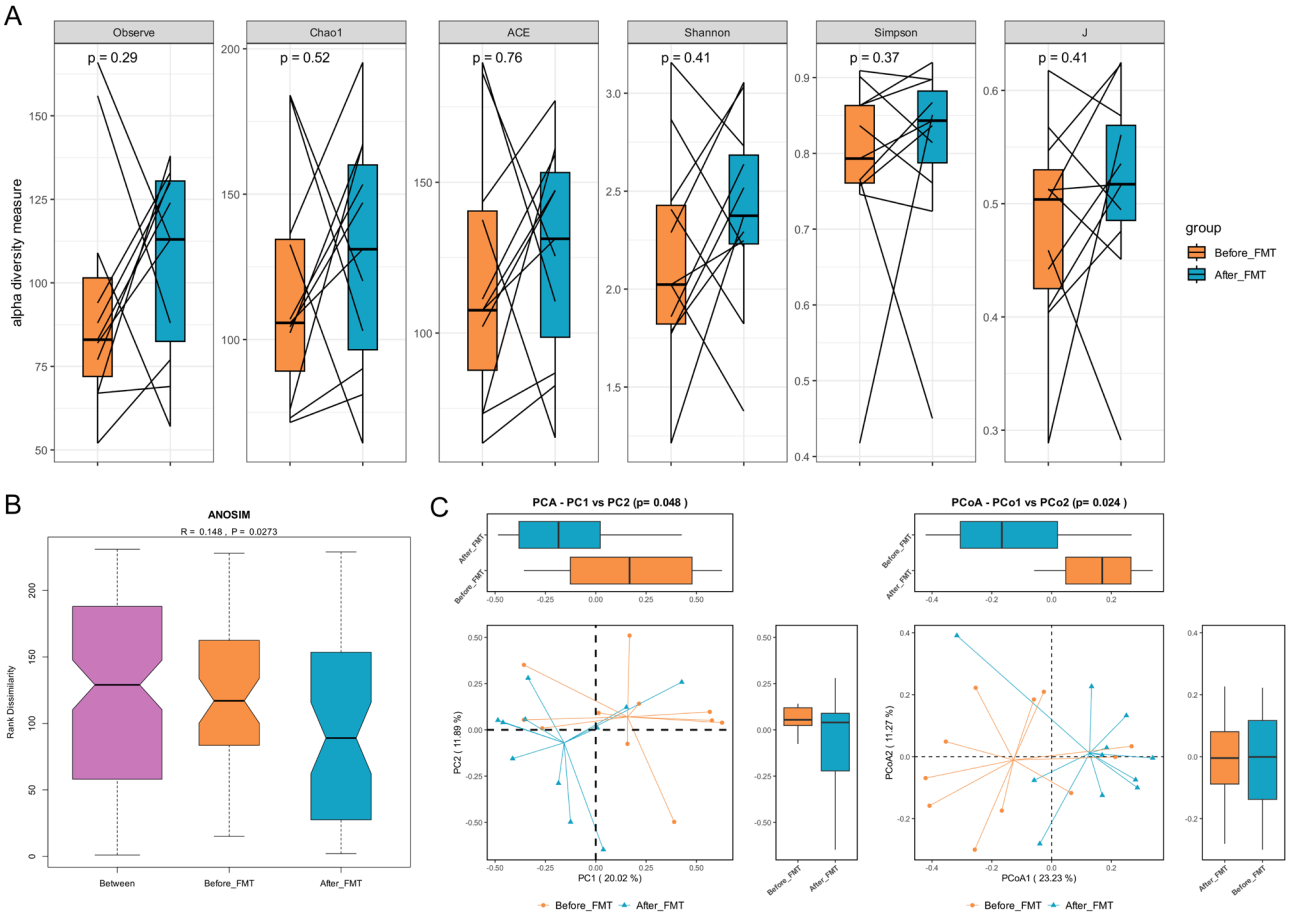


Fig. 3 Diversity of intestinal microbiota in patients before and after FMT. (**A**-**C**) The α-diversity, ANOSIM index, and β-diversity of intestinal microbiota before and after FMT in patients (*N* = 11). Sample scatter points in A showing alterations before and after transplantation. FMT, fecal microbiota transplantation

