# Intestinal Mucosal Characteristic Flora in Diarrhea with Intestinal Dampness-Heat Syndrome

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**Research Article**

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## Abstract

# Background

With the change of lifestyle and diet structure in our daily life, diarrhea with intestinal dampness-heat syndrome (IDHS) has become a multiple disease. Emerging evidence indicates that intestinal flora contributes to the occurrence and development of diarrhea. However, little is known about the effects of the intestinal mucosal characteristic flora and specific mechanism on diarrhea with IDHS. To evaluate the characteristic biomarkers role of intestinal mucosal flora in diarrhea with IDHS.

# Results

Our study manifested that diarrhea with IDHS had the modulated effect on the diversity of intestinal mucosal flora, presented by the increasing Chao 1 and Observed species indexes and decreasing Simpson and Shannon indexes in the model group. The apparent dispersion between the control and model group indicated that diarrhea with IDHS altered the overall structure of the intestinal mucosal flora. The composition of the dominant bacteria was basically similar both at the phylum level and genus level before and after modelling, while the modeling led to change in the relative abundance of dominant bacteria at the species level, specially reflected in a significant decrease in the abundance of *Lactobacillus gasseri* and *Lactobacillus salivarius* in the model group.

# Conclusions

To sum up, *Lactobacillus gasseri* and *Lactobacillus salivarius* could serve as potential biomarkers for diarrhea with IDHS of the intestinal mucosal flora in mice and the mechanism of diarrhea with IDHS might be attributed to the inhibition of the growth of *Lactobacillus gasseri* and *Lactobacillus salivarius.*

## Background

Diarrhea is a common gastrointestinal disorder characterised by increased frequency of stools and loose or watery stools[1]. According to the clinical manifestations, it can be divided into diarrhoea caused by functional or organic pathologies of the digestive system such as irritable bowel syndrome, acute and chronic enteritis, gastrointestinal dysfunction, malabsorption syndrome, intestinal flora disorders, etc. Epidemiological surveys showed that the prevalence of diarrhea is on the rise worldwide, reaching 7–25%, and seriously affecting the physical, mental, work and life of patients[2]. Due to the different etiology and clinical manifestations of diarrhea, there are differences in the syndrome type of Traditional Chinese Medicine (TCM) they exhibit. Among them, intestinal dampness-heat syndrome (IDHS) is one of the common syndrome type, mostly caused by damp heat attacking the large intestine, with the fluid being forced down by the heat. The main clinical symptoms include abdominal pain and diarrhea, urgent diarrhea, yellowish brown and smelly stools[3]. With changes in lifestyle and diet, the occurrence of

diarrhea with IDHS is often accompanied by the diabetes, chronic liver disease, kidney disease and ulcerative colitis[4–6], so strengthening the study of diarrhea with IDHS have a useful role to expanding the understanding of the prevention and treatment of these major diseases.

The establishment of animal models of IDHS diarrhoea is the basis and prerequisite for the study of the pathogenesis and treatment of these diseases. At present, the diagnosis and treatment mode of "disease differentiation" in western medicine combined with "syndrome differentiation" in TCM has been commonly accepted in clinical practice[7]. Clinical research, however, is limited by many factors such as case selection and control of influencing factors, making it difficult to make significant progress in the short term in the integration of disease and syndrome. Animal disease model overcomes the characteristics of slow development, long incubation period and various causes of human diseases, and plays an outstanding role in the study of the development and prevention mechanism of various diseases[8]. As such, the replication of the animal model of diarrhea with IDHS can not only realistically simulate the characteristics of patients and more closely resemble TCM syndrome differentiation and treatment, but provide objective and powerful support for subsequent screening of Chinese medicine and mechanism research.

Disease research using intestinal flora as a biomarker is now coming into the public view. There is growing evidence of the potential benefits of intestinal flora in diarrhea with IDHS and that changes in certain intestinal specific flora may be the main cause of diarrhea with IDHS[9]. Clinical studies made points that diarrhoeal irritable bowel syndrome patients with dampness-heat syndrome of spleen and stomach, the relative abundence of the *Enterobacteria* and *Enterococcus* increased, while *Bifidobacteria*, *Lactobacillus* and *Peptococcus* decreased significantly[10]. Ding et al. have illustrated that significant differences in intestinal flora between the model group and the healthy group when they investigated the diversity of intestinal flora in ulcerative colitis patients with IDHS[11]. *Lactobacillus*, *Lactobacillaceae*, *Erysipelotrichaceae* and *Erysipelotrichales* were predominant in the model group and there was markedly enriched in *Akkermansia*, suggesting that these bacteria might be the target characteristic markers of intestinal flora in ulcerative colitis patients with IDHS. Our previous study confirmed that diarrhea with IDHS caused changes of intestinal mucosal flora structure in mice, which altered the diversity and the relative abundance of intestinal mucosal flora, especially the inhibition of *Muribaculum* intestine and the promotion of *Neisseria mucosa* [12]. It is thus clear that intestinal flora has been identified as being closely related to the development of diarrhea. Analysis of the changes in intestinal flora of diarrhea with IDHS attribute to elucidate its pathogenesis, accelerate the targeted regulation of intestinal flora and provide effective solutions for the diagnosis and treatment of diarrhea with IDHS.

