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A study on the efficacy and safety of fecal microbiota transplantation as an adjunctive therapy for treating depressive episodes

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Abstract

Objective: This study aimed to evaluate the efficacy and safety of fecal microbiota transplantation (FMT) as an adjunctive therapy for depressive episodes.

Methods: This study recruited 46 participants aged 18-65 from January 2022 to December 2023 who were diagnosed with depression according to the International Classification of Diseases, 10th edition. They were randomly divided into two groups to receive different treatments, including FMT combined with medication group (test group, n=23) and the medication-only group (control group, n=23). Assessments were performed before and two weeks after treatment. Ten predominant gut microbiota species were analyzed, and the Hamilton's Depression Scale-24(HAMD-24) was used to evaluate depressive symptoms. Adverse events related to treatment were assessed using an adverse event scale and laboratory tests. The main evaluation indicators included the reduction rate of HAMD-24 scores, treatment efficacy rate, and changes in the indicators of the ten predominant intestinal bacteria before and after transplantation. Safety assessment indicators included adverse events, blood routine, biochemistry, electrocardiogram, immunological parameters (immunoglobulins and complement), hypersensitive C-reactive protein(hs-CRP), thyroid function, and glycated hemoglobin. The rank-sum test was performed to compare differences in microbiota before and after FMT treatment. The relationship between gut microbiota and depression severity was

examined by means of correlation analysis.

Results: The baseline HAMD-24 scores showed no significant difference between the test and control groups (P>0.05). After two weeks treatment, the reduction in HAMD-24 scores (P=0.048) and the HAMD-24 reduction rate (P=0.016) were significantly higher in the test group than control group (P<0.05). Following FMT treatment, Enterococcus, Lactobacillus, Bifidobacterium, and Butyricicoccus levels significantly increased compared to baseline (P<0.05). Linear discriminant analysis(LDA) revealed a significant post-treatment increase in Enterococcus relative abundance (P=0.02). In the test group, baseline-enriched Clostridium prausnitzii (P=0.040), Butyricicoccus (P=0.029), and Eubacterium rectale (P=0.004) showed significant negative correlations with HAMD-24 scores, whereas post-treatment Enterococcus was correlated with HAMD-24 scores (P=0.030). Adverse event incidence was 28.6% in the test group and 30% in the control group, with no significant difference (P=0.928). Reported discomforts during FMT treatment—nausea, vomiting, and nasopharyngeal discomfort—were mild and self-resolving, with no serious adverse events observed.

Conclusion: The administration of FMT as an adjunctive therapy demonstrates superior improvement in depressive symptoms and is deemed safe with no apparent adverse reactions. There was no change in the composition of gut microbiota structure before and after FMT in patients with depression. Enterococcus showed a significant relative abundance increase in the gut after FMT. The post-treatment Enterococcus was correlated with HAMD-24 scores.

Key words Fecal microbiota transplantation；depressive episodes；efficacy；safety；gut microbe

Introduction

Depressive disorder (DD) is a group of mood disorders the aetiology of which is multifactorial, and which is characterised clinically by a cluster of significant and persistent depressive symptoms. The core symptoms of depressive disorder are depressed mood and loss of interest that are disproportionate to the situation. The COVID-19 pandemic triggered a significant surge in depression cases globally, with one major study estimating over 53 million new cases of major depressive disorder in 2020 alone[1].According to model-based estimates from the World Health Organization (WHO) in 2022, the global prevalence of depression increased by approximately 28% during the first year of the COVID-19 pandemic (2020)[2]. Furthermore, based on the 2019 Global Burden of Disease study, major depressive disorder is the leading cause of disability worldwide[3]. The disease is characterised by high morbidity, high relapses and a high suicide rate, which causes a significant burden to families and society and has become a serious social and medical problem. The prevailing focus of neurobiochemical studies of depression has been on three major monoamine neurotransmitters: serotonin (5-HT), norepinephrine (NE), and dopamine (DA)[4, 5]. Nevertheless, a significant proportion of depressed patients (1/3 to 1/2) continue to demonstrate poor outcomes following treatment with such antidepressants[6, 7]. The monoamine neurotransmitter hypothesis is not a complete explanation of the pathogenesis of depression[8]. Therefore, a safe and effective drug is urgently needed to treat DD.

In recent years, the evidence from epidemiological, clinical, and animal studies supports that immune-inflammatory mechanisms play an important role in the development of depression, and that the closest link to immune-inflammation is our

gastrointestinal tract, which is the largest immune organ in the body. There are currently more than 1,000 species of intestinal flora known to humans. They are involved in many physiological activities of the human body, including the absorption and metabolism of nutrients, the development and maturation of the immune system, and resistance to invasion by foreign pathogens. They have been called the "second brain." In recent years, a new concept of "microbial gut-brain axis (MGB)" [0]has been proposed. The role of gut microbes in the MGB has been gradually recognized and has become a hot research topic. The interaction between the brain and gut microbiota is bidirectional. The gut microbiota can influence emotions and brain activity through neural networks, endocrine, immune, and metabolic pathways[0]. Conversely, the brain can also regulate the structural composition of the gut microbiota, adapting to environmental changes and maintaining the balance of the gut microecology. This mutual influence is achieved through three main mechanisms: the impact of gut microbiota on the immune system, the interaction between gut microbiota and the hypothalamic-pituitary-adrenal (HPA) axis, and the ability of gut microbiota to affect neurotransmitters. This relationship is closely linked to a range of neuropsychiatric disorders, including depression, autism, anxiety, and Parkinson's disease[0].

Advances in the study of the correlation between intestinal flora and depression have demonstrated the presence of disturbed intestinal flora[0] and significant differences in the composition of the flora[0] in depressed patients compared to healthy controls. Furthermore, the intestinal microbial fractions of depressed patients tend to undergo bacterial translocations[0]. Nannan Li et al. conducted a study in which they transplanted the intestinal flora of

depressed mice into the intestines of healthy mice. They observed that mice receiving intestinal flora transplants from depressed mice also developed depressive symptoms. Furthermore, the transplants had higher numbers of Proteobacterium and Verrucomicrobiae, Verrucous bacteria whereas beneficial bacteria such as Bifidobacteriaceae and Lactobacillusceae were significantly reduced. These findings suggest that depression is caused by a disturbance in the intestinal flora[15]. Mechanistically, this phenomenon may be associated with elevated levels of HPA axis-related hormones (adrenocorticotropic hormone, cortisol, and adrenocorticotropic hormone), overactivation of the HPA axis, heightened levels of TNF-alpha, IL-6, and IL-1, and reduced levels of hippocampal neurotransmitters (5-HT, DA, and NE) in murine models[16]. In patients with depression, intestinal flora disorders compromise the intestinal mucosa, leading to lesions and abnormal enteric nervous system activity[0]. This further disrupts intestinal secretion and immune defense. Metabolites and bacteria passing through the damaged intestine intensify systemic inflammation[0]. Bacterial products like lipopolysaccharides and other inflammatory factors enter the brain via the blood-brain barrier, causing neuroinflammation, brain damage, and triggering depression[0]. While the proposed mechanism of MGB appears to complement existing depression mechanisms and offer a potential solution to the existing treatment dilemma, further research is necessary to fully elucidate the mechanism of action of MGB in depression. Current approaches to enhance intestinal microecology encompass the use of oral probiotics and fecal microbiota transplantation (FMT), which is regarded as the most efficacious method of restoring intestinal flora.