of the gut microbiota in 11 of these kidney transplant recipients before and after FMT. The results indicated that, following FMT treatment, there was no significant change in α-diversity, but a notable shift was observed in β-diversity, which could potentially underlie the improvement in clinical symptoms. Additionally, our data revealed a significant decrease in the relative abundance of bacterial genera including *Veillonella*, *Enterococcus*, and *Oribacterium*, while a notable increase was observed in the relative abundance of *Faecalibacterium*, *Roseburia*, *Sutterella*, *Parasutterella*, and *Ruminiclostridium 5* post-FMT in the patients. Moreover, significant changes were observed in the metabolic pathways of the gut microbiota following FMT, with notable enrichment in pathways involved in Flavone and flavonol biosynthesis, Cytoskeleton proteins, Chromosome-related processes, NOD-like receptor signaling pathway, Progesterone-mediated oocyte maturation, and Antigen processing and presentation. Correlation analyses of intestinal microbiota composition before and after FMT demonstrated significant associations between the relative abundances of specific bacterial taxa. Notably, positive correlations were observed between *Fusobacterium* and *Lachnospira*, *Streptococcus* and *Sutterella*, *Streptococcus* and *Lactobacillus*, as well as *Sutterella* and *Lactobacillus*. In contrast, a marked negative correlation was identified between *Bacteroides* and *Veillonella*. These findings suggest potential ecological interactions, including synergistic relationships among co-abundant taxa and competitive exclusion between inversely associated genera, which may reflect functional reorganization of the gut microbiome following FMT intervention. Overall, FMT proved to be an effective treatment for managing rUTI and diarrhea resulting from immunosuppressant use after kidney transplantation.

Prior research has elucidated the prevalence of intestinal microbiota dysbiosis among recipients of solid organ transplants, encompassing kidney and liver transplantation [38–40]. A comprehensive investigation employing shotgun metagenomic sequencing, encompassing fecal samples from 1,370 liver transplant recipients and 415 kidney transplant recipients, revealed