In this study, we constructed the mice model of diarrhea with IDHS combined with disease and syndrome, analyzed intestinal mucosal flora by using the 16S rRNA gene high-throughput sequencing, in order to systematically characterize overall differences in intestinal mucosal microbial communities of diarrhea with IDHS and identify the characteristic flora biomarkers associated with the development of diarrhea with IDHS. This is important for studying the relationship between intestinal homeostasis and human

health, constructing a spectrum of characteristic intestinal bacteria related to TCM syndrome, realizing the objectivity of TCM syndrome diagnosis and revealing the scientific connotation of syndrome differentiation and treatment.

## Results

# General behavioral changes in diarrheal mice with IDHS

The mice in the ccm group have fine and shiny hair, good mental state, clean crissum, full stools particles with moderate moisture. Compared with the ccm group, the cmm mice showed reduced activity, poor mental state, dirty crissum, increased defecation frequency, yellowish-brown loose stools and smelly smell.

# Bacterial DNA sequence and OTU number of intestinal mucosal flora in diarrheal mice with IDHS

Based on the PacBio Sequel sequencing platform and after quality control, a total of 89, 175 usable high-quality sequences were obtained from two groups of 10 samples with an average effective sequence length in the range of 1452 bp-1469 bp. Table 1 showed that the coverage index was above 0.9846 for all groups of samples, indicating that the sequencing depth was sufficient to effectively reflect the true picture of the microorganisms in the samples for subsequent analysis. Using QIIME software, valid sequences were divided by 97% sequence similarity and Venn diagram was plotted using R software. As can be seen from Fig. 1, there were 618 OTUs in the cmm group, 639 OTUs in the ccm group, and 342 identical OTUs were shared in the two groups. The results manifested that after intervention with model, the number of OTU in intestinal mucosa of mice was decreased.

# Alpha diversity and Beta diversity of intestinal mucosal flora in diarrheal mice with IDHS

Alpha diversity, including a series of statistical indices, is regularly adopted to summarize the structure of an ecological community concerning its richness (number of taxonomic groups), evenness (distribution of abundance of the groups), or both [13]. To illustrate the diversity within a particular region or ecosystem, alpha diversity index can reflect the richness and diversity of microbial community. The richness in each sample was calculated by using the Chao1 index and the Observed species index. Shannon index and Simpson index were used to calculate the diversity of each sample. From the calculation results of richness and diversity indexs, the Chao 1 and Observed species indexes in the cmm group had the mild increase in comparison with the ccm group ($p > 0.05$; $p > 0.05$) (Fig. 2A, B), and the Shannon and Simspon indexes were decreased slightly ($p > 0.05$; $p > 0.05$) (Fig. 2C, D). There was uneven colony distribution led to a increase richness and decrease diversity of the cmm group, suggesting that diarrhea with IDHS caused an imbalance in the proportion of intestinal mucosal flora structure in mice.

Beta diversity, which could be evaluated in many different ways, was broadly used to analyze the partitioning of biological diversity of environments or along a gradient[14]. Principal Coordinates Analysis (PCoA) is a most classic unconstrained ranking analysis method. PCoA takes the sample distance as a whole and projects the sample distance into a low-dimensional space, which can preserve the distance relationship of the original sample to the greatest extent and is more in line with the characteristics of ecological data[15]. As shown in the Fig. 3, all groups exhibited an obviously distinct clustering of microbiota composition, indicating a high degree of parallelism in the samples of the each group. The distribution of 5 samples in the ccm group was relatively concentrated, which reflected that there was a high degree of bacterial similarity in the ccm group samples. Some of the samples in the cmm group appeared scattered, especially in cmm2 and cmm5, which might be due to the considerable individual differences among the mice. Specially, the distance between the ccm and cmm groups was relatively far, suggesting a pronounced difference between the groups. Taken together, these results performed that diarrhea with IDHS evidently altered the overall structure of the intestinal mucosal flora.

# The relative abundance of intestinal mucosal flora in diarrheal mice with IDHS

To better understand the changes in the composition of the intestinal mucosal flora after modeling, the relative abundance of each group was determined at the phylum, genus and species levels respectively. Meanwhile, the relative abundance of different phyla, genera and species in each group was also explored to illustrate the impact of diarrhea with IDHS. The relative abundance histograms showed the dominant bacteria at the phylum, genus and species level, respectively (Fig. 4, 5, 6). The abscissa represented different groups and the ordinate represented the relative abundance at the phylum, genus and species level, with different colored squares manifesting different bacteria and the length of the squares informing the relative abundance of the bacteria they represented.

A total of 16 phyla were detected in 10 samples of the two groups. The abundance of Bacteroidetes, Firmicutes and Proteobacteria was relatively high, of which Bacteroidetes and Firmicutes occupied a dominant advantage, Proteobacteria followed closely. Firmicutes was the first dominant phylum in the ccm group, followed by Bacteroidetes and Proteobacteria. In the cmm group, Bacteroidetes was the first dominant bacteria, followed by Firmicutes and Proteobacteria (Fig. 4). Among the 16 known phyla identified in all samples, Verrucomicrobia was unique to the ccm group, as well as Elusimicrobia was unique to the cmm group. Among them, Firmicutes presented a significant difference in the detected phyla between the two groups.