FMT is a procedure in which faecal matter from a healthy donor is prepared into an enterobacterial fluid through a process of treatment and subsequently transplanted into the intestinal tract of the patient via various routes. The purpose of this procedure is to allow the transplanted flora to colonize the patient's body and restore intestinal flora to a state of functionality, thereby treating diseases[0]. In 2013 and 2014, the American Gastroenterological Association and the European Society for Clinical Microbiology and Infectious Diseases, respectively, incorporated FMT into their clinical guidelines for the treatment of recurrent Clostridium difficile infection (RCDI)[21-22]. In recent years, FMT has demonstrated efficacy in the treatment of inflammatory bowel disease, hepatic encephalopathy, autism, and Alzheimer's disease, among other conditions.

This study explores FMT as a supportive treatment for patients with depressive episodes. It examines the relationship between post-transplant intestinal flora composition and antidepressant efficacy, as well as changes in gut microbiota after FMT response. The objective of this study is to identify bacterial communities associated with antidepressant response and develop safe and effective non-drug treatments.

Results

Comparison of general information at baseline

A total of 46 cases were enrolled in the study, including 23 cases in the FMT group. However, three cases were not completed due to patient discharge, three cases were dislodged due to family opposition, two cases were not complied with due to long distance, one case was feared, and 14 cases were finally completed. In the drug group, 23 cases were enrolled, and three cases were not

completed due to discharge, while 20 cases were finally completed. Participant flow diagram see Fig.1. Independent samples t-tests were performed for age, BMI, and HAMD-24 scores in both groups. Independent samples rank sum test was performed on the years of education of the two groups. The $\chi 2$ test was performed for gender, whether it was the first episode, whether there was a family history, and whether there were gastrointestinal symptoms at the onset of the disease in both groups. The findings indicate an absence of statistically significant variation in the demographic characteristics examined. Refer to Table 1 for specific details. General information on the experimental group and dropouts from the experimental group is shown in Supplementary Files 1. General characteristics of the control group and those who dropped out of the control group are shown in Supplementary Files 2.

Comparison of clinical outcomes between the two groups

At the end of the second week of treatment, the HAMD-24 scores of both the experimental and control groups decreased from the baseline values, and the differences were statistically significant (both $P<0.01$), as shown in Table 2.

At the end of 2 weeks of treatment, the HAMD-24 score reduction and HAMD-24 reduction rate of the experimental group was higher than that of the control group, and the difference was statistically significant (both $P<0.05$). The treatment efficacy rate of the experimental group was 71.4% (10/14), and the treatment efficacy rate of the control group was 35% (7/20), and the difference between the 2 groups was statistically significant ($Z=4.371$, $P=0.037$). See Table 3.

Analysis of 10 dominant flora of human intestinal tract before and after fecal microbiota transplantation in experimental group

Changes of 10 human intestinal flora before and after FMT in the experimental group.After FMT treatment, Enterococcus, Lactobacillus, Bifidobacterium, and Clostridium butyricum increased significantly, and the difference was statistically significant ($P < 0.05$), see Table 4.

In order to visualize the relative abundance of the ten dominant human intestinal flora before and after treatment in the experimental group, the grouped percentage stacked bar graphs of intestinal flora decanalysis data before and after FMT in the experimental group are shown in Fig. 2.

Significant difference test between groups: Microbial taxa with significant impacts were calculated using LDA discriminant histograms. The greater the impact of species abundance, the greater the LDA score. The relative abundance of Enterococcus spp. in the intestine after FMT treatment was found to be significantly increased from the LDA discriminant bar graph (P=0.02), and no significant difference was observed for the remaining 9 dominant intestinal flora (Fig. 3).

Correlation analysis between gut flora and depression level

Spearman correlation analysis was performed on the intestinal flora abundance and HAMD-24 scores before and after FMT treatment in the experimental group. It was found that the enrichment of Faecalibacterium prausnitzii (F. prausnitzii)(P=0.040), Clostridium butyricum(C. butyricum) (P=0.029), and Eubacterium rectum (P=0.004) in the experimental group before FMT was significantly negatively correlated with the total HAMD-24 score, and the other differential microorganisms showed different correlations with the HAMD-24 score, but were not statistically significant. After FMT treatment, enterococci were significantly positively correlated with HAMD-24 scores (P=0.030),

while the other 9 dominant bacterial species had no significant correlation with HAMD-24 scores.See Table 5.

Comparison of safety between the two groups

In the experimental group, 4 patients (28.6%, N=14) experienced mild adverse events during treatment, including 1 case of nasopharyngeal discomfort (7.1%), 2 cases of nausea (14.3%), and 1 case of abdominal distension (7.1%).In the control group, there were 6 cases (30.0%, N=20), 3 cases of nausea (15.0%), 2 cases of mild headache (10%), and 1 case of constipation (5%).There was no significant difference in the incidence of adverse events between the two groups ($\chi 2=0.008$, P=0.928), see Table 6 for details. The remaining safety items were subjected to paired t-test (see Table 7), and the P values were all greater than 0.05, indicating that the differences were not statistically significant.

Discussion

The efficacy and safety of intestinal bacterial transplantation in patients diagnosed with depression remains to be reported, both domestically and internationally. The objective of this study was to evaluate the efficacy and safety of FMT as an adjunctive therapy for depressive episodes. The study revealed no statistically significant differences between the depressed patients included in this study with respect to gender, age, education, BMI, whether the episode was first-time or recurrent, and association with the gastrointestinal tract, indicating that the two samples were comparable. The findings of a substantial multicentre clinical trial demonstrated that following the initial monotherapy phase with citalopram, one of the selective serotonin reuptake inhibitors (SSRIs), approximately 47% of patients diagnosed with depression exhibited efficacy, with 33% attaining clinical remission. It is

noteworthy that two-thirds of these patients achieved clinical remission following a four-phase sequential treatment approach, incorporating strategies such as switching, augmentation, or combination therapy[03-25].Overall, compared to other antidepressants, ESC demonstrates superior efficacy, lower discontinuation rates, and greater patient acceptance[26]. This is why ESC was selected for this study, as it is more suitable for practical clinical application. The results of the study indicated that at the conclusion of the second week of treatment, the HAMD-24 scores of both the experimental and control groups underwent a decrease from their respective baselines, suggesting that the patients' depressive symptoms exhibited a substantial improvement in response to both FMT and ESC treatment. Following the conclusion of the second week of treatment, it was evident that the experimental group exhibited superior improvement in HAMD-24 scores, score reduction, and score reduction rate when compared to the control group. This suggests that the combination of FMT and ESC treatment demonstrated enhanced efficacy in the experimental group at the end of the second week.