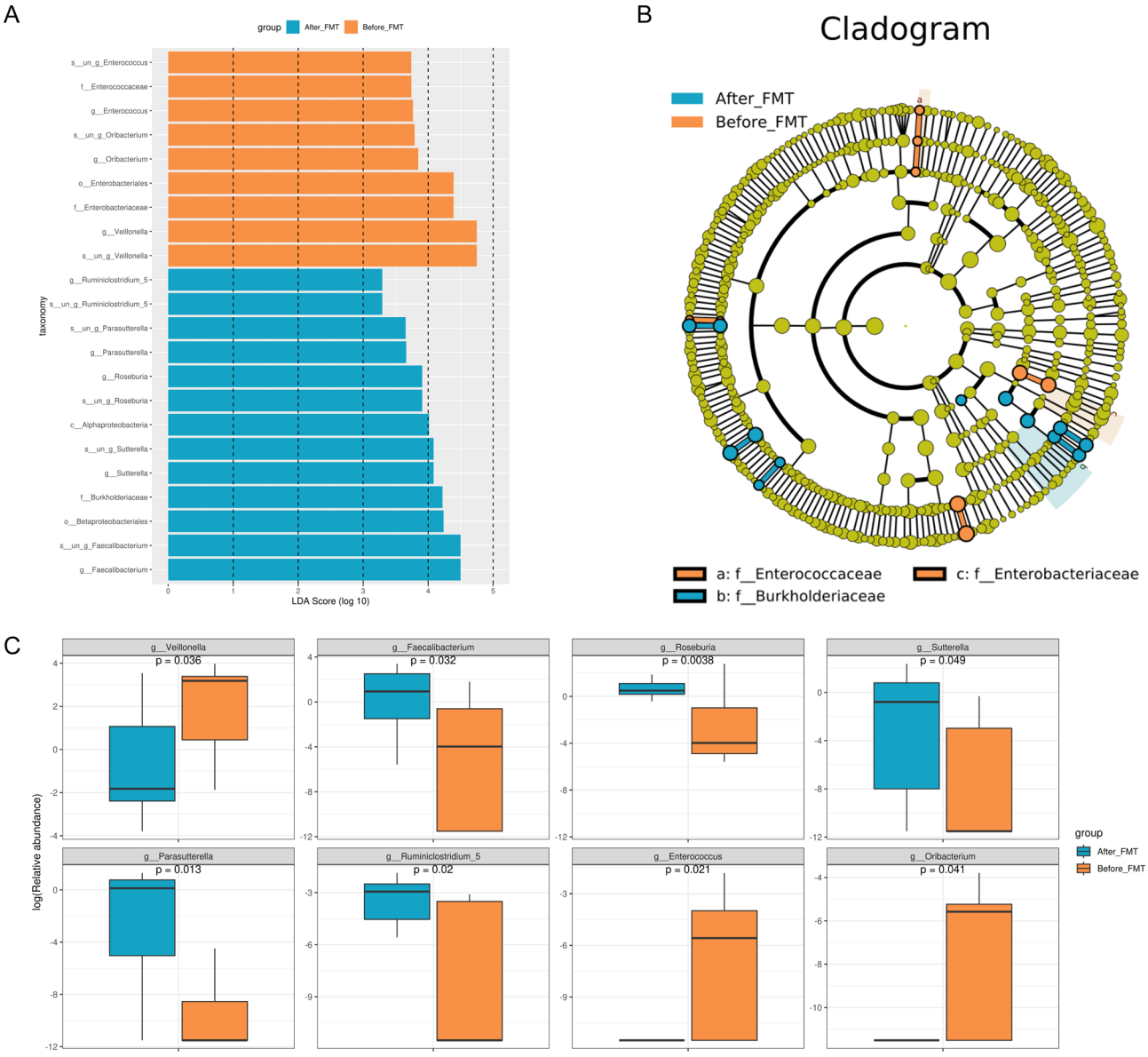
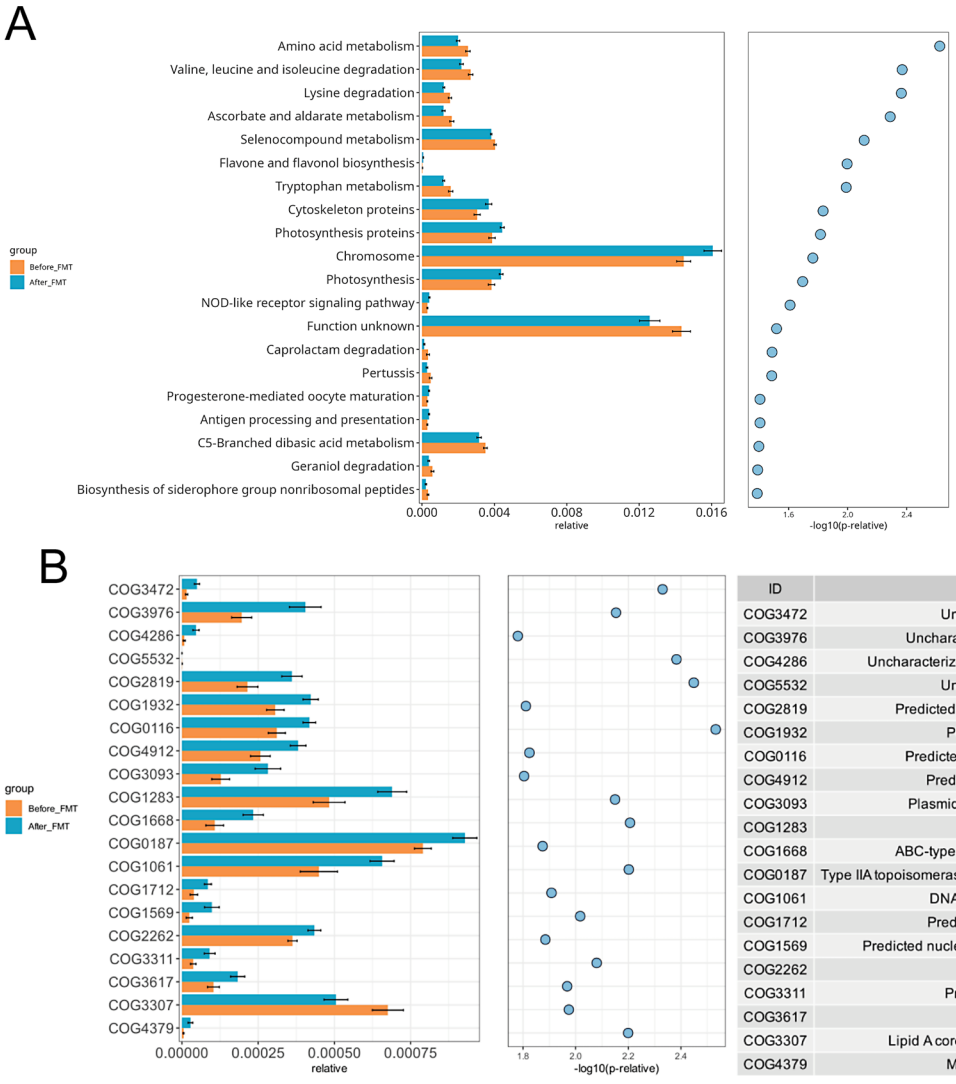