There were 224 genera detected in 10 samples of the two groups, of the 224 identified genera, 182 genera were detected in the ccm group, and 165 genera were detected in the cmm group. Furthermore, 42 genera were unique to the cmm group and 59 genera were unique to the ccm group (Fig. 5). The predominant genera with abundance more than 3% of the samples in the ccm group were *Prevotella, Streptococcus, Lactobacillus, Veillonella, Porphyromonas, Neisseria, Capnocytophaga* and *Selenomonas*, which accounted for 9.74%, 9.04%, 6.95%, 5.53%, 4.37%, 4.32%, 4.15% and 3.94% of the total bacteria,

respectively. *Muribaculum, Streptococcus, Neisseria, Porphyromonas, Capnocytophaga, Prevotella and Haemophilus*,which accounted for 13.97%, 9.64%, 9.50%, 7.19%, 5.21%, 5.15% and 3.41% of the total bacteria, respectively, were the dominant genera with abundance more than 3% of the samples in the cmm group (Table 2). In general, diarrhea with IDHS altered the relative abundance of genera, but the differences between the groups were not significant among the dominant genera with abundance more than 3% except for *Lactobacillus.*

Figure 6 presented the relative abundance of intestinal mucosal flora in mice at the species level. There were 319 species detected in 10 samples of the two groups, including 251 species in the ccm group and 242 species in the cmm group. Moreover, 68 species were unique to the cmm group, and 77 species to the ccm group. The dominant species with abundance more than 3% in the ccm group were *Veillonellaparvula* (5.44%), *Porphyromonasgingivalis* (3.25%), *Capnocytophaga granulose* (3.18%) and *Prevotellaoris* (3.13%). However, *Muribaculumintestinale* (13.97%), *Porphyromonaspasteri* (5.39%), *Neisseria perflava* (3.67%), *Neisseria mucosal* (3.62%) were the dominant species with abundance more than 3% of the samples in the cmm group (Table 3). Taken together, diarrhea with IDHS caused the changes in the relative abundance of dominant species. Among them, the *Porphyromonas Gingivalis, Lactobacillus salivarius* and *Streptococcus anginosus* showed obvious difference between the two groups.

# The characteristic flora of intestinal mucosal flora in diarrheal mice with IDHS

In order to identify the characteristic flora of intestinal mucosal flora in mice, LEfSe analysis was carried out in ccm group. Usually, the abscissa in the Linear discriminant analysis (LDA) score chart represented the LDA score, and the ordinate consisted of the taxonomic units that differ significantly between groups. The higher the LDA value, the greater the difference. Figure 7 showed that the abundance of genus *Lactobacillus* was significantly different between the two groups. Combine with the relative abundance in the species level, the abundance of *Lactobacillus Gasseri* and *Lactob*acillus *Salivarius* were obviously reduced in the cmm group in comparison with ccm group, illustrating that diarrhea with IDHS inhibited the growth of species *Lactobacillus gasseri* and *Lactobacillus salivarius.*

# Discussion

The etiology of diarrhea with IDHS is complex and varied, and a weakened spleen and stomach is a prerequisite for the formation of diarrhea with IDHS. Based on the traditional understanding of TCM, this study established high sugar and high fat, high temperature and high humidity environments, and received with wine and ice water to simulate the pathogenesis of IDHS. High sugar and high fat diet has been identified to damage the function of spleen and stomach, disrupt the qi flow, and cause the internal humidity and internal heat by stagnating water and dampness[16]. High temperature and high humidity environments attribute to the dysfunction of the spleen and stomach, causing "external dampness to induce internal dampness", with dampness turning into heat over time[16]. Wine, as a hot and damp

product, can help both dampness and heat. Ice water is cold and stimulate internal dampness and heat, resulting in the abdominal cold pain, diarrhea and other symptoms[17]. During the establishment of the model, the mice in the cmm group were depressed, crouched and lazy, sparse and rough hair, loss of appetite, dirty crissum, increased defecation frequency, yellowish-brown loose stools and smelly smell, which were consistent with the symptoms of IDHS, indicating that the model was successfully replicated[3, 18].

Studies have demonstrated that the occurrence of digestive tract related diseases is largely a change in the structure of the normal flora of origin[19]. At present, researchers are working to reveal the relationship between different intestinal microbes and diseases, and predict the likelihood of illness and the severity of diseases based on the types and abundance of intestinal microbes. In this study, the characteristics of intestinal mucosal flora was discussed after diarrhea with IDHS in the intestinal mucosal environment. The results made points that the OTU number of intestinal mucosal flora in the cmm mice slightly decreased after the intervention of modeling. The Chao 1 and the Observed species indexes increased slightly, while the Shannon and the Simpson indexes presented a decreased trend, which indicated that diarrhea with IDHS was characterized by increasing richness and decreasing diversity. In the context of the analysis of PCoA, it was implied that diarrhea with IDHS exerted the modulating effect on the community structure of intestinal mucosal flora was changed after diarrhea with IDHS. However, there were still discrete individual samples in the cmm group, which might be caused by the differences between individual mice as well. Overall, diarrhea with IDHS both altered the diversity and community structure of the intestinal mucosal flora, additionally we speculated that this might be one of the main causes of intestinal mucosal flora disorders in diarrhea with IDHS.

