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REVIEW ARTICLE

16S rDNA analysis of the effect of fecal microbiota transplantation on pulmonary and intestinal flora

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Abstract This study aims to explore the effect of FMT on regulations of dysbacteriosis of pulmonary and intestinal flora in rats with 16S rDNA sequencing technology. A total of 27 SPF rats (3–4 weeks old) were randomly divided into three groups: normal control group (K), model control group (MX), and fecal microbiota transplantation group (FMT); each group contained nine rats. The OTU values of the pulmonary and intestinal flora of the MX group decreased significantly compared with the normal control group. After FMT, the OTU value of pulmonary flora increased, while the value of OTU in intestinal flora declined. At the phylum level, FMT down-regulated *Proteobacteria*, *Firmicutes*, and *Bacteroidetes* in the pulmonary flora. At the genus level, FMT down-regulated *Pseudomonas*, *Sphingobium*, *Lactobacillus*, *Rhizobium*, and *Acinetobacter*, thus maintaining the balance of the pulmonary flora. Moreover, FMT could change the structure and diversity of the pulmonary and intestinal flora by positively regulating the pulmonary flora and negatively regulating intestinal flora. This study may provide a scientific basis for FMT treatment of respiratory diseases.

Keywords Fecal microbiota transplantation · Pulmonary flora · Intestinal flora · 16S rDNA

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Introduction

Fecal microbiota transplantation (FMT) is a kind of treatment method for transferring the functional bacteria from normal feces to the gastrointestinal tract, reconstructing the new intestinal flora, and restoring the host function. ZhouHouBeiJiFang in the Chinese Yellow Dragon Soup might be the first record of fecal transplant therapy, suggesting that FMT could treat intestinal and pulmonary diseases. In 1958, for the first time, Eiseman et al. reported that FMT was used to treat patients with severe pseudomembranous colitis, who were not treated with conventional antibiotics, and the satisfactory treatment effects in all the patients (Eiseman et al. 1958). Khoruts et al. treated the refractory *Clostridium difficile* infection diarrhea patients by FMT and finally found that FMT improved the symptoms of diarrhea and promoted the recovery of intestinal flora function (Khoruts et al. 2010). In 1983, for the first time, Schwan et al. reported that FMT was effective in the treatment of CDI by enema (Schwan et al. 1983). At present, FMT has been widely applied in the treatment of recurrent or refractory CDI enteritis and more mature treatment norms and guidelines have been developed. In addition, in recent years, with the extensive application of sequencing technology and the further studies between flora and diseases, FMT has achieved good treatment effects in many other diseases, such as IBD, IBS, and metabolic syndrome (Colman and Rubin 2014; Zoller et al. 2015; Marotz and Zarrinpar 2016).

The human intestinal tract has the largest surface area as well as the largest contact area with the external environment. There are abundant bacteria in the intestinal tract. Intestinal microflora and its metabolites (short chain fatty acids, etc.) and intestinal mucosal immunity form the

intestinal microenvironment, which is generally in a dynamic balance. The damaged balance may lead to various diseases. Intestinal flora is an important part of the intestinal microenvironment, so it is important to balance the intestinal flora (Mcknite et al. 2012; Van den Elsen et al. 2017). In the treatment of chronic respiratory diseases, antibiotics are often used (Suresh et al. 2013). Although antibiotics can relieve diseases, they cannot avoid the repeated occurrence of these diseases. The extensive application of antibiotics and hormone could induce dysbacteriosis and cause diseases. Therefore, the mechanisms of the repeated occurrence of chronic respiratory diseases might involve the regulation of the flora balance (Chakhava et al. 1985; Tsuei et al. 2014; Herbert 2015).

In this study, with 16S rDNA sequencing technology, we explored the influences of FMT on the pulmonary and intestinal flora which could provide a scientific basis for FMT treatment of respiratory diseases.