The gut microbiota is comprised of a variety of microorganisms, including bacteria, viruses, archaea, and fungi. These can be categorised into beneficial bacteria, harmful bacteria, and neutral bacteria. These elements coexist in specific proportions, exerting a reciprocal influence that is integral to their respective functions in nutrition, metabolism, immune development, and host defence. The relative abundance of specific bacterial genera has been demonstrated to serve as an indicator of gut health. A high abundance of genera such as Bifidobacterium and Lactobacillus is typically associated with beneficial gut function, whereas overrepresentation of genera like Clostridium or Escherichia may

be linked to certain intestinal disorders. In this study, we detected the changes of 10 intestinal dominant flora by qPCR. Enterococci, Lactobacillus, Bifidobacterium, and Clostridium butyricum increased significantly after 2 weeks of FMT treatment, which indicated that the proportion of beneficial flora increased significantly after FMT treatment, and it could effectively improve the microecology of the intestinal tract.

In particular, Enterococcus showed a significant increase in relative abundance in the intestinal tract and met the LEfSe program biomarker screening criteria for dominant flora. Enterococci have been shown to possess a variety of beneficial properties as commensal bacteria. These properties include participation in nutrient metabolism, maintenance of intestinal pH, assistance in the synthesis of essential vitamins, and utilization as probiotic food additives or in the treatment of intestinal dysbiosis[0].Enterococcus supplementation has also been reported in the literature to promote neurological recovery and alleviation of depressive symptoms in stroke patients**Error! Reference source not found.**.Enterococci are also opportunistic pathogens that cause infectious diseases and promote inflammation in the organism when antibiotics are used in large quantities or when the host is immunocompromised. A particular study revealed that the presence of enterococci had a substantial negative impact on the progression of colitis, neuroinflammation, and depression-like behaviors, while concomitantly increasing the incidence of flora translocation in murine subjects**Error! Reference source not found.**.Enterococci are usually in excess in depressed patients compared to healthy controls and are negatively correlated with 5-HT levels in the gut[0].One plausible mechanistic explanation for our observation involves the potential pathogenic potential of

specific Enterococcus strains, particularly E. faecalis, which is a well-documented and potent biofilm former[31-32].While biofilms are a natural mode of bacterial growth, the formation of dysbiotic biofilms by opportunistic pathogens in the gut lumen represents a significant threat to intestinal homeostasis.From a psychiatric pathophysiology perspective, this is highly relevant. The formation of a robust biofilm by a translocated or pathobiont Enterococcus strain could destabilize the delicate architecture of the intestinal epithelial barrier[33-34].This disruption compromises gut integrity, increasing intestinal permeability[35]—a condition colloquially referred to as "leaky gut." This breach in the primary defensive barrier of the gut facilitates the translocation of bacteria and their microbial-associated molecular patterns (MAMPs), such as lipopolysaccharide (LPS), into the systemic circulation[33].The ensuing activation of the host's innate immune system triggers a state of chronic, low-grade systemic inflammation[34-35],characterized by elevated levels of pro-inflammatory cytokines (e.g., IL-6, TNF-α, and CRP).This mechanistic pathway is of direct consequence to the neurobiology of depression. These circulating inflammatory mediators can access the central nervous system, where they disrupt key processes implicated in mood regulation, including:1.Monoamine Metabolism[36-37]:Inflaming cytokines downregulate the expression of enzymes critical for synthesizing serotonin, dopamine, and norepinephrine.2.Neuroendocrine Function[36-37]:They hyperactivate the hypothalamic-pituitary-adrenal (HPA) axis, a system frequently dysregulated in depression.3.Neuroplasticity[37-38]:They inhibit neurotrophic factors like BDNF and can contribute to excitotoxicity, impairing synaptic resilience and hippocampal function.Therefore, we hypothesize that

the expansion of a biofilm-forming Enterococcus strain post-FMT, while potentially beneficial in some contexts, could in our cohort have acted as a pro-inflammatory pathobiont[39].Its presence may have inadvertently sustained or amplified the inflammatory component of depression in a subset of patients, thereby attenuating the overall therapeutic response to the FMT intervention and manifesting as a positive correlation with depression severity scores.This hypothesis underscores the necessity for future FMT studies in psychiatry to employ deep metagenomic sequencing to characterize not just microbial abundance, but also the virulence gene profiles (e.g., biofilm-forming genes like esp in E. faecalis) of transplanted microbiota. This will be crucial for optimizing donor selection and ensuring the safety and efficacy of microbial-based interventions for mental disorders. Consequently, the rise or fall in the relative abundance of enterococci in the human gut flora cannot be attributed merely to their classification as either "beneficial" or "harmful."

Concurrently, a correlation analysis was conducted between 10 predominant enterobacteria and the severity of depression. The analysis revealed a statistically significant negative correlation between F. prausnitzii, C. butyricum, and Eubacterium and the symptoms associated with depression. These findings are analogous to the results reported by foreign scholars Knuesel[0], which suggests that these enterobacteria may play a pivotal role in the pathophysiology of depression. F. prausnitzii produces a number of substances with anti-inflammatory properties that inhibit the production of IL-8 immune proteins and activate the production of IL-10 mediated T cells to help fight inflammation[0]. C. butyricum has the function of balancing the intestinal flora and

promoting the proliferation of beneficial intestinal flora and has been found to alleviate depressive-like behavior in stressed mice[0]. Eubacterium rectale represents a distinctive gut microbial signature in patients with major depressive disorder (MDD). Fecal samples from these patients have higher levels of Bacteroides and lower levels of Eubacterium and Blautia[0]. SSRIs have been shown to increase the abundance of Eubacterium rectale in the gut[0].The short-chain fatty acids (SCFAs)(acetate, propionate and bu tyrate) produced by the metabolism of these three enterobacteria a re important substances in the intestinal system. They maintain the integrity of the intestinal wall barrier, regulate the pH of the intest inal lumen, promote the production of anti-inflammatory factors an d inhibit the production of pro-inflammatory factors. In particular, butyrate stimulates villous growth and promotes the production of mucins, antimicrobial peptides, and the synthesis of T-junctional pr oteins, which contribute to host resistance to colonization by intesti nal pathogens and to immunomodulation[0]. Thus, an increase in thei r abundance may have an adjuvant effect on antidepressant drug th erapy. In animal studies, it was also found that depression model mice had dysbiosis of the intestinal flora, altered levels of SCFAs, and a significant reduction in neurotransmitters compared to normal controls[0], and that supplementation with butyrate and short-chain fatty acids had a mitigating effect on symptoms of depression and anxiety.