By comparing the relative abundance of intestinal mucosal flora in ccm group and cmm group, we further understood how diarrhea with IDHS changed intestinal microbial environment. At the phylum level, the relative abundance of Bacteroidetes, Firmicutes and Proteobacteria was relatively high, among which Bacteroidetes and Firmicutes occupied the dominant positions. After modeling, the relative abundance of Bacteroidetes and Proteobacteria increased, Firmicutes decreased significantly, and Actinobacteria was basically the same in the two groups. At the genus level, among the 16 genera with relatively higher abundance in the two group samples, the abundance of *Muribaculum, Neisseria, Porphyromonas, Capnocytophaga* and *Rothia* showed the increased trend after modeling, while *Prevotella, Streptococcus, Lactobacillus, Veillonella* and *Selenomonas* decreased. However, there were only significant differences in *Lactobacillus*. At the species level, the abundance of *Lactobacillus gasseri* and *Lactobacillus salivarius* exhibited a distinct reductionin in the cmm group. *Lactobacillus* was a general term for bacteria that could ferment carbohydrates and produce large amounts of lactic acid, which was widely found in the human oral cavity, gastrointestinal tract and genitourinary tract, and had the functions of inhibiting pathogenic bacteria, anti-infection, regulating the balance of flora and enhancing the immunity of the body[20, 21]. Specifically, the adhesion of *Lactobacillus* to the cell surface of the intestinal mucosa was the primary condition for its colonisation and maximum probiotic effect (inhibition of pathogenic bacteria colonisation, regulation of intestinal flora balance and promotion of the immune response). *Lactobacillus*

*gasseri* could antagonize helicobacter pylori infection[22], its strain *Lactobacillus gasseri PA3 had a definitive regulating effect of* reducing purine and balancing serum uric acid level[23], and strain *Lactobacillus gasseri APC 678 revealed a potential effect of* inhibiting the growth of *clostridium difficile*[24]. Emerging evidence elucidated that *Lactobacillus salivarius* existed a certain adsorption effect which could colonize and grow well on the intestinal surface to exert its immunomodulatory effect and improved local mucosal immunity of small intestine. These results suggested that the significant decrease in the abundance of *Lactobacillus gasseri* and *Lactobacillus salivarius* might be the one of the reasons for the occurrence of diarrhea in IDHS.

# Conclusions

This study revealed that diarrhea with IDHS modulated the changes of diversity and community structure of intestinal mucosal flora in mice. Besides, *Lactobacillus gasseri* and *Lactobacillus salivarius* could serve as potential biomarkers for diarrhea with IDHS in the intestinal mucosal flora of mice. We therefore speculated that the mechanism of diarrhea with IDHS might be attributed to the inhibition of the growth of *Lactobacillus gasseri* and *Lactobacillus salivarius*, which provide the basis for future intestinal flora-targeted treatment of diarrhea with IDHS.

# Methods

### Experiment materials and reagents preparation

The basal feed used in our study was purchased from Hunan Slaccas Jingda Laboratory Animal Company (Hunan, China). The high-sugar and high-fat feed (80% basal feed mixed with 12% lard and 8% honey) was purchased from Jiangsu Synergetic Pharmaceutical Biological Engineering Co., LTD with license number Su Feeding Certificate (2014) 01008. Hongxing Erguotou (56 Degrees) was produced by Beijing Hongxing Co., LTD.

### Animals

Ten one-week-old specific pathogen free (SPF) male Kunming mice, weighing 18–20 g, were purchased from Hunan Slaccas Jingda Laboratory Animal Company (Hunan, China) with license number SCXK (Xiang) 2016-0002. Experimental animals were kept in the Experimental Animal Center of Hunan University of Chinese Medicine (Experimental Unit Use License Number: SYXK (Xiang) 2019-0009) under the controlled conditions (temperature 23-25°C, humidity 50-70%). All procedures involving animals were performed according to protocols approved by the Institutional Animal Care and Use Committee of Hunan University of Chinese Medicine. The trial procedures for the care and use of experimental animals were carried out in accordance with the European Community guidelines (directive 2010/63/EU).

### Animal experimental process

After a 3-days acclimatization period, 10 mice were randomly distributed into the control (ccm) group and model (cmm) group, with 5 mice in each group. We have improved the modeling method of diarrhea with IDHS construction in reference literature[25]: mice in the cmm group were fed with high sugar and high fat feed continuously 11 days, whereas the ccm mice were fed with basal feed. Starting from day 12, mice in the cmm group were placed in an artificial climate chamber at 31.5 °C-32.5 °C and 95 % relative humidity for 8 h/d. At the same time, the cmm group were gavaged 0.35 mL of Hongxing Erguotou at 9 am and 8 pm every day, and gavaged 0.35 mL of ice water at 3 pm, lasted 4 days. The ccm group was administered with equal amount of distilled water.

### Model evaluation criteria

We referred to the "Consensus Opinions of Diarrhea Traditional Chinese Medicine Diagnosis and Treatment Experts (2017)" on the main points of the differentiation of the diarrhea with IDHS, and formulated the general behavioral diagnostic criteria of the diarrhea with IDHS model[3]: ①increased defecation frequency; ②yellowish-brown loose stools; ③foul-smelling stools; ④perianal soiling.

### Collection of mice intestinal mucosa

Mice in each group were sacrificed by cervical dislocation, immediately placed on an ultra-clean bench and whole sections of small intestine were taken. The small intestine was dissected, the contents were washed from the intestinal wall with saline and the water was blotted dry with sterile filter paper. We scraped the intestinal mucosa with a coverslip, weighed and labelled it in the sterile eppendorf (EP) tubes, added 2 times the weight of the intestine in saline and stored it at -80°C in the refrigerator.