Materials and instruments

Animals

A total of 27 SPF wistar rats (100 ± 10 g), 3–4 weeks old male, were obtained from Chengdu Dashuo Experimental Animal Co. Ltd. All rats were kept under standard environmental conditions with free access to rodent diet and water.

Experimental drugs

Antibiotics

Cefradine capsules were purchased from Shijiazhuang Pharmaceutical Group Ouyi Pharma Co.Ltd., and gentamicin sulfate was obtained from Shanghai Shen Guang Company.

Hormone

Dexamethasone sodium phosphate was purchased from Shanxi, Ruicheng Kelon Veterinary Medicine Co. Ltd.

Methods

Grouping

The animals were randomly divided into normal control group (K), model control group (MX), and fecal transplantation group (FMT), with nine rats in each group.

Drug preparation

Preparation of mixed suspension of hormone and antibiotics

Cefradine capsules, gentamicin sulfate, and dexamethasone sodium phosphate injection were combined to a mixture of 22.6 g/L in the proportion of 1:5:6, which was administered intraperitoneally to rats for 2 ml per day.

Preparation of fecal suspension

On the ninth day, 15 g of fresh feces was obtained from the rats of the normal control group, placed in a sterilized beaker containing 150 mL of the saline solution, and mixed fully. After sterile filtration, the filtrate was placed in a 10-mL centrifuge tube and centrifuged at 5000 r/min and 0 °C for 15 min. In addition, the supernatant was then collected and stored at − 4 °C for 3 h. In the seven consecutive days, the supernatant was prepared once a day (Tian et al. 2017).

Induction of dysbacteriosis of pulmonary and intestinal florae in rats and treatment with FMT

Construction of the model of dysbacteriosis

From the ninth day, the mixed suspension of antibiotics and hormone was injected to the model control group and the FMT group once a day for eight consecutive days. Meanwhile, normal saline was injected to the normal control group in the same way.

Treatment with FMT

The rats in the FMT group were fixed on the fixed plate, upside down, and catheters were inserted into the anus up to 5–8 cm. 5 mL of the fecal suspension was injected into the rats by enema and maintained for 3 min.

Index detection

On the 16th day, the rats were killed and the intestinal contents and lung tissues were taken under aseptic conditions and stored at − 80 °C.

High-throughput sequencing

Sample collection

From the K group, MX group, and FMT group, three samples (lung: FK2, FK4, FK6; FMX2, FMX4, FMX6; FFMT2, FMT4, FFMT6; gut: CK2, CK4, CK6; CMX2,

CMX4, CMX6; CFMT2, CFMT4, CFMT6) of lung tissues and intestinal contents were randomly selected from each group, to follow up the analysis of flora.

MetaVx^{TM} library preparation and illumina MiSeq sequencing

Next-generation sequencing library preparations and Illumina MiSeq sequencing were conducted at GENEWIZ, Inc. (Suzhou, China). DNA samples were quantified using a Qubit 2.0. Fluorometer (Invitrogen, Carlsbad, CA, USA). 30–50 ng DNA was used to generate amplicons using a MetaVx^{TM} Library Preparation kit (GENEWIZ, Inc., South Plainfield, NJ, USA). V3and V4 hypervariable regions of prokaryotic 16S rDNA were selected for generating amplicons and following the taxonomy analysis. The v3 and v4 regions were amplified using forward primers containing the sequence "CCTACGGRRBGCASCA GKVRVGAAT" and reverse primers containing the sequence "GGACTACNVGGGTWTCTAATCC". The v4 region was amplified using forward primers containing the sequence "GTGYCAGCMGCCGCGGTAA" and reverse primers containing the sequence "CTTGTGCGGKCC CCCGYCAATTC". The first round PCR products were used as templates for the second round amplicon enrichment PCR. At the same time, indexed adapters were added to the ends of the 16S rDNA amplicons to generate indexed libraries ready for downstream NGS sequencing on Illumina Miseq. DNA libraries were validated by Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA, USA) and quantified by Qubit 2.0 Fluorometer. DNA libraries were multiplexed and loaded on an Illumina MiSeq instrument according to the manufacturer's instructions (Illumina, San Diego, CA, USA). Sequencing was performed using a 2 × 300/250 paired-end (PE) configuration; image analysis and base calling were conducted by the MiSeq Control Software (MCS) embedded in the MiSeq instrument.