Indeed, there is an absence of precise research data concerning the efficacy of FMT in the treatment of depression. Preliminary studies have demonstrated that FMT can alleviate depression-like behaviours in murine models, as well as inhibit neuroinflammation, correct imbalances in the gut microbiota, and repair intestinal barrier damage[0]. Furthermore, a range of

preclinical experiments, case reports, meta-analyses, and systematic evaluations have also substantiated the antidepressant effect of FMT[047-51]. In studies on different intestinal bacteria, it was found that a variety of intestinal flora would affect the stress hormone levels of animals and patients with depression[52-55]. One study found that germ-free mice, lacking intestinal flora, exhibited stress responses and maladaptive behaviors. These behaviors returned to normal after the mice were transplanted with probiotics[0].A study by Akkasheh et al. [0]also found a decrease in Beck Depression Scale scores after 8 weeks of FMT in depressed patients. However, the study by Romijn et al.[0] did not find an antidepressant effect of intestinal bacterial transplantation, which may be related to the number of transplanted intestinal colonies, the severity of the condition of the study participants, genetics, and dietary structure, among other factors[0]. It is equally crucial to recognize the dynamic nature of gut flora, influenced by a variety of factors such as genetics, gender, age, diet, geographic location, and health status[0]. Jingjing Rao et al. employed chronic unpredictable mild stimulation (CUMS) to induce depressive behaviors in rats and treated them with FMT for a period of 14 days. The researchers ascertained that by inhibiting the activation of NLRP3 inflammatory vesicles and suppressing the release of pro-inflammatory cytokines, they were able to inhibit inflammation, which ultimately manifested itself in increased neurotransmitter secretion and improved symptoms of depression in a clinical study[61]. The "microbe-gut-brain" axis is a concept that has been postulated to explain the mechanism by which FMT can improve depression symptoms[62]. Among patients with gastrointestinal diseases accompanied by manifestations of depression and anxiety, FMT treatment has also achieved significant therapeutic effects.

This study attempts to continue to explore the efficacy and safety of FMT in assisting first-line antidepressants in the treatment of depression from the perspective of clinical application, and to explore in depth the changes in the structure of the intestinal flora of patients with depression who respond to FMT, and to search for the flora associated with FMT treatment. Our study also has several limitations. Firstly, the sample size was limited, and the study exclusively examined the regression of depressed patients two weeks after FMT treatment, lacking a longitudinal study. Secondly, due to the complexity of practical implementation and ethical considerations, we were unable to employ autologous faecal microbiota transplantation (FMT). The invasive nature of the FMT procedure itself may generate a significant placebo effect, meaning that some of the clinical improvements observed may stem from patients' expectations of the treatment and the act of receiving it, rather than solely from the specific effects of the donor microbiota. It is recommended that future confirmatory studies, if feasible, utilise autologous FMT or simulated colonic irrigation as controls in order to more robustly dissect the respective contributions of FMT-specific biological effects versus non-specific placebo effects. However, a significant increase in the levels of Lactobacillus, Bifidobacterium, and Clostridium butyricum was observed in the treatment group, indicating a substantial rise in beneficial microbiota following FMT that effectively improves gut microbiome composition. Furthermore, only 10 dominant human intestinal flora commonly used in clinical practice were tested, and the smaller sample size is unconvincing in the face of such a huge total number of human intestinal microorganisms. This study was conducted at a singlecentre and the participants were recruited exclusively from within the

province and surrounding areas. The implications for cross-cultural, dietary, and ethnic differences when compared with multi-center and foreign studies remain unclear. In view of this, it is proposed that the number of subjects enrolled in the study will be increased when conditions permit, that collaboration will be initiated with multiple centres, that the region from which subjects originate will be expanded, and that a randomised control will be designed to exclude confounding factors and to render the study conclusions more reliable. In the future, the results of analysing intestinal flora using 16SrRNA gene sequencing technology will be more comprehensive. The present study will undertake long-term follow-up on changes in intestinal microbial abundance after FMT.In addition, it will assess the prognosis of depression and its influencing factors, and evaluate the safety of the treatment. The study will also explore the optimal frequency and dosage of FMT for the treatment of depression. Furthermore, it will establish a precise intervention model from multiple levels of research, such as biological characteristics. The ultimate aim of the study is to provide a more reliable basis for the short-term and long-term effects of FMT in the treatment of depression.

FMT has been demonstrated to be well tolerated and safe, consistent with its use as a treatment for recurrent C. difficile infection. Mild gastrointestinal symptoms and nasopharyngeal discomfort often occur during FMT treatment. In this study, we transplanted flora through the upper gastrointestinal route, and these subjective discomforts generally occurred during FMT treatment. These discomforts may be attributable to patient anxiety, as well as to the endoscopic procedure and nasoenteric tube. It was observed that these discomforts generally did not require special treatment, and all of them could be alleviated within two days at

the conclusion of the FMT treatment. No serious adverse events were reported. Notwithstanding the occurrence of certain adverse effects and complications associated with FMT, the future of this treatment remains promising**Error! Reference source not found.**. Presently, dry powder capsules comprising a comprehensive range of intestinal bacteria are available for clinical application. It is anticipated that oral capsules will emerge as a safer and more convenient modality for flora transplantation.

In conclusion, the present study demonstrated that there was a significant increase in the levels of Enterococcus, Lactobacillus, Bifidobacterium, and Clostridium butyricum after combined FMT treatment. This increase was especially significant in the case ofEnterococcus , and there was a more pronounced improvement in patients' depressive symptoms. The incidence of adverse effects was also lower. These results showed a favourable benefit-risk profile, providing evidence for the potential of intestinal microbe-targeted therapy for depressive episodes. The safety and cost-effectiveness of FMT as an intervention has been empirically demonstrated to be safe and cost-effective in other indications, which contributes to the potentially beneficial effects of clinical practice in rapid implementation.

Methods

Study design and Participants

This study enrolled patients with depressive episodes who were admitted to the outpatient or inpatient department of the Department of Mental Health at Shulan Hospital (Hangzhou) from January 2022 to December 2023. Participants were randomly assigned to either the FMT plus medication group (experimental group) or the medication-only group (control group) using a random number table. The randomisation process was executed by

a single, independent professional. The experimental group received one course of FMT in addition to medication therapy. The experimental group underwent testing for 10 dominant human gut microbiota at baseline and at the end of the second treatment week. The Hamilton Depression Rating Scale (HAMD-24) was utilised to evaluate depressive symptoms, while the TESS and laboratory indicators were employed to assess adverse events.The study was approved by the Ethics Committee of Shulan Hospital (Ethics Number: KY2024024) and it was conducted in accordance with the principles of the Declaration of Helsinki. All subjects provided written informed consent prior to participation.