### 16S rRNA gene high-throughput sequencing

Total metagenomic DNA of intestinal mucosal bacteria were extracted with CTAB and SDS. Then, the quantity and quality of the extracted DNA were measured using a NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) and 3.0 Flurometer Qubit (Life Technologies, CA, USA) and agarose gel electrophoresis method. The full length of bacterial 16S rDNA gene sequence was amplified using the extracted DNA as template. The primers used for amplification were 27F (5'-AGRGTTYGATYMTGGCTCAG-3') and 1492R (5'-RGYTACCTTGTTACGACTT-3'). PCR amplification system was prepared as follows (25 L): 5 μL of KAPA HiFi Buffer (5 ×), 0.75μL (1 U/ μL) of KAPA HiFi Hot Start DNA Polymerase, 0.75 μL (10 mM) of dNTPs, 0.75 μL (10 uM) each of the forward and reverse primers, 2 μL of DNA template, and 15 μL of ddH2O. PCR amplification conditions were as follows: initial denaturation at 95 °C for 5 sec, followed by denaturation at 95 °C for 30 sec, annealing at 57 °C for 30 sec, extension at 72 °C for 60 sec, a total of 25 cycles, and a final extension at 72 °C. PCR amplicons were purified with Agencourt AMPure Beads (Beckman Coulter, Indianapolis, IN) and quantied using the PicoGreen dsDNA AssayKit (Invitrogen, Carlsbad, CA, USA). After the individual quantification step, amplicons were pooled in equal amounts. The pooled sample was then used to generate a library by using SMRTbell Template Prep Kit 1.0-SPv3, and the PCR products were then sequenced by PacBio Platform using DNA/Polymerase Binding Kit 3.0 (PacBio). The extraction, amplification, database

construction and sequencing of the sample DNA were all completed by Wuhan Frasergen Bioinformatics Co., LTD.

### Bioinformatics and statistical analysis

### Bioinformatics analysis

Based on the SMRT (Single Molecule Real-Time) single molecular real-time sequencing technology and the PacBio Sequel sequencing platform, the raw data were stripped of the adapter sequences to obtain validated inserts. The software SMRT Link v8.0 was used to pre-process and filter the raw sequencing output data to obtain Circular Consensus Aequences (CCS), i.e. raw reads. Finally, clean reads were obtained for subsequent analysis by primer removal and length filtering (1300-1600bp) performed on raw read (the corresponding raw data has been uploaded to National Center for Biotechnology Information (NCBI, https://www.ncbi.nlm.nih.gov/) database and the accession number is PRJNA673123). Qiime software (v.1.8.0, http://www.qiime.org/) was used to conduct OTU clustering with 97% identity for Clean CCS sequences of all samples. The longest sequence in each OTU was selected as the representative sequence of the OTU, and the sequence with the similarity over 97% with the representative sequence of the OTU was selected to generate the original OTU table. Qiime was used to flatten the original OTU table according to the sample with the lowest data volume, so that all the samples had the same data volume after flattening, and the final OTU table was obtained. Community diversity was reflected by a variety of different indexes, which measured the diversity with different emphasis. Chao 1 and Observed species index focused on estimating the abundance of bacteria, while Simpson and Shannon indexes were usually used to describe the diversity of the community. In this study, Chao 1, Observed species, Simpson and Shannon indexes were calculated by MOTHUR (version v.1.30.1, http://www.mother.org/). Beta diversity was used to investigate the structural variation of microbial communities across samples using UniFrac distance metrics and used R software to analyze Principal coordinate analysis (PCoA). Linear discriminant analysis effect size (LEfSe) was used to screen key biomarkers based on linear discriminant analysis (LDA) effect size.

### Statistical analysis

SPSS 24.00 software (IBM Corp., Armonk, NY, USA) was used for statistical analysis, and data were expressed as mean ± standard deviation. Data were tested for normality using Shapiro-Wilk test and for variance homogeneity by Levene test. Means between the two groups conformed to normality and chi-squared were compared using the independent samples t-test, otherwise the non-parametric Mann-Whitey test was applied. Then, $p<0.05$ was regarded as a significant difference, $p<0.01$ as the extremely significant difference.

# Declarations

### Ethics approval and consent to participate

All animal work was carried out in accordance within the guidelines of the Institutional Animal Care and Use Committee of Hunan University of Chinese Medicine (NO.20171202). The animal experiments were performed in accordance with Animal Research: Reporting In Vivo Experiments guidelines approved by SYSU IACUC and were conducted in a laboratory designed to ensure biosafety. All authors knew and approved of this animal experiments.

### Consent to publication

Not applicable.

### Availability of data and materials

All data generated or analyzed during this study were included in this published article. The datasets used and analysed during the current study were available from the corresponding author on reasonable request.

### Competing interests

The authors declared that they have no competing interests.


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### Authors’ contributions

XL analyzed the data and drafted the manuscript. CL performed most of the experiments and guided the performance of animal experiment. ZT was responsible for studying the design and collecting fund. All authors reviewed and approved the final manuscript.


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# Tables

**Table 1** Statistics table of data preprocessing results

| Sample | CCS | Coverage | Filtered | Average Length |
|---|---|---|---|---|
| ccm1 | 8,742 | 0.9850 | 8,067 | 1,469 |
| ccm2 | 9,202 | 0.9984 | 8,639 | 1,456 |
| ccm3 | 9,803 | 0.9983 | 9,165 | 1,457 |
| ccm4 | 10,313 | 0.9991 | 9,525 | 1,452 |
| ccm5 | 11,068 | 0.9988 | 10,282 | 1,453 |
| cmm1 | 6,359 | 0.9846 | 5,913 | 1,457 |
| cmm2 | 9,854 | 0.9966 | 9,257 | 1,454 |
| cmm3 | 9,890 | 0.9883 | 9,186 | 1,459 |
| cmm4 | 9,571 | 0.9929 | 8,936 | 1,458 |
| cmm5 | 10,860 | 0.9948 | 10,205 | 1,459 |

Note: CCS: Circular consensus sequence; Filtered: Number of effective CCS after removing primers and length filtering; Coverage: Calculate sequencing depth index; Average Length: Effective sequence average length. ccm1-5: control group sample 1-5; cmm1-5: model group sample 1-5.