Data analysis

The 16S rDNA data analysis was used by the QIIME package data and R programming language. The forward and reverse reads were joined and assigned to samples based on barcode and truncated by cutting off the barcode and primer sequence.

Results

Behavioral changes

The rats in the K group showed no abnormal intake, shiny hair, fecal morphology, loose stools, or other abnormal conditions. In the MX group, the intake decreased, the hair lost luster, the shapes of feces were changed, and sneezing, nasal discharge, and other symptoms occurred. Compared with the MX group, the intakes of rats in the FMT group was increased and hair luster and fecal morphology were slightly improved, but sneezing or nasal secretions were not observed.

Table 1 Filtered data quality statistics

Samples	#PE_reads	#Nochimera	AvgLen (nt)	GC (%)	Effective (%)
CFMT2	43138	32848	461.21	51.63	76.15
CFMT4	252584	186310	460.46	52.34	73.76
CFMT6	69040	55359	461.06	52.93	80.18
CK2	67902	49032	453.63	53.13	72.21
CK4	70416	49807	454.83	52.6	70.73
CK6	67081	48314	455.06	52.98	72.02
CMX2	64579	44820	456.67	52.84	69.4
CMX4	80856	58467	454.47	53.45	72.31
CMX6	67245	47065	458.68	52.81	69.99
FFMT2	78609	58976	451.85	52.72	75.02
FFMT4	70965	53345	451.35	52.7	75.17
FFMT6	74921	56016	451.9	52.65	74.77
FK2	63854	47940	452.59	52.67	75.08
FK4	73497	55780	450.57	53.15	75.89
FK6	77270	58904	451.93	52.75	76.23
FMX2	67107	49658	452.29	52.68	74
FMX4	48050	34673	451.23	52.77	72.16
FMX6	52788	38156	452.02	52.73	72.28

16S rDNA gene sequence and operational taxonomic units (OTUs)

The effective sequences were determined by Illumina platform (Table 1). The OTU values of the groups (CK, CMX, CFMT, FK, FMX, and FFMT) were 300, 247, 178, 144, 97, and 121(Table 2).

Changes of Chao1 and Shannon indices

Chao1 and Shannon are important components of alpha diversity index. Chao1 index is used to estimate the total number of OTUs contained in the sample. Shannon index is used to estimate the diversity of microbial communities in the samples. Compared with Chao1 and Shannon indices of the CK and FK group, the two indices in the groups of CMX, FMX, and CFMT were decreased, but the two indices in the FFMT group increased (Figs. 1, 2).

Compositions and changes at the levels of phylum and genus

According to the sequencing results, the composition of each sample could be obtained (Fig. 3, Table 3). At the phylum level, the groups of CK, CMX, CFMT, FK, FMX, and FFMT were mainly composed of *Bacteroidetes*, *Firmicutes*, *Proteobacteria*, *Actinobacteria*, *Tenericutes*, *Spirochaetae*, *and Saccharibacteria* (Fig. 4, Table 4). At the genus level, the groups of CK, CMX, and CFMT were mainly composed of *Lactobacillus*, *Prevotellaceae_Ga6A1_group*, and *Allrevotella*. The groups FK, FMX, and FFMT were mainly composed of *Pseudomonas*, *Sphingobium*, *Lactobacillus*, *Rhizobium*, and *Acinetobacter* (Fig. 5 and Tables 5 and 6).