Inclusion criteria: (1) meet the diagnostic criteria for single or recurrent depression in the International Classification of Diseases, 10th edition (ICD-10); (2) age 18-65 years old, gender is not limited; (3) HAMD24 total score ≥14 at the time of enrollment; (4) have sufficient audiovisual level and comprehension ability to cooperate and understand the assessment of the scale; (5) treated with a single antidepressant; (6) participated voluntarily and signed an informed consent form. (7) No use of medications and foods that affect intestinal flora, such as yogurt and antibiotics, in the last 2 weeks.

Exclusion criteria: (1) severe physical illness or organic brain disease; (2) mental illness such as mental retardation, bipolar disorder, personality disorder, alcohol or substance use disorder; (3) current severe suicide risk (HAMD-24 suicide item ≥ 3 points); (4) receiving or planning to receive MECT in the past 6 months and MECT is ineffective. (5) current or past severe, active physical illness that may interfere with the study treatment: a. patients receiving thrombolytic therapy; b. patients with progressive neurological exacerbation; c. patients with intracranial infection,

intracranial hemorrhage, intracranial tumor, or any other brain disease that is not suitable for inclusion; d. patients with uncontrolled severe hypertension (systolic blood pressure > 180 mmHg or diastolic blood pressure > 110 mmHg after drug treatment); e. laboratory test indicators are unqualified: aspartate aminotransferase or alanine aminotransferase is higher than 3 times the upper limit of normal, creatinine clearance < 0.6 ml/s or blood creatinine > 265 umol/l (□3.0mg/dl), platelets□100×10^9/L; f. Patients with severe blood system diseases or severe coagulation dysfunction, retinal hemorrhage or internal hemorrhage; g. Patients who have undergone or are expected to undergo major surgery during the study (including stent implantation, angioplasty or other related medical device treatments); h. Patients with contraindications to enterobacteria transplantation; i. Any systemic disease that is not suitable for inclusion in the study; (6) Pregnant and lactating women; (7) Patients who are currently participating in or have participated in other clinical studies within 3 months before inclusion in the study, or have participated in this study, or other situations that the researchers believe are not suitable for inclusion in the study.

Exclusion and dropout criteria: (1) Serious adverse events occurred during FMT treatment or the study. (2) The subject refused to continue participating in the study for various reasons during the study. (3) The subject developed a serious physical illness during the study period. (4) The subject could not tolerate drug treatment during the study. (5) The subject's medication compliance during the study was <80% or >120%.

Procedures

Our hospital is equipped with a standard donor, which is recommended by the guidelines to prioritize the use of standard donors. All potential fecal donors underwent a rigorous multi-step screening process in accordance with the international standards outlined by the European consensus conference[63]. The screening criteria were also consistent with the recommendations from the Chinese expert consensus [64] to ensure suitability for the local population.(1) Physiological eligibility was primarily determined through scientific measurements and laboratory tests. Physical examinations in internal medicine and surgery yielded negative findings. Donors were aged between 18 and 30 years, with a body mass index (BMI) ranging from 18.5 to 23.9 kg/m². All hematological, pathogenetic, and stool tests returned negative results. Serological screening for monogenic genetic disorders was also negative.(2) A structured interview conducted by a psychiatrist confirmed the donor's sound psychological status. Scores on the Self-Rating Depression Scale (SDS), Self-Rating Anxiety Scale (SAS), and Pittsburgh Sleep Quality Index (PSQI) were within the normal ranges. There was no family history of psychiatric disorders within two generations across three lineages.(3) Medical and personal history assessment indicated no gastrointestinal discomfort within the preceding two weeks, and no use of antibiotics, immunosuppressants, chemotherapeutic agents, or similar medications within the past three months. There was no history of digestive system diseases, surgeries, infectious diseases, or contact with infectious sources. Additionally, donors had no history of allergic diseases, autoimmune disorders, malignancies, metabolic diseases, cardiovascular and cerebrovascular diseases, neurological disorders, or relevant family history of these conditions.(4) Donors underwent regular re-evaluations based on

the above criteria. A stool sample was archived following each donation event.

Preparation of Gut Microflora Solution:(1) Stool collection: the donor of the bacteria in the morning of the operation in the sterilization of fresh feces in the container, weighing which formed 100g of soft stool.(2) Sterilization: Place in a sterilized beaker, add 250mL of 0.9% NaCl and stir well with a glass stirring rod.(3) Filtration: placed in the ultra-clean bench, respectively, with a diameter of 2.0mm, 1.0mm, 0.5mm, 0.25mm steel wire mesh from coarse to fine filtration step-by-step, to remove large particles of solid impurities will be transferred to the bacterial liquid in a 50ml centrifuge tube. (4) Centrifugation: centrifuge at 6000 rpm for 15 minutes at room temperature, and collect 80-120mL of supernatant as fresh fecal fluid.(5) Storage and thawing: The pre-prepared Enterobacteriaceae liquid can be stored in the refrigerator at -20℃ for short-term storage, with a shelf life of about 1 week; for long-term storage, it must be stored in the refrigerator at -80℃, with a shelf life of 6 months. Thaw in a 37℃ constant temperature water bath.

FMT Clinical Operations:(1) Confirm that the transplant recipient has signed an informed consent form and that the recipient has a nasoenteric tube.(2) Recipients underwent bowel preparation: oral amoxicillin 0.5g BID + levofloxacin 0.5g QD + metronidazole 0.2g BID 3 days before enterobacteria transplantation, bowel cleansing was performed with 2 boxes of Compound Polyethylene Glycol Electrolyte Dispersion (I) (Hengkang Zhengqing) (Manufacturer: Jiangxi Hengkang Pharmaceutical Co., Ltd, Batch No.: National Drug License No. H20020031) on the day before transplantation.(3) Start of

Enterobacteriaceae transplantation: The transplant recipient stands and the treating physician slowly injects Enterobacteriaceae liquid through a nasoenteric tube behind the recipient back. This is done at a fixed time each day for three days. On the first day, 80 ml of Enterobacteriaceae liquid is administered, and on the second and third days, 120 ml of Enterobacteriaceae liquid is administered. After completion of the bacterial fluid injection each day, the patient was required to stand for 0.5 hours and was allowed to eat after 2 hours. The nasoenteric tube was removed after completion of enterobacterial transplantation. Operational procedures for fecal microbiota transplantation see Fig.4.