Fig. 4 Microbiota characterization at the genus level in patients before and after FMT. (**A**) LEfSe analysis of bacterial genera in patients before and after FMT. (**B**) Phylogenetic tree with circles radiating from the center to the outside representing taxonomic levels from phylum to genus. The diameter of the small circles was proportional to their relative abundance. The differentiated bacteria were colored according to their respective groups. (**C**) Differential bacterial genera in patients before and after FMT. FMT, fecal microbiota transplantation; LEfSe, linear discriminant analysis effect size

noteworthy alterations in both Shannon index diversity and β-diversity within the intestinal microbiota of these transplant cohorts [41]. In addition, previous work has delineated substantive alterations in the structural composition of intestinal microbiota in individuals who manifested post-kidney transplantation diarrhea while under the influence of immunosuppressive agents, implying a potential nexus between changes in intestinal microbiota and the etiology of post-kidney transplantation diarrhea [8]. Besides, Biehl et al. has documented the ameliorative effects of FMT on symptoms associated with rUTI in post-kidney transplantation patients [25], providing preliminary evidence supporting the alleviative potential of FMT in addressing symptoms of diarrhea and rUTI concomitant with immunosuppressive exposure following kidney transplantation. In this study, we employed 16 S rRNA gene sequencing to assess the intestinal microbiota of kidney transplant recipients prior to and following fecal microbiota transplantation. Our findings indicate that there was no noteworthy alteration in α-diversity, but a significant modification in β-diversity was observed in kidney transplant patients after FMT. This discrepancy may be attributed to the limited sample size, suggesting that the significance of these two



ID	description
COG3472	Uncharacterized conserved protein
COG3976	Uncharacterized protein conserved in bacteria
COG4286	Uncharacterized conserved protein related to MYG1 family
COG5532	Uncharacterized conserved protein
COG2819	Predicted hydrolase of the alpha/beta superfamily
COG1932	Phosphoserine aminotransferase
COG0116	Predicted N6-adenine-specific DNA methylase
COG4912	Predicted DNA alkylation repair enzyme
COG3093	Plasmid maintenance system antidote protein
COG1283	Na+/phosphate symporter
COG1668	ABC-type Na+ efflux pump, permease component
COG0187	Type IIA topoisomerase (DNA gyrase/topo II, topoisomerase IV), B subunit
COG1061	DNA or RNA helicases of superfamily II
COG1712	Predicted dinucleotide-utilizing enzyme
COG1569	Predicted nucleic acid-binding protein, contains PIN domain
COG2262	GTPases
COG3311	Predicted transcriptional regulator
COG3617	Prophage antirepressor
COG3307	Lipid A core - O-antigen ligase and related enzymes
COG4379	Mu-like prophage tail protein gpP