Table 2 OTU values of all the groups

Groups	OTU
CFMT	178
CK	300
CMX	247
FFMT	121
FK	144
FMX	97

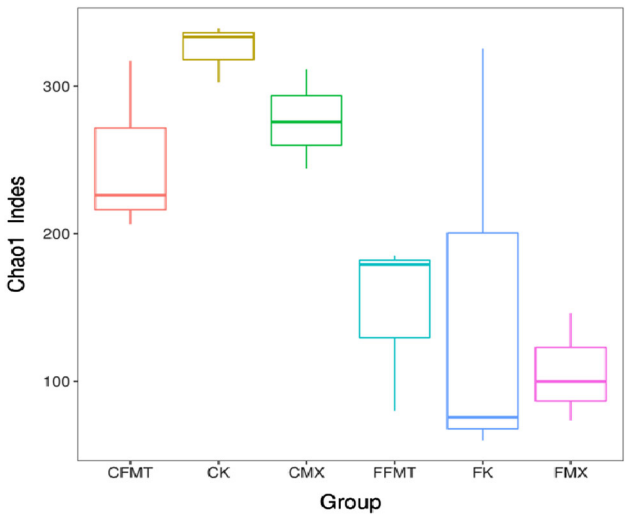

Fig. 1 Chao1 Index of all the six groups

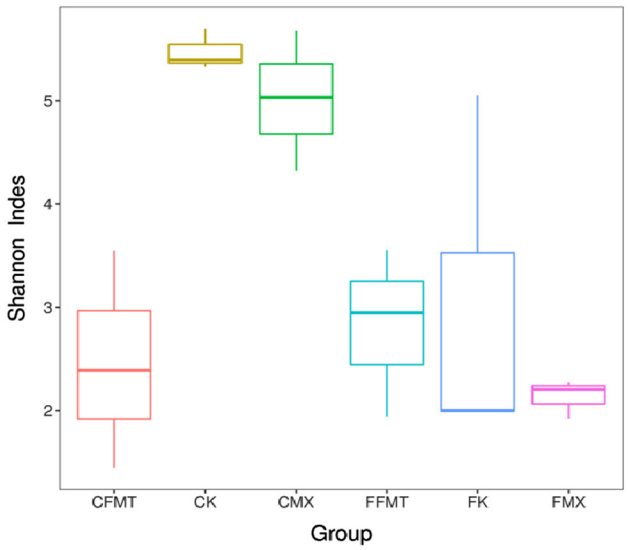

Fig. 2 Shannon Index of all the six groups

Principal component analysis (PCA)

In the coordinate system, the closer the distance between two points, the higher was the similarity. The analysis results showed that the relative dispersion between different groups was relatively concentrated in the same group. According to the results of PCA analysis, there were significant differences between the pulmonary flora and the intestinal flora (Fig. 6).

Discussion

Through the analysis of the combined influence of antibiotics and hormone on the behaviors of rats, we found that in the MX group, the rats showed some changes including mental fatigue and irregular stool shape. In this study, the pulmonary and intestinal florae were sequenced. The OTU values of the CK and FK groups were relatively large and the pulmonary and intestinal florae in the normal rat group showed the highest diversity. The OTU values of the pulmonary and intestinal florae in the MX group decreased