**Table 2** Relative abundance of dominant bacteria in the ccm group and cmm group samples at genus level

| Genus | ccm group | cmm group |
|---|---|---|
| *Muribaculum* | 0.0252±0.4309 | 0.1397±0.2381↑ |
| *Prevotella* | 0.0974±0.0851 | 0.0515±0.0301↓ |
| *Neisseria* | 0.0432±0.0164 | 0.0950±0.0646↑ |
| *Porphyromonas* | 0.0437±0.0098 | 0.0719±0.0561↑ |
| *Lactobacillus* | 0.0695±0.0448 | 0.0133±0.0135*↓ |
| *Streptococcus* | 0.0434±0.0070 | 0.0030±0.0046↓ |
| *Veillonella* | 0.0553±0.0517 | 0.0238±0.0137↓ |
| *Capnocytophaga* | 0.0415±0.0198 | 0.0521±0.0336↑ |
| *Rothia* | 0.0074±0.0046 | 0.0282±0.0268↑ |
| *Selenomonas* | 0.0394±0.0149 | 0.0207±0.0162↓ |
| *Haemophilus* | 0.0058±0.0046 | 0.0341±0.0253↑ |
| *Clostridium* | 0.0269±0.0223 | 0.0041±0.0074↓ |
| *Leptotrichia* | 0.0093±0.0024 | 0.0254±0.0206↑ |
| *Ottowia* | 0.0272±0.0133 | 0.0096±0.0084↓ |
| *Fusobacterium* | 0.0105±0.0053 | 0.0212±0.0153↑ |
| *Granulicatella* | 0.0059±0.0058 | 0.0211±0.0134↑ |

Note: ccm group stand for control group, cmm group stand for model group; *stand for $p<0.05$, **stand for $p<0.01$

**Table 3** Relative abundance of dominant bacteria in the ccm group and cmm group samples at species level

| Species | ccm group | cmm group |
|---|---|---|
| *Muribaculum intestinale* | 0.0252±0.0431 | 0.1397±0.2381↑ |
| *Veillonella parvula* | 0.0544±0.0505 | 0.0232±0.0136↓ |
| *Porphyromonas pasteri* | 0.0045±0.0042 | 0.0539±0.0493↑ |
| *Prevotella oris* | 0.0313±0.0501 | 0.0040±0.0040↓ |
| *Neisseria mucosa* | 0.0148±0.0085 | 0.0362±0.0317↑ |
| *Streptococcus anginosus* | 0.0222±0.0322 | 0.0017±0.0033*↓ |
| *Lactobacillus gasseri* | 0.0197±0.0290 | 0.0019±0.0034↓ |
| *Neisseria perflava* | 0.0167±0.0080 | 0.0367±0.0256↑ |
| *Prevotella histicola* | 0.0159±0.0235 | 0.0019±0.0013↓ |
| *Lactobacillus salivarius* | 0.0184±0.0222 | 0.0010±0.0017*↓ |
| *Capnocytophaga granulosa* | 0.0318±0.0160 | 0.0241±0.0159↓ |
| *Porphyromonas gingivalis* | 0.0325±0.0113 | 0.0109±0.0126*↓ |
| *Selenomonas noxia* | 0.0191±0.0174 | 0.0118±0.0113↓ |
| *Aggregatibacter aphrophilus* | 0.0006±0.0008 | 0.0150±0.0204↑ |
| *Capnocytophaga leadbetteri* | 0.0023±0.0023 | 0.0190±0.0189↑ |
| *Haemophilus parainfluenzae* | 0.0051±0.0039 | 0.0255±0.0194↑ |
| *Ottowia beijingensis* | 0.0272±0.0133 | 0.0095±0.0083↓ |
| *Fusobacterium nucleatum* | 0.0105±0.0054 | 0.0210±0.0153↑ |
| *Prevotella melaninogenica* | 0.0099±0.0050 | 0.0174±0.0139↑ |
| *Lactobacillus crispatus* | 0.0229±0.0149 | 0.0095±0.0126↓ |
| *Rothia mucilaginosa* | 0.0038±0.0024 | 0.0139±0.0098↑ |
| *Streptococcus gordonii* | 0.0063±0.0046 | 0.0137±0.0100↑ |
| *Curvibacter lanceolatus* | 0.0170±0.0095 | 0.0036±0.0025↓ |

Note: ccm group stand for control group, cmm group stand for model group; *stand for $p<0.05$, **stand for $p<0.01$