Fig. 5 PICRUSt analysis of intestinal microbiota metabolic pathways in patients pre- and post-FMT. (**A**, **B**) KEGG-based and COG-based analysis of metabolic pathways of intestinal microbiota in patients before and after FMT. The X-axis represents relative abundance. FMT, fecal microbiota transplantation. PICRUSt, Phylogenetic Investigation of Communities by Reconstruction of Unobserved States

diversities in disease progression is dissimilar. Moreover, it is plausible that the alteration in β-diversity is responsible for the amelioration of clinical symptoms in patients.

Intestinal microbiota plays a pivotal role in fostering the maturation of both the "mucosal immune system" and the "systemic immune system" [42] but also acts as a reservoir for UTI [43–45]. The susceptibility to recurrent UTIs was partly influenced by the gut-bladder axis, which encompassed gut dysbiosis and a varied immune response to bacterial colonization of the bladder, leading to the manifestation of symptoms [45]. It was emphasized that the potential of modulating the gut microbiota represents a novel strategy to prevent rUTI. Additionally, the successful use of FMT in resolving recurrent *C. difficile* infections (rCDI) had improved gut microbiota and reestablish immune system [46–48]. Although this literature directly addressed rCDI, similar principles could apply to the treatment of rUTI. Recent research has provided additional evidence supporting the potential efficacy of FMT in managing rUTI, where FMT significantly decreased the incidence of rUTI and improved the antibiotic susceptibility of the causative pathogens [49, 50]. In this study, our data revealed a significant decrease in the relative abundance of bacterial genera *Veillonella*, *Enterococcus*, and *Oribacterium* and a notable increase in the relative abundance of *Faecalibacterium*, *Roseburia*, *Sutterella*, *Parasutterella*, and *Ruminiclostridium 5* post-FMT in the patients received immunosuppressant administration after kidney transplantation. *Veillonella* was significantly increased within the intestinal of individuals afflicted with IBD as comparison to healthy controls [51], and has a positive correlation with gut