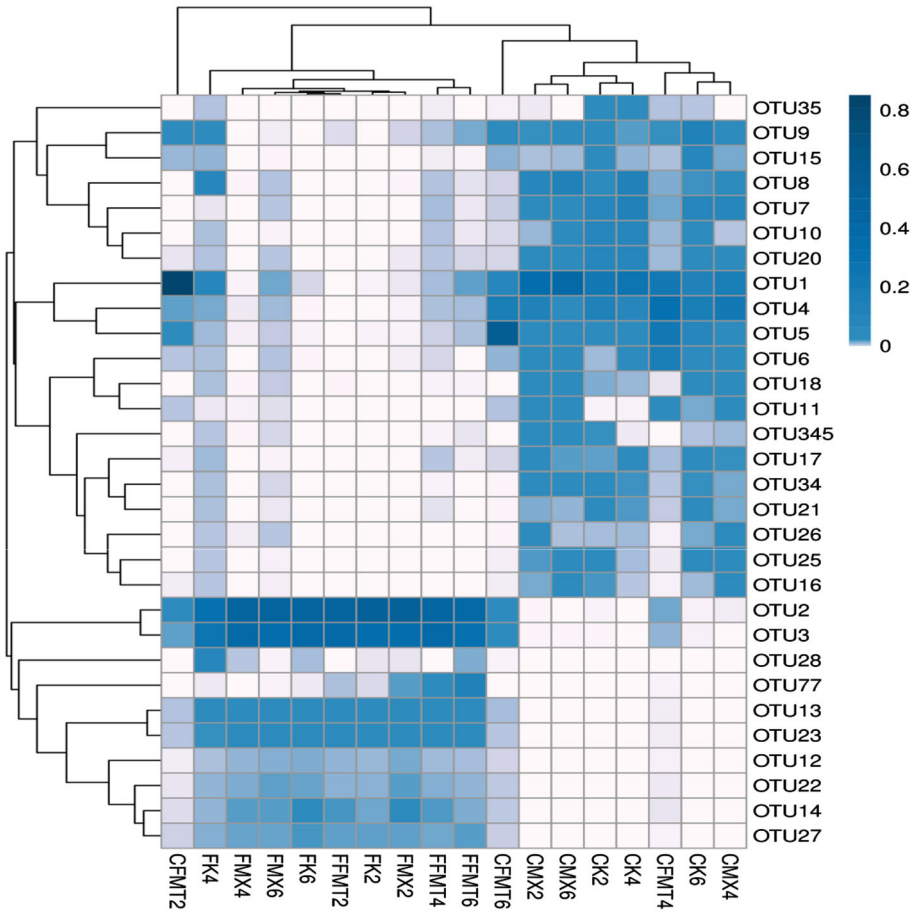

Fig. 3 OTU heatmap of all the samples. Thermal analysis provides abundant information of the selected OTU, further demonstrating the similarity and differences among these data, including the 30 OTUs with the highest default abundances. The left names are OTU ID, and the color value of each square for each row indicates the relative abundance of OTU

significantly compared with the normal control group. After FMT, the OTU value of pulmonary flora increased, while the value of OTU in intestinal flora decreased. Chao1 is often used to estimate the total number of species in ecology, and Shannon index is used to estimate the diversity of microbial communities. The larger Chao1 and Shannon values mean a larger total number of species and higher diversity (Valverde and Mellado 2013; Michelle et al. 2016).

The results of OTU, Chao1, Shannon indices, and the distribution and abundance of flora showed that FMT positively regulates the pulmonary flora and negatively regulates the intestinal flora. This change in the intestinal flora may be interpreted as follows. The variation of intestinal microflora could lead to the resistance phenomenon and self-reaction due to the direct effect of FMT, but FMT had no direct effect on the respiratory tract flora. The changes in the main compositions at the phylum and genus level indicated that the changes in the structure and

Table 3 The numbers at the phylum and genus levels of all the groups

Taxon	Phylum	Genus
CK	8	77
CMX	8	70
CFMT	7	85
FK	10	93
FMX	7	74
FFMT	11	94

abundance of the pulmonary and intestinal flora in rats were induced by antibiotics and hormone. At the phylum level, FMT down-regulated the levels of *Proteobacteria*, *Firmicutes*, and *Bacteroidetes*, which were the dominant phyla in the pulmonary flora and could maintain the balance of the bacterial flora (Pragman et al. 2012; Poroyko et al. 2015). At the genus level, FMT down-regulated *Pseudomonas*, *Sphingobium*, *Lactobacillus*, *Rhizobium*,