All subjects were treated with a single antidepressant (escitalopram oxalate tablets, ESC), manufacturer: Shantung Jingwei Pharmaceutical Co. Ltd, Batch No: NDT H20103327), and no antidepressant adjustments were made during the course of combined FMT treatment. In the presence of severe anxiety, agitation, or insomnia, non-benzodiazepines may be used for a short period of time.

Observation indicators：General demographic data such as sex, age, body mass index (BMI), years of education, whether it was the first time or not, and whether there was a family history were collected from the enrolled patients. Patients were assessed for depressive symptoms using the HAMD-24 at baseline and at the end of week 2 in both groups, and for treatment-emergent adverse events using the Treatment Emergent Symptom Scale (TESS) and laboratory tests. In the observation group, feces were collected on the same day, one day before, and at the conclusion of the second week of FMT treatment for testing of ten dominant flora (hereinafter referred to as the "ten-combination test"). In the control group, the test was performed at baseline. Ten-combination

test: Detection of 10 dominant bacterial species in the intestinal tract by real-time fluorescence quantitative PCR (Realtime qPCR). These include Lactobacillus and Bifidobacterium, Enterobacteriales, Enterococcus, Bacteroides, Atopobium, F. prausnitzii, C. butyricum, C. leptum and Eubacterium rectum. These ten representative bacteria were selected from a large cohort of healthy individuals and are considered potential indicators for evaluating the entire human gut microbiome. Fecal bacterial DNA samples were subjected to qPCR using a PCR instrument (ABI-7500, ABI, USA). qPCR was performed in units of copies per microliter of total fecal microbial DNA (Copies/uL) for each dominant bacterial group.

Evaluation indicators: The primary assessment indicators include the deduction value and deduction rate of HAMD-24, treatment efficiency, and changes in indicators before and after the transplantation of ten dominant bacteria in the human gut.The safety assessment indicators encompassed a wide range of parameters, including adverse drug events, serious adverse events, routine blood tests, biochemistry, electrocardiogram, immune indicators (immunoglobulin + complement), Hypersensitive C-reactive protein(hs-CRP), thyroid function, glycosylated hemoglobin, and an array of additional laboratory tests and examinations. Adverse events were solicited and documented throughout the study, with subjects providing a subjective assessment of severity (categorized as mild, moderate, or severe). The relationship of these events to treatment was also queried (categorized as none, rarely, possibly, probably, very probably, and definitely), and the regression of adverse events was meticulously monitored during the study. The flow chart of the study is shown in Fig.5.

Statistical analysis

SPSS 25.0 was used for statistical analysis. When the measurement data met the normal distribution and the variance was homogeneous, it was represented by mean ± standard deviation (x±s) and independent sample t test was used. When the data distribution did not meet the normal distribution or the variance was uneven, Wilcoxon rank sum test was used, and Md (P25, P75) was used. Statistical data were measured by $\chi 2$ test. $P<0.05$ was statistically significant. In order to visualize the changes of ten dominant flora in human intestinal tract before and after FMT, the data of intestinal flora decanalysis before and after FMT of the experimental group were grouped into percentage stacked bar charts. Linear discriminant analysis effect size (LEfSe) to analyze the differential flora between groups. Correlation test between intestinal flora and depressive symptoms: the severity of depressive symptoms was expressed by HAMD-24 score, and the correlation between the overall abundance of intestinal flora and depressive symptoms before and after treatment in the FMT group was examined using the Spearmen correlation analysis, and it was significant at $P<0.05$.

Ethics approval and consent to participate

The study adhered to the ethical standards established by the Ethics Committee of Shulan Hospital (Ethics Number: KY2024024), and it was conducted in accordance with the principles of the Declaration of Helsinki. All subjects provided written informed consent prior to participation.

Conflict of interests

The authors declare no conflict of interest.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Author's contribution

Linlin Wang, Sijia Zhang, Xujuan Li and Guoqiang Tian designed the study. Linlin Wang, Sijia Zhang and Yiyun Liu completed the experiment and data collection. Linlin Wang, Sijia Zhang and Deqiang Li contributed to data statistics. Linlin Wang and Sijia Zhang wrote the first draft of the manuscript. Xujuan Li and Yufeng Li reviewed and revised the manuscript. All authors read and approved the manuscript.

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Figure legends

Fig.1 Participant flow diagram

The flowchart illustrates the progression of participants through each stage of the trial. Out of 46 randomized participants (23 to the experimental group and 23 to the control group), 9 in the experimental group and 3 in the control group dropped out during the intervention period. Consequently, 14 participants in the

experimental group and 20 in the control group were included in the final analysis.

Fig. 2 Distribution map of the relative abundance of intestinal flora before and after treatment in the experimental group

The ten combined test data of intestinal flora before and after FMT treatment in the experimental group were grouped into percentage accumulation bar charts. From a to n represents the intestinal flora of the experimental group before FMT treatment, and from a1 to n1 represents the intestinal flora of the experimental group after FMT treatment.

Fig. 3 LDA Discriminant Bar Chart

The relative abundance of Enterococcus spp. in the intestine after FMT treatment was found to be significantly increased from the LDA discriminant bar graph (P=0.02), and no significant difference was observed for the remaining 9 dominant intestinal flora.

Note: The Linear discriminant analysis effect size (LEtSe) revealed that Enterococcus was the only genus with significant differences in the intestinal microbiota community structure after FMT treatment for depression.

Fig. 4 This figure outlines the standardized operation procedure for fecal microbiota transplantation.

Fig. 5 Flow chart of the study.

Table 1 Comparison of general information of the two groups $\bar{x} \pm s$ / N(%)

	Experimental group N=14	control group N=20	$t/Z/\chi^2$	P

Sex				
Men	8（57.1）	7（35.0）	1.638	0.201
Women	6（42.9）	13（65.0）		
Age(Years)	46.29±14.568	39.25±14.628	-1.383	0.176
Body mass index (kg/m3)	22.30±3.899	21.793±2.930	0.435	0.666
Education Years(Years)	13.5（6 16）	12（8.5 16）	1.956	0.582
first episode				
Yes	0（0）	5（25）	4.103	0.063
No	14（100）	15（75）		
family history				
Yes	1（7.1）	2（10）	0.084	0.773
No	13（13）	18（90）		
Gastrointestinal symptoms				
Yes	4（28.6）	6（23.1）	0.147	0.718
No	10（71.4）	20（76.9）		
HAMD-24	21.43±2.065	21.05±2.417	0.476	0.637

Table 2 Comparison of HAMD-24 before and after treatment within the two groups（x±s）

	Baseline HAMD-24	2 Weekend HAMD-24	*t*	*p*	*95%CI*
Experimental group	21.36±2.170	9.86±1.916	14.865	0.000	（9.910 13.090）
control group	21.30±2.473	11.90±2.075	13.022	0.000	（7.939 10.861）