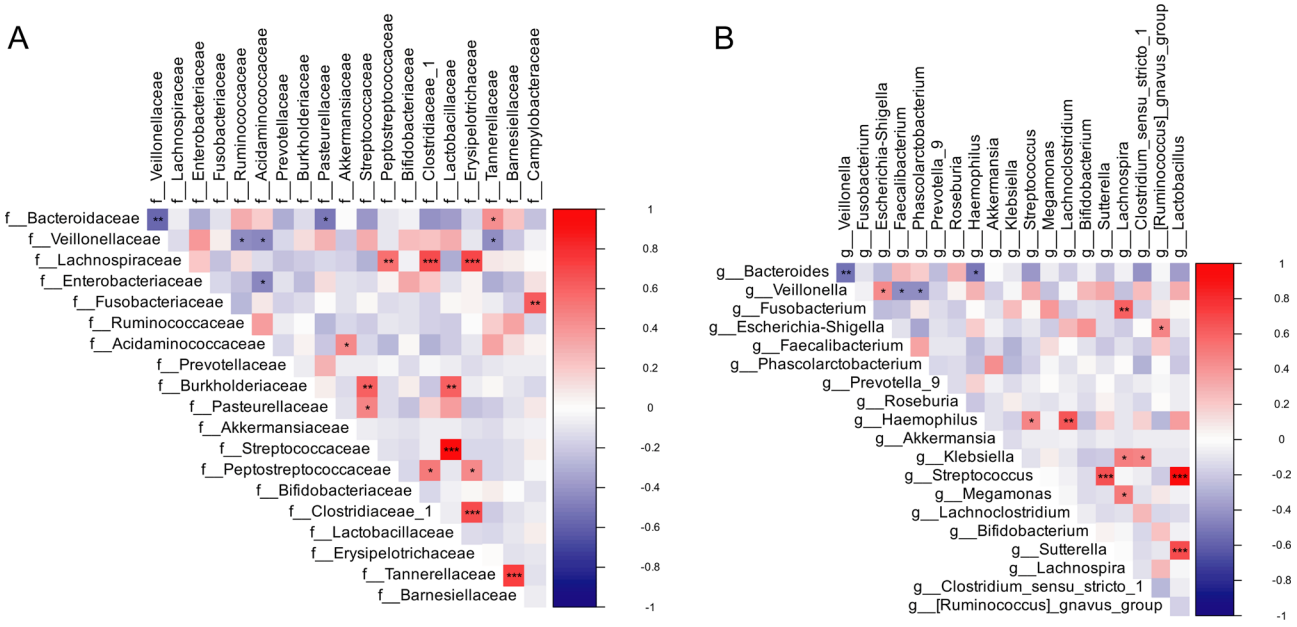


Fig. 6 Correlation analysis among intestinal bacterial taxa in patients before and after FMT. (**A**, **B**) correlation analysis of intestinal microbiota of at the family and genus levels in patients before and after FMT. FMT, fecal microbiota transplantation

microbiota metabolite cadaverine that exhibited in vitro cytotoxicity on the intestinal cell line HT29 [52]. *Enterococcus* constitutes a widespread group of Gram-positive bacteria [53], often implicated as opportunistic pathogens in UTI [54]. Oribacterium was significant increase in patients with Systemic Lupus Erythematosus (SLE) in activity [55]. Conversely, *Faecalibacterium*, *Roseburia*, *Sutterella*, *Parasutterella*, and *Ruminiclostridium 5* may as a bacterial genus with promising human health applications [56–60]. Through the application of FMT, there was a marked reduction in the abundance of pathogenic bacteria within the gut, leading to the reestablishment of intestinal microecology and subsequent alleviation of rUTI symptoms. Our study provides preliminary evidence supporting the efficacy of FMT in the management of rUTI.

Regarding the molecular mechanisms, our investigation revealed a significant enhancement in the enrichment of metabolic pathways associated with flavone and flavonol biosynthesis post-FMT, concomitant with a substantial relative decrease in pathways associated with Pertussis among patients subjected to FMT. The antioxidant [61, anti-inflammatory [62, 63], and antibacterial [64, 65] properties attributed to flavone and flavonol biosynthesis have been documented [66]. Consequently, we posit that FMT may elicit an upregulation in the synthesis of flavones and flavonols, thereby potentially mitigating local inflammatory responses within the gastrointestinal tract. The NOD-like receptor signaling pathway, a crucial component of the innate immune system [67], exhibits altered enrichment after FMT, suggesting that FMT may modulate this pathway to affect the host's immune response [68]. Additionally, KEGG analysis indicated a significant enrichment of pathways related to cytoskeletal proteins, chromosome-associated processes, the NOD-like receptor signaling pathway, progesterone-mediated oocyte maturation, and antigen processing and presentation following FMT. Despite the observed significant alterations in certain signaling pathways post-FMT, our comprehension of the specific mechanisms and interconnections of these pathways in influencing gastrointestinal health and disease states remains inadequate.

In this study, the definition of indications may have been overly broad. The study considered rUTI and chronic diarrhea. Although this broad definition facilitated the exploration of the potential applications of FMT in various diseases, it may also have limited the generalizability of the study findings. The retrospective design, relying on existing medical records and data, may introduce limitations such as information bias and selection bias. To more rigorously infer the efficacy of FMT, future studies should expand the cohort size and could employ prospective randomized controlled trials (RCTs), which are the gold standard for evaluating the effectiveness of medical interventions, to minimize the influence of bias and confounding factors. Furthermore, this study primarily inferred the therapeutic benefits of FMT based on the observation of clinical outcomes. While this approach can reflect the actual effects of FMT to some extent, it also has its limitations. Patient individual differences, concomitant therapies, and fluctuations in the natural course of the disease may all interfere