## Figures

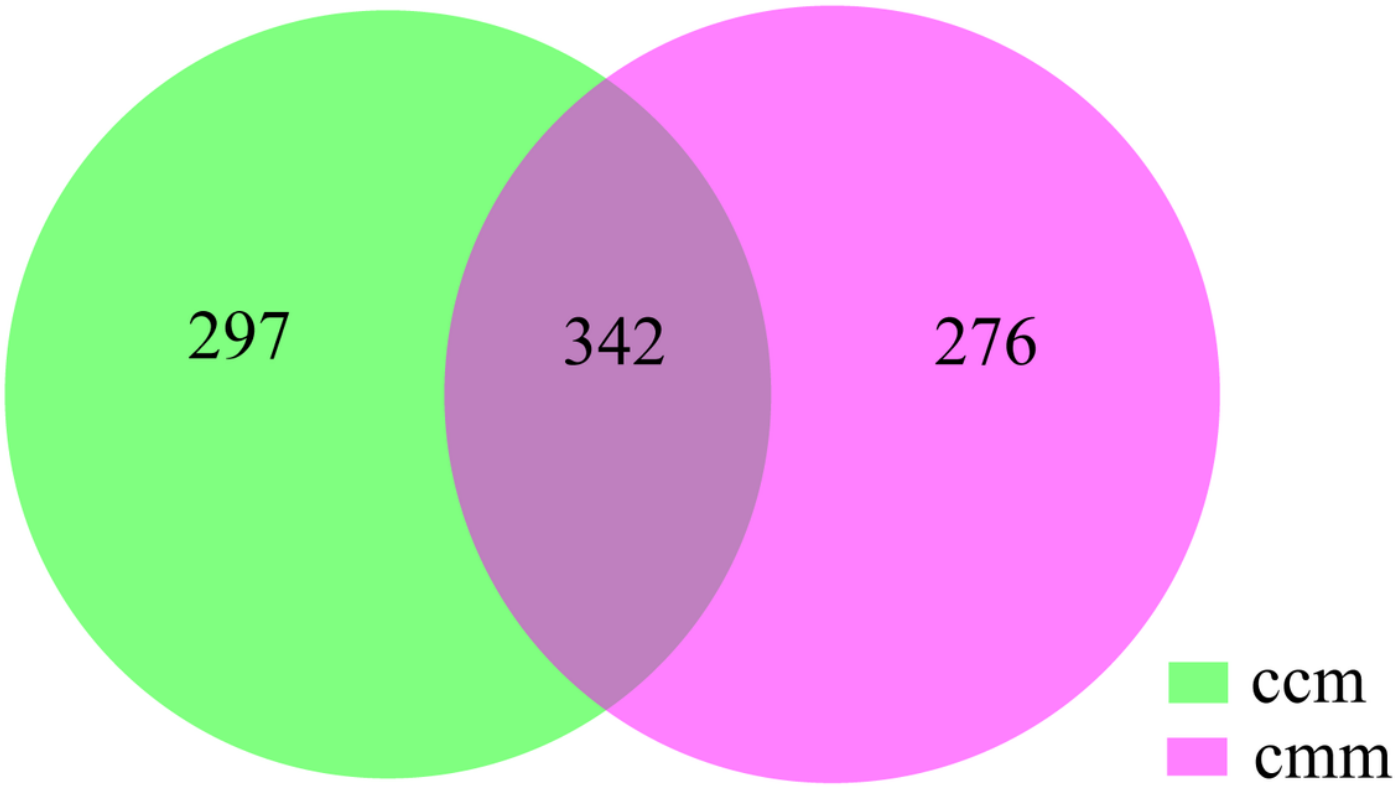



**Figure 1**

OTU number of intestinal mucosal flora in the ccm group and cmm group. The green circle represented the total number of OTU in the intestinal flora of mice in the ccm, and the purple circle represented the total number of OTU in the intestinal mucosal flora of mice in the cmm. The intersecting part between the two groups showed the total number of OTU in the two groups. ccm group: control group; cmm group: model group.