Note: HAMD-24 is Hamilton’s Depression Scale-24

Table 3 Comparison of HAMD-24 Score Reduction and Reduction Rates Between the Experimental and Control Groups at 2 Weeks Post-Treatment

Group	Cases	ΔHAMD-24 （x±s）	Reduction Rate（%） （x±s）	Treatment efficacy rate N(%)
Experimental	14	11.50±3.057	56.2±11.4	10（71.4）
Control	20	9.40±2.644	43.7±9.8	7（35.0）
t/Z		2.138	2.610	—
p		0.040	0.014	0.04**
95%CI		（0.099 4.101）	（2.090 16.945）	—

Note: HAMD-24 score reduction rate = (Initial score - Final score)/Initial score ×100% ； Treatment efficacy = HAMD-24 score reduction rate ≥ 50% ,Treatment efficacy rate = Number of effective cases / Total number of treated cases × 100%

***P values were calculated using the Fisher's exact test for categorical variables.*

—, not applicable.

Table 4 Changes of 10 intestinal flora in humans before and after FMT in the experimental group

Flora	Before FMT M(P_{25} P_{75}）	After FMT M(P_{25} P_{75}）	Wilcoxon rank sum test Z	P
F. prausnitzii	1700000.00	469000.00	-0.760	0.447

	（423250.00， 2582500.00）	（117675.00， 2750000.00）		
Enterococcus	2725.00（392.500， 13275.00）	70500.00 （2355.00， 523250.00）	-2.552	0.011
Bacteroides	15800000.00 （6352500.00， 31650000.00）	6410000.00 （80100.00， 98500000.00）	-0.930	0.352
Lactobacillus	5190.00（1530.00， 60650.00）	189000.00 （25300.00， 816250.00）	-2.563	0.010
Bifidobacterium	45900.00 （6030.00， 283500.00）	366500.00 （37825.00， 5799000.00）	-1.996	0.046
Eubacterium	46450.00 （1162.50， 679500.00）	493500.00 （4055.00， 3567500.00）	-1.270	0.204
Atopobium	96650.00(16375.00,550000.00)	163000.00 （95425.00， 1355000.00）	-1.316	0.188
Enterobacteriales	678000.00 （24425.00， 2725000.00）	581500.00 （39575.00， 2747500.00）	-.272	0.785
C. butyricum	34100.00（944.00， 211500.00）	2115000.00 （528250.00， 13725000.00）	-3.062	0.002
C. leptum	4125000.00 （110600.00,8790000.00）	385500.00 （35797.50， 7097500.00）	-1.361	0.174

Table 5 Correlation analysis between 10 intestinal flora and HAMD-24 in the experimental group before and after FMT

	Pre-FMT vs. baseline	Post-FMT & 2 Weekend

	HAMD-24 R	*P*	HAMD-24 R	*P*
F. prausnitzii	-0.554	0.040	0.149	0.611
Enterococcus	-0.073	0.803	0.578	0.030
Bacteroides	-0.227	0.435	-0.477	0.085
Lactobacillus	-0.307	0.310	0.228	0.432
Bifidobacterium	-0.002	0.994	0.319	0.267
Eubacterium	-0.714	0.004	-0.301	0.296
Atopobium	-0.033	0.910	0.267	0.356
Enterobacteriales	-0.109	0.711	0.095	0.747
C. butyricum	-0.583	0.029	0.183	0.531
C. leptum	-0.137	0.639	-0.115	0.695

Table 6 Comparison of safety indicators [%]

Adverse Events	experimental group	control group	χ^2	*p*
Nasopharyngeal discomfort	1[7.1]	0[0]	1.472	0.412
nausea	2[14.3]	3[15]	0.003	0.954
abdominal distension	1[7.1]	0[0]	1.472	0.225
Headache	0[0]	2[10]	1.488	0.223
constipation	0[0]	1[5]	0.721	0.396
Total incidence	4[28.6]	6[30]	0.008	0.928

Table 7 Comparison of Laboratory-Related Safety Indicators

Security Indicators	Before FMT treatment	After FMT treatment	*t*	*p*
blood routine test				
leucocyte	5.621±1.504	5.557±1.288	0.149	0.884
erythrocyte	4.552±0.870	4.513±0.916	0.443	0.665
platelet count	204.285±68.051	215.500±71.141	-1.117	0.284
glycosylated hemoglobin	5.321±0.617	5.229±0.572	0.457	0.655
thyroid functions				
TT3	1.431±0.265	1.456±0.369	-0.287	0.779
TT4	96.957±22.379	99.585±24.393	-0.321	0.754
FT3	4.536±0.722	4.468±0.532	0.265	0.795
FT4	14.575±2.507	14.680±2.962	-0.085	0.933
TSH	2.569±4.369	2.186±1.765	0.278	0.786
immune system				
Immunoglobulin M	1.204±0.449	0.955±0.378	1.995	0.067
Immunoglobulin G	11.923±.723	11.576±4.256	0.420	0.681
Immunoglobulin A	2.291±0.810	2.22±0.744	0.356	0.728
Complement C3	1.039±0.157	0.983±0.288	1.079	0.300
Complement C4	0.254±0.109	0.278±0.142	-0.659	0.522
hsCRP	1.379±1.166	0.786±0.729	1.878	0.083