with the assessment of efficacy. To provide a reasonable basis for decision-making regarding FMT, we need to delve deeper into the severity of patient symptoms and implement stratified treatment decisions. According to the Bristol Stool Form Scale [69], it is possible to quantify the daily frequency and consistency of bowel movements. In alignment with the Infectious Diseases Society of America (IDSA) guidelines, the number of UTI episodes and the characteristics of pathogen resistance over the past six months can be documented. For patients with milder symptoms, prioritizing conservative treatment or a watchful waiting strategy is advisable to mitigate the risks associated with unnecessary interventions. Conversely, for patients experiencing severe or recurrent symptoms, and after the exclusion of organic diseases, FMT may be a viable option worth considering. Clinicians should conduct a comprehensive assessment of the potential risks and benefits associated with FMT and provide patients with detailed explanations of its principles, procedures, possible risks, and anticipated outcomes to facilitate informed decision-making. A long-term follow-up plan should be established, which includes assessing the stability of the gut microbiota through shotgun metagenomics, evaluating short-chain fatty acid (SCFA) levels—indicative of significant intestinal and immunomodulatory functions [70]—using fecal metabolomics, and monitoring the dynamics of peripheral blood immune cells and inflammatory factors in patients. The 16S rRNA gene sequencing of gut microbiota has certain limitations in bacterial classification and identification, limiting species-level identification. It was not possible to determine which bacterial species were responsible for alleviating diarrhea and improving rUTI in patient after FMT. Moreover, different bacterial strains within the same genus can exhibit significant differences in their metabolic capabilities, physiological characteristics, and responses to environmental stimuli. Relying solely on genus-level taxonomic information to infer the functional roles of microbial communities may result in the neglect of essential biological processes that are crucial to community function but are carried out by only a few or specific strains. Despite these limitations, this study represents the largest cohort to date evaluating FMT for post-kidney transplantation complications. Our findings provide preliminary evidence supporting FMT's role in managing immunosuppression-related morbidity after kidney transplantation.

Conclusions

Following episodes of severe diarrhea or rUTI, recipients of renal transplants underwent fecal microbiota transplantation, resulting in notable alleviation of clinical symptoms. FMT represents a promising therapeutic modality targeted at ameliorating complications arising from the administration of immunosuppressants in individuals who have undergone renal transplantation.

Abbreviations

FMT	Fecal microbiota transplantation
rUTI	Recurrent urinary tract infection
ANOSIM	Analysis of similarity
LEfSe	Linear discriminant analysis effect size
CDI	Clostridioides difficile infection
BMI	Body mass index
PCoA	Principal Coordinate Analysis

Supplementary Information

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Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

Supplementary Material 4

Supplementary Material 5

Supplementary Material 6

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Not applicable.

Author contributions
Yongguang Liu, Ming Zhao, Jianmin Hu, and Ding Liu conceived and supervised the study; Jianmin Hu and Ding Liu designed the experiments; Jianmin Hu, Ding Liu, Guorong Liao, Ying Guo, Min Li, Jun Liao, Hua Chen, Song Zhou, Siqiang Yang, and Shichao Li performed the experiments; Jianmin Hu and Ding Liu analyzed the data; Jianmin Hu wrote the manuscript; Ding Liu, Guorong Liao, Ying Guo, Min Li, Jun Liao, Hua Chen, Song Zhou, Siqiang Yang, Shichao Li, Yongguang Liu, and Ming Zhao revised the manuscript. All the authors have read and approved the final manuscript.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
All participants provided written consent to partake in the research. The study was approved by the Ethics Commitment of Zhujiang Hospital, Southern Medical University (approval number 2023-KY-049-02) and conducted with adherence to the Declaration of Helsinki.

Consent for publication
All authors read and approved the submission of this manuscript.

Competing interests
The authors declare no competing interests.

Author details
[1]Department of Organ Transplantation, Zhujiang Hospital, Southern Medical University, No.253 Industrial Avenue, Haizhu District, Guangzhou 510282, China

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