Changes in Intestinal Flora Following Fecal Microbiota Transplantation in Patients with Multidrug-Resistant organisms Infection

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Abstract

Incidence of antimicrobial-resistant infections has increased dramatically worldwide in the past few decades. To combat this alarming problem, strategies preventing colonization with resistant pathogens have been proposed. Fecal microbiota transplantation (FMT) may help in decolonizing multidrug-resistant organisms (MRO) and in preventing recurrent infections. In this study, efficacy of FMT against multidrug resistance was assessed. Three patients that suffered from multidrug resistance complicated with diarrhea were enrolled from January 2019 to September 2020. All had severe pulmonary infections. Standard FMT was given, and whole metagenome sequencing of stool was conducted before and after FMT. All patients accepted FMT, and 12 samples were harvested. All treatments were successful, and there were no adverse events. All patients showed a decrease in species of intestinal flora, but FMT could recover some of the diversity. Normal people had the lowest abundance of antimicrobial-resistant genes (ARGs), but the patients had the highest abundance before FMT. FMT decreased ARGs to some extent. Nineteen ARG subtypes were significantly different between the normal group and the patients. Patients with MRO infections had increased abundance of ARGs and low bacterial diversity. FMT eradicated ARGs and restored microbial diversity, and there were no serious adverse events.
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Keywords: fecal microbiota transplantation, multidrug-resistant infection, antimicrobial-resistant genes, bacterial diversity, intestinal flora

1. Introduction

Infection caused by antibiotic-resistant bacteria is one of the main causes of death in patients in intensive care units. Such patients need high doses of antibiotics, which disturb intestinal flora and cause antibiotic-related diarrhea. With disturbance of intestinal flora, mucosal immunity decreases and some drug-resistant bacteria migrate, leading to multidrug-resistant (MDR) bacterial infection. Thus, a vicious circle begins. Intestinal flora protect the intestinal mucosal barrier, inhibit the growth of pathogenic microorganisms, secrete antimicrobial peptides, and reduce colonization of the intestine by MDR bacteria [1-5]. Moreover, fecal microbiota transplantation (FMT) has excellent curative effect in the treatment of Clostridium difficile [6]. However, whether intestinal flora have a therapeutic effect in MDR bacterial infections and by what mechanisms remain unclear.

2. Materials and Methods

This study was an uncontrolled observational clinical trial. Three patients that suffered from MDR bacterial infection were enrolled from January 2019 to September 2020 with consent to apply FMT. Multidrug-resistant bacteria are pathogens simultaneously resistant to three or more antibiotics. On the basis of drug susceptibility tests, patients accepted antibiotics alone or combination, including Tigecycline, Tienam, and polymyxin. Fecal bacterial liquid was obtained from the Shanghai 10th Hospital (Shanghai Human Intestinal Flora Development and Engineering Technology Center, Shanghai). Selection of donors was rigorous. Briefly, screening of donor candidates included stool culture for bacterial enteric pathogens, a C. difficile cytotoxin assay, ova and parasite ELISA (Giardia lamblia, Entamoeba histolytica, and Cryptosporidium sp.), and serology for HIV, syphilis, hepatitis A (HAV IgM), hepatitis B (HBsAg), hepatitis C (HepC total Ab), and human T-cell lymphotropic virus (HTLV-1/2 antibody). In addition, donors reported no acute illness, no diarrhea, no functional bowel disorders, no receipt of antibiotics within one month, and no immunosuppressive therapy within six months of stool donation.

Fecal microbiota transplantation was performed. Briefly, 300 ml of fecal fluid was delivered via a nasal jejunal tube once a day for three consecutive days. Patients needed no additional bowel preparation before FMT. Subjects were assessed on days 0, 1, and 7 after FMT by collecting stool for whole metagenome sequencing. Acquisition of an antimicrobial-resistant gene (ARG) was defined when a particular gene was in the donor at the beginning of FMT and then was in the recipient at any subsequent visit after FMT. Deletion of an
ARG was defined when a resistant gene was in the patient on day 0 but was not in the donor and then was absent in the recipient on all subsequent visits [1].

Microbial DNA was extracted from fecal samples using an E.Z.N.A.® DNA Kit (Omega Bio-tek, Norcross, GA, USA) according to the manufacturer’s protocols. Metagenomic shotgun sequencing libraries were constructed and sequenced at the Shanghai Biozeron Biological Technology Co. Ltd. (China). Briefly, from each sample, 1 μg of genomic DNA was sheared by a Covaris S220 focused-ultrasonicator (Woburn, MA, USA), and sequencing libraries were prepared with a fragment length of approximately 450 bp. All samples were sequenced on an Illumina HiSeq X instrument (Illumina, Inc.San Diego, California USA) in paired-end 150-bp (PE150) mode. Raw sequence reads underwent quality trimming using Trimmomatic (http://www.usadellab.org/cms/uploads/supplementary/Trimmomatic) to remove adaptor contaminants and low-quality reads. After quality control, reads were mapped against the human genome (version: hg19) by the Biometrics for Web Authentication (BWA) mem algorithm (parameters: -M -k 32 -t 16; http://bio-bwa.sourceforge.net/bwa.shtml). All analyses were performed in R (v 3.3)(https://www.r-project.org).