Fig.1

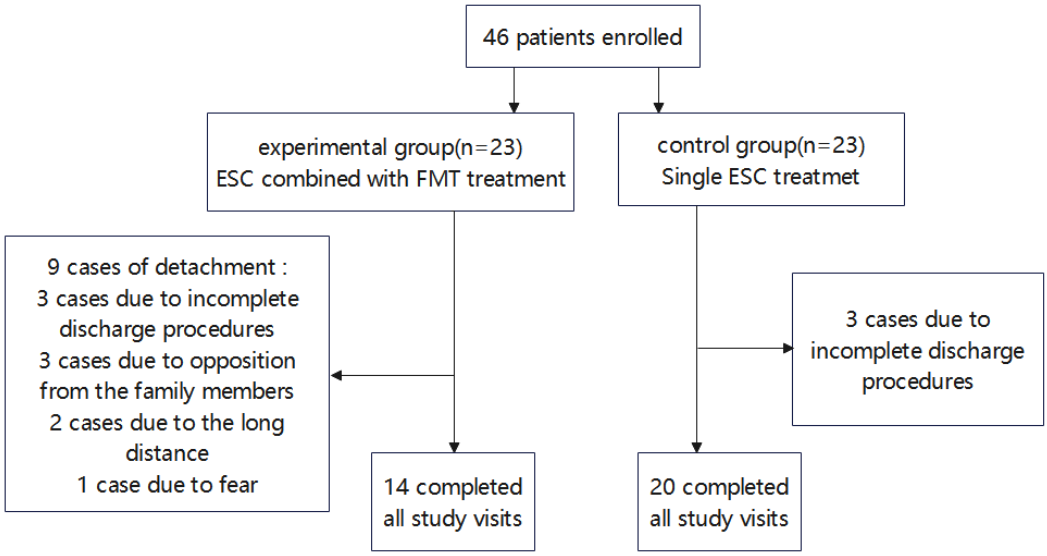

Fig.2

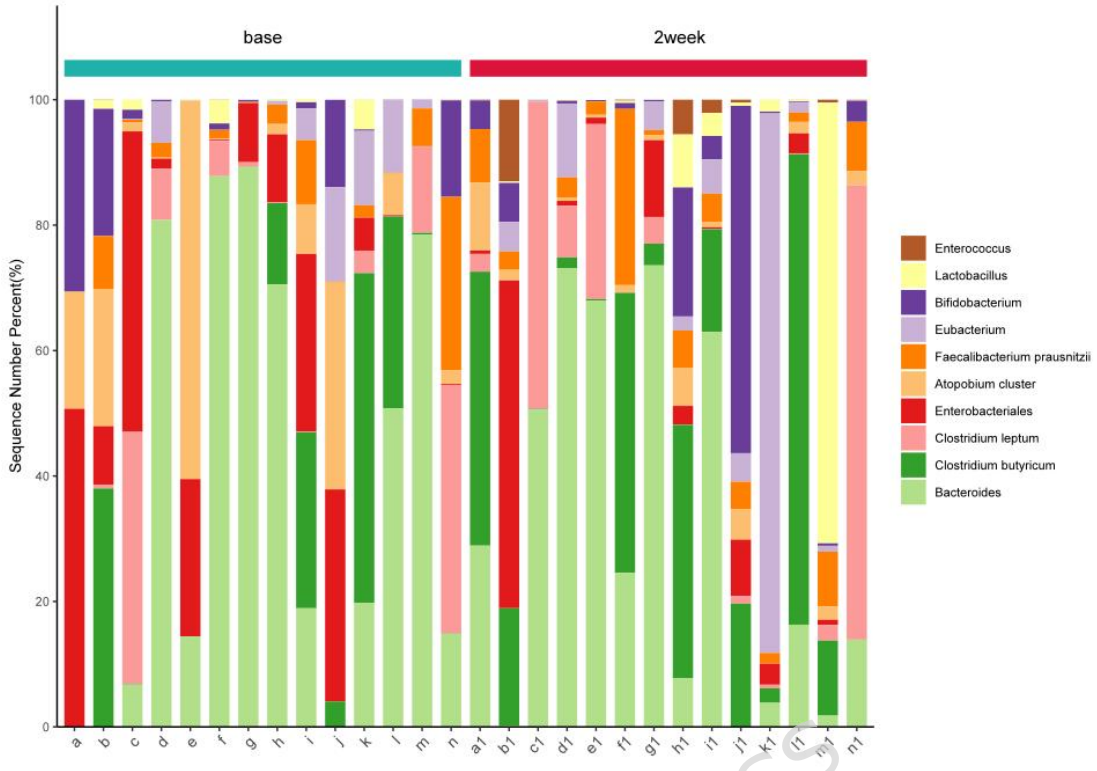

Fig.3

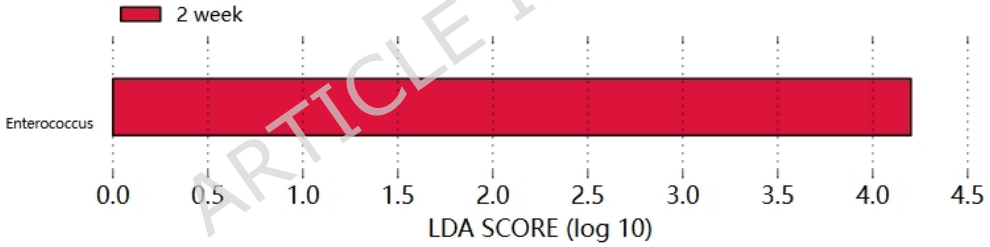

Fig.4

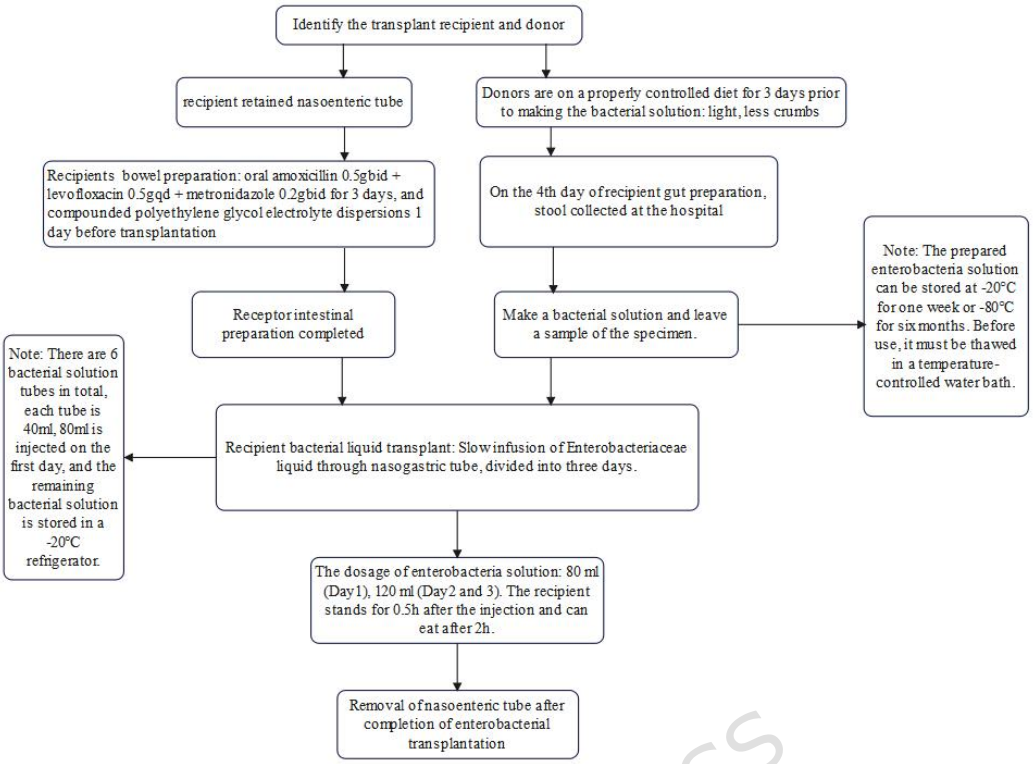

Fig. 5