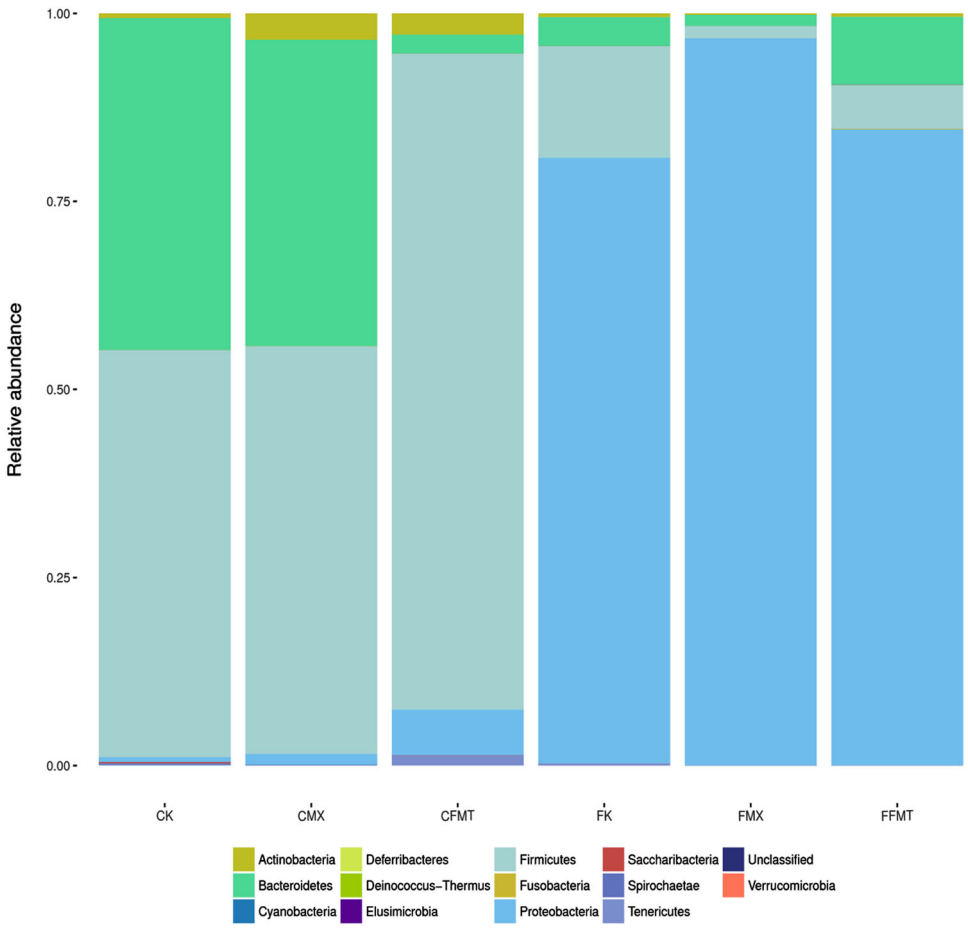

Fig. 4 Phylum composition of all the six groups. At the phylum level, the groups of CK, CMX, CFMT, FK, FMX, and FFMT were mainly composed of *Bacteroidetes*, *Firmicutes*, *Proteobacteria*, *Actinobacteria*, *Tenericutes*, *Spirochaetae*, and *Saccharibacteria*

Table 4 The top seven percentages at the phylum level of all groups (%)

Taxon	CK	CMX	CFMT	FK	FMX	FFMT
Firmicutes	54.09	54.17	87.20	14.87	1.61	5.82
Bacteroidetes	44.14	40.75	2.50	3.81	1.47	8.99
Proteobacteria	0.65	1.45	6.05	80.51	96.71	84.56
Actinobacteria	0.61	3.49	2.81	0.49	0.18	0.44
Tenericutes	0.17	0.02	1.38	0.22	0.00	0.00
Spirochaetae	0.15	0.10	0.00	0.00	0.00	0.00
Saccharibacteria	0.17	0.00	0.03	0.02	0.00	0.01

and *Acinetobacter*, which might cause lung infection, inflammation, and metabolic disorders, and maintained the balance of the pulmonary flora (Dickson et al. 2014; Hu et al. 2015; Lo et al. 2015; Souto et al. 2014; Datta et al. 2017). After FMT, the pulmonary flora in the levels of phylum and genus was restored, suggesting that FMT might be one of the effective ways to prevent and treat chronic respiratory diseases.