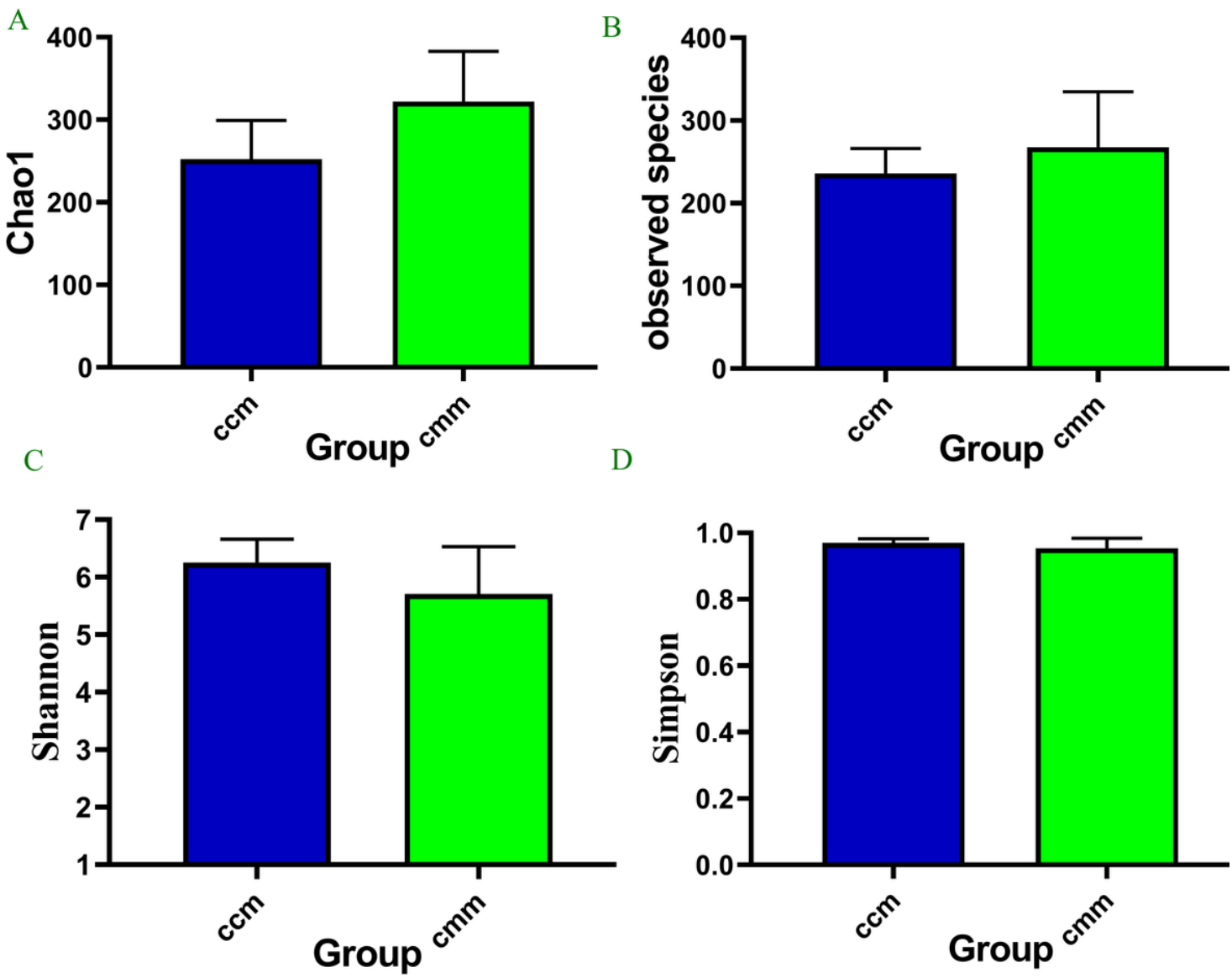
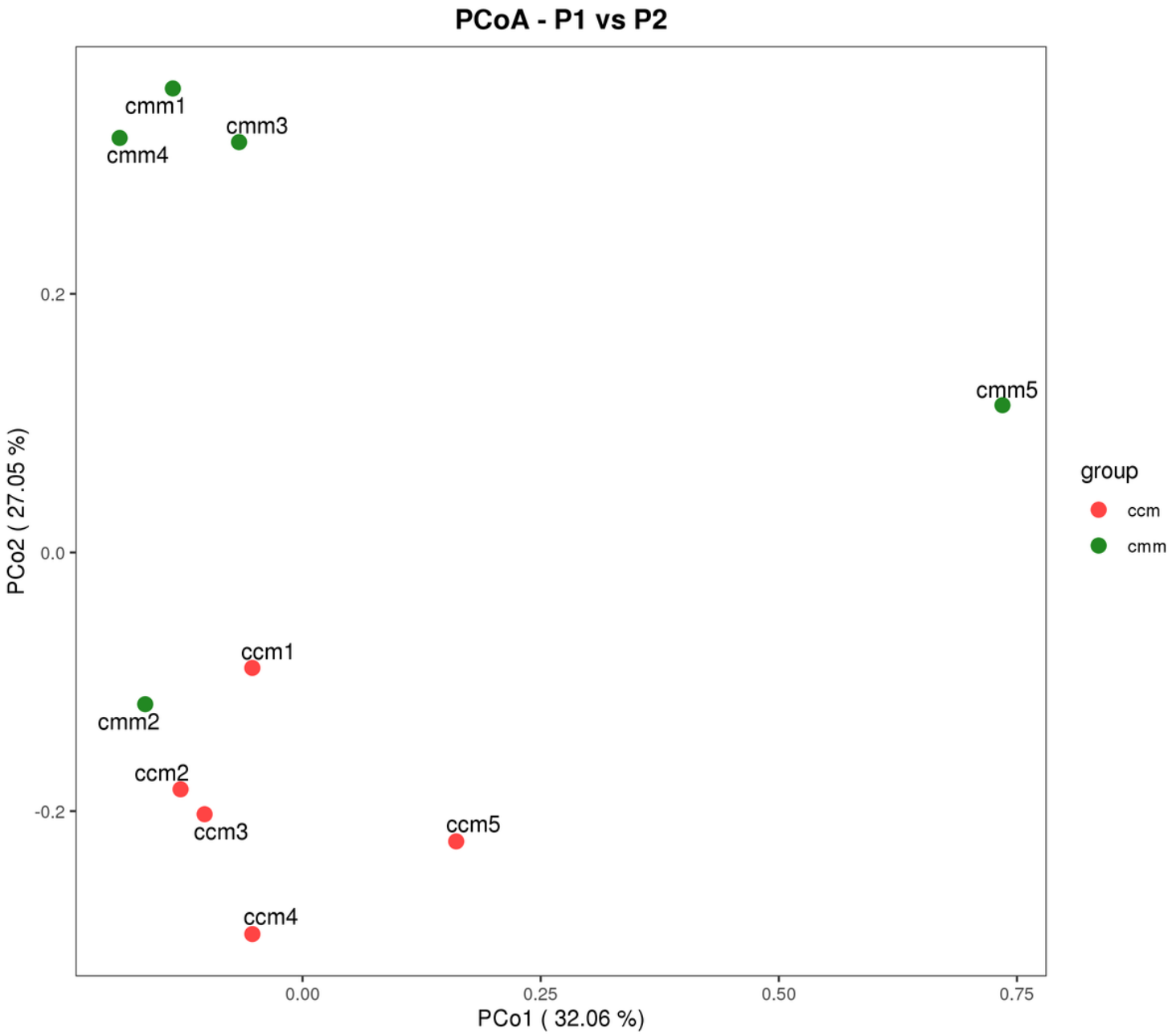