3. Results

In general, the three patients accepted FMT, and 12 samples were harvested. All FMTs were successful, and there were no adverse events. The 12 samples included four groups: normal control (CKF), pretreatment (TFF), one day after FMT (TPF1), and seven days after FMT (TPF7). Each group consisted of three biological replicates.

3.1 Genetic and Species-Level Diversity

Genetic and species-level diversity were measured in each group of samples. Normal control samples had high diversity and a good balance of bacteria, indicating a healthy intestinal community. However, diversity in the three samples before treatment was very low, indicating serious imbalance in the bacterial community. After FMT treatment, diversity of bacterial communities gradually increased, with diversity higher seven days after treatment than after one day. Thus, FMT had a significant effect (Figure 1).

3.2 Composition of Intestinal Flora

Floral composition of samples was relatively similar within one group. However, one sample was an exception, TPF7-2 had a high virus rate seven days after FMT, which should be explored further (Figure 2).

3.3 Differences in Species

There were significant differences in species composition among the four groups. The abundance of many taxa was significantly higher in the control group than in the other groups, including that of Eubacteriales, Oscillatoriales, Flavobacteria, Erysipelotrichaceae, Erysipelotrichia, and Negativicute. By extension, this result indicated that intestinal flora of the patients was unbalanced. Abundance of Pipldae increased significantly in the TFF group, whereas the taxon was not in the CKF group. Abundance of Hafniae and Morganellaceae increased significantly in the TPF7 group (Figure 3). The roles of these strains in the different study groups need to be studied further.

3.4 Functions of Flora and Differences in KEGG Pathways

Functional differences in flora among the four groups were explored in Kyoto Encyclopedia of Genes and Genomes (KEGG)(including KEGG levels 1, 2, and 3). Overall, in normal controls (red), gene expression was significantly enriched in pathways involved in cellular motility, environmental adaptation, and amino acid anabolism. By contrast, expression in pathways involved in primary and secondary bile acid synthesis, drug metabolism, and staphylobacterium was higher in patients than in normal controls (green) (Figure 4).

3.5 Antibiotic-Resistant Genes

Increases in ARGs are the direct cause of bacterial resistance, and the gut microbiome is a reservoir of ARGs [7]. Thus, differences in total abundance of ARGs were explored in healthy subjects and in patients before
treatment and one and seven days after FMT. Normal subjects had the lowest abundance of ARGs, whereas the highest abundance was in patients before FMT. After FMT, abundance of ARGs decreased to some extent, but there was no significant difference between days one and seven (Figure 5).

Antibiotic-resistant genes are divided into types and subtypes. The inner ring represents the type of ARG and the outer ring the subtype. Node size indicates relative abundance, and color indicates whether there was a difference. Yellow nodes indicate no difference, whereas red, green, blue, and purple nodes indicate significantly higher abundance in CKF, TFF, TPF1, and TPF7 groups, respectively. Nineteen ARG subtypes were significantly different between the normal group and patients. One patient had a high amount of virus at seven days after FMT, leading to low abundance of ARGs, which was consistent with species composition. Compared with other samples, aminoglycoside ACC (6')-I was significantly more abundant in TFF, and Bacitracin bacA was significantly more abundant in CKF (Figure 6).

Bacteria that produce the most ARGs included enterococci, Staphylococcus aureus, Klebsiella, Acinetobacter, Streptococcus, and enterobacter (Figure 7). This result is consistent with observations in clinical practice. In sepsis patients, especially those with severe lung infection, sputum culture is often composed of Klebsiella pneumoniae, Acinetobacter baumannii, and Staphylococcus aureus, of which most are MDR. Of the ARG subtypes, the abundance of ami AAC(6')-I, van vanS, multidrug transporter, mac ermB, bac bacA, sul sul1, fos fosX, and tet tetA increased. Normal people had much greater bacterial diversity than that in patients and therefore also carried more ARGs. However, the overall abundance of ARGs was the highest among patients. A few strains highly expressed ARGs [Figures 8, 9].

4. Discussion

High biodiversity of microbiota is essential for good health [8, 9], and diseases are often associated with dysbiosis. With dysbiosis and destruction of the gut barrier, pathogens propagate in the spleen and lung where there are many immune cells [8-11]. Then, inflammation-induced organ failure occurs.

According to Chinet (Chinese Bacterial Resistance Monitoring Network, 2020), Staphylococcus aureus, Streptococcus pneumoniae, excrement enterococci, epidermis staphylococcus, and enterococci are the top five MDR gram-positive bacteria. Over 80% of Staphylococcus aureus isolates are methicillin-resistant. Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Acinetobacter baumannii, and Haemophilus influenzae are the top five MDR gram-negative bacteria. The detection rate of carbapene-resistant Klebsiella pneumoniae (CR-KP) continues to increase, from 4.9% in 2013 to 10.9% in 2019 [12]. Infections caused by MDR bacteria lead to increased morbidity, longer hospital stays, higher health care costs, and worse outcomes. To combat this alarming problem, one therapeutic option targets colonization with MDR organisms. However, how to effectively slow production of MDR bacteria and block their spread remains uncertain. Therefore, new nonantibiotic treatments need to be developed. Because of the large role of intestinal flora in immune regulation and biological antagonism, FMT could be a candidate [13, 14].