In addition, FMT-related adverse reactions have been widely reported (Li et al. 2015; Patel et al. 2013; De Leon et al. 2013), including abdominal distension, intestinal peristalsis, and other symptoms, which may be related to the changes in the intestinal flora in this experiment. FMT is a new non-standardized treatment, and its adverse effects, potential risks, or long-term safety are unknown (Kelly et al. 2015; Paramsothy et al. 2015). Therefore, the

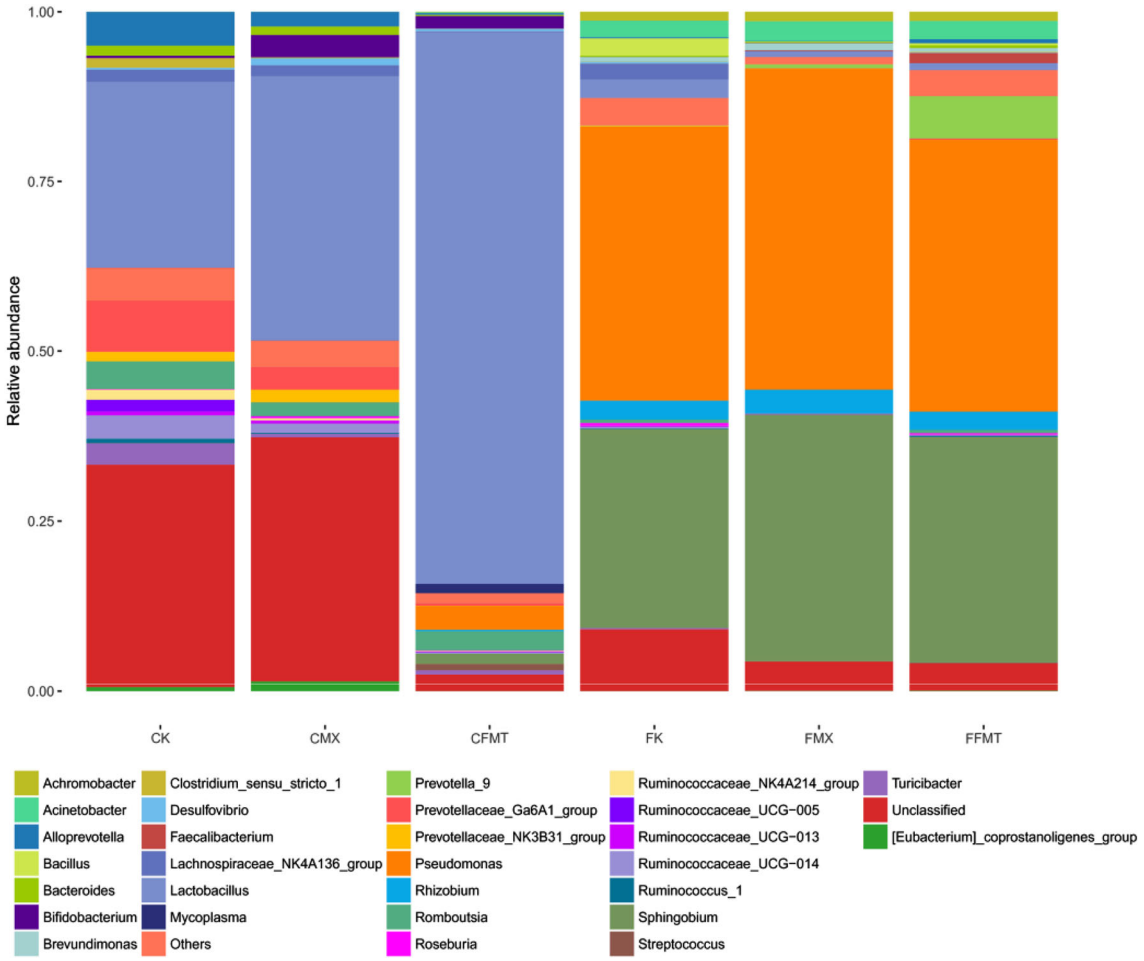

Fig. 5 Genus composition of all the six groups. At the genus level, the groups CK, CMX, and CFMT were mainly composed of *Lactobacillus*, *Prevotellaceae_Ga6A1_group*, and *Alloprevotella*; the groups FK, FMX and FFMT were mainly composed of *Pseudomonas*, *Sphingobium*, *Lactobacillus*, *Rhizobium*, and *Acinetobacter*

Table 5 The top three percentages at the genus level in the intestinal flora (%)

Taxon	CK	CMX	CFMT
Lactobacillus	27.4	38.95	81.24
Prevotellaceae_Ga6A1_group	7.57	3.38	0.42
Alloprevotella	5	2.17	0.25

Table 6 The top five percentages at the genus level in the pulmonary flora (%)

Taxon	FK	FMX	FFMT
Pseudomonas	40.4	47.29	40.04
Sphingobium	29.27	36.3	33.2
Rhizobium	2.79	3.53	2.69
Acinetobacter	2.45	2.91	2.71
Lactobacillus	2.71	0.8	1

treatment of respiratory tract infections by FMT needs to be further studied.