In patients treated with combined antibiotics, bacterial diversity was greatly reduced and drug-resistant genes developed, which might explain why some patients did not respond well to antibiotics or even showed no effect. After fecal bacterial transplantation, diversity of intestinal flora in patients gradually recovered and converged with that of the donor. However, TBF7-2 (seven days after taking the drugs) had a very high virus rate, which was inconsistent with the composition of donor bacteria. However, the proportion of Bacteroidetes in that patient after transplantation was almost the same as that of the donor, which might be an important reason for the relief of clinical symptoms. It was also a concern that although the donor community was almost devoid of Klebsiella, the genus was abundant in the patients. Klebsiella is also a pathogenic bacteria of lungs, and whether translocation of intestinal flora caused lung infections remains unknown. However, recent studies on the lung–gut axis show that intestinal flora can influence immune response of the lungs [15-18].

Donor screening is a very important consideration. In this study, donors were carefully selected, as described previously. Samples were collected and analyzed for diversity after preparation to ensure quality. The samples were also stored long term for later traceability. It might be antagonism of mucosal and luminal
flora or differences in the viability of flora that lead to microbial rearrangement. Additional samples and longer follow-up times are needed to further clarify changes in microflora.

A study at Peking University’s School of Pharmacy showed that AAC3-II is the most important mechanism of resistance to aminoglycoside in *Klebsiella* in China. In another study [19], 15 antibiotic effluent pump coding-resistant genes were detected, including *crp*, *baeR*, *HNS*, *patA*, *emrB*, *msbA*, *acrA*, *acrB*, *emrR*, *mdtC*, *mdtB*, *mdtG*, *kdpE*, *mdfA*, and *msrB*. In this study, in general, normal people had the lowest abundance of drug-resistant genes, whereas the patients had the highest abundance before FMT. The abundance of drug-resistant genes decreased to some extent after FMT (the change was not significant one and seven days after treatment). However, because of the limited number of samples, there was no significant difference in drug-resistant genes between normal subjects and patients before and after treatment.

Currently, the development of new antibiotics is limited, and therefore, existing antibiotics will continue to be used. Antibiotic-resistant genes persist and are widespread in various microecosystems. As a reservoir of resistant genes, the human intestine facilitates the transfer of various resistant genes. Thus, regulation of intestinal flora has become an important direction in the treatment of infection and resistance to antibiotics. Fecal microbial transplantation is an infusion of fecal preparation from a healthy donor into the gastrointestinal tract of a patient in order to treat intestinal or extra-intestinal diseases [14, 20-22]. At present, FMT has successfully treated *C. difficile* infection, which is difficult to treat with conventional antibiotics [6]. Because of the unique structure of intestinal flora, it is essential to understand and control antibiotic-resistance in the intestine. Antibiotics can also lead to the progression of noninfectious diseases by affecting intestinal flora. Thus, relations between antibiotics and intestinal flora deserve further study, and intestinal flora should be given special consideration in the discovery of new antibiotics.

Antibiotic treatment of multidrug-resistant bacteria infection requires combination of drugs, and easily lead to increased drug resistance rate. Other treatments need to be sought. Patients with MRO infections had increased abundance of ARGs and low bacterial diversity. FMT eradicated ARGs and restored microbial diversity, and there were no serious adverse events. FMT may be a new direction but need further exploration.

**Declarations**

This work was approved by the Institutional Ethics Committee of the First Affiliated Hospital of Soochow University. Consent for publication were obtained from the three patients.

**Availability of Data and Materials**

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

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**Authors' contributions**

Yao Wei and Hang Yu drafted the manuscript. Jindan Kong and chenyan Zhao carried out the FMT process. Jun Jin and Hui Chen participated in the design of the study and performed the statistical analysis. Xueke Liu helped to draft the manuscript and recorded patient data. All authors read and approved the final manuscript. All authors contribute to the completion of the article.

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**Competing Interests**
The authors declare that they have no competing interests.

Highlights

The incidence of multi-drug resistant bacteria infection was increasing gradually, and gram-negative bacteria were the main ones. Antibiotic treatment faces challenges and difficulties.

FMT maintain intestinal barrier function, reduce translocation of resistant bacteria, and reduce expression of antibiotic resistance genes.

Antibiotics combined with FMT may become a new method for the treatment of multi-drug resistant infections.

Reference


12. CHINET http://www.chinets.com/Data/Map


Hosted file

Figure.docx available at https://authorea.com/users/656218/articles/661683-changes-in-intestinal-flora-following-fecal-microbiota-transplantation-in-patients-with-multidrug-resistant-organisms-infection