In this study, FMT regulated the intestinal bacterial flora imbalance effectively in rats, which could provide a scientific basis for clinical prevention and treatment of chronic respiratory diseases by FMT. Chakradhar also reported the same treatment method (Chakradhar 2017; Tamburini and Clemente 2017). Respiratory tract flora and intestinal flora play an important role in maintaining the ecological balance in the human body. For the first time in this study, we found that the intestinal flora could regulate the respiratory tract flora. These 16S rDNA analysis results

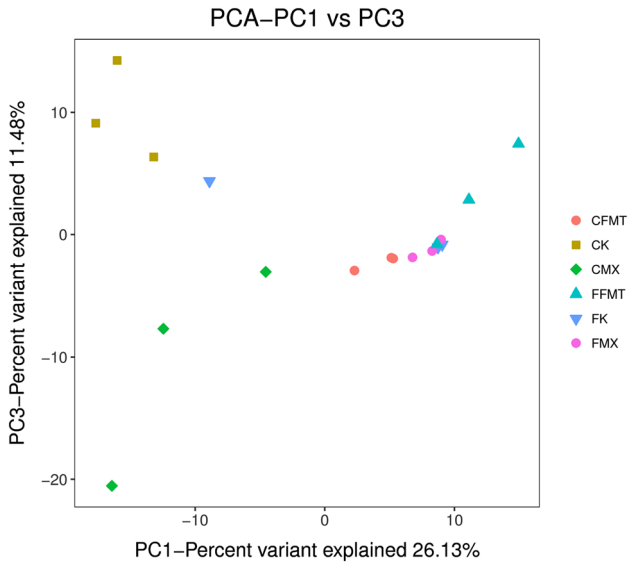

Fig. 6 Principal component analysis results of all the six groups. Principal component analysis (PCA) is a technique to simplify data analysis and can effectively identify the most important elements and structures in the data. PC1, PC2, and PC3 represent the first, second, and third principal components, respectively. The percentage of the principal component represents the contribution rate of this component to the sample difference, and it measures how much the principal component extracts from the original information. The distance between the sample points represents the similarity of the function classification distribution in the samples. The closer the distance, the higher is the similarity

verified the theory "pulmonary lung diseases could be treated via the intestinal regulation" recorded in Chinese ancient books "HuangDiNeiJing" and provided a scientific basis for FMT treatment of respiratory diseases.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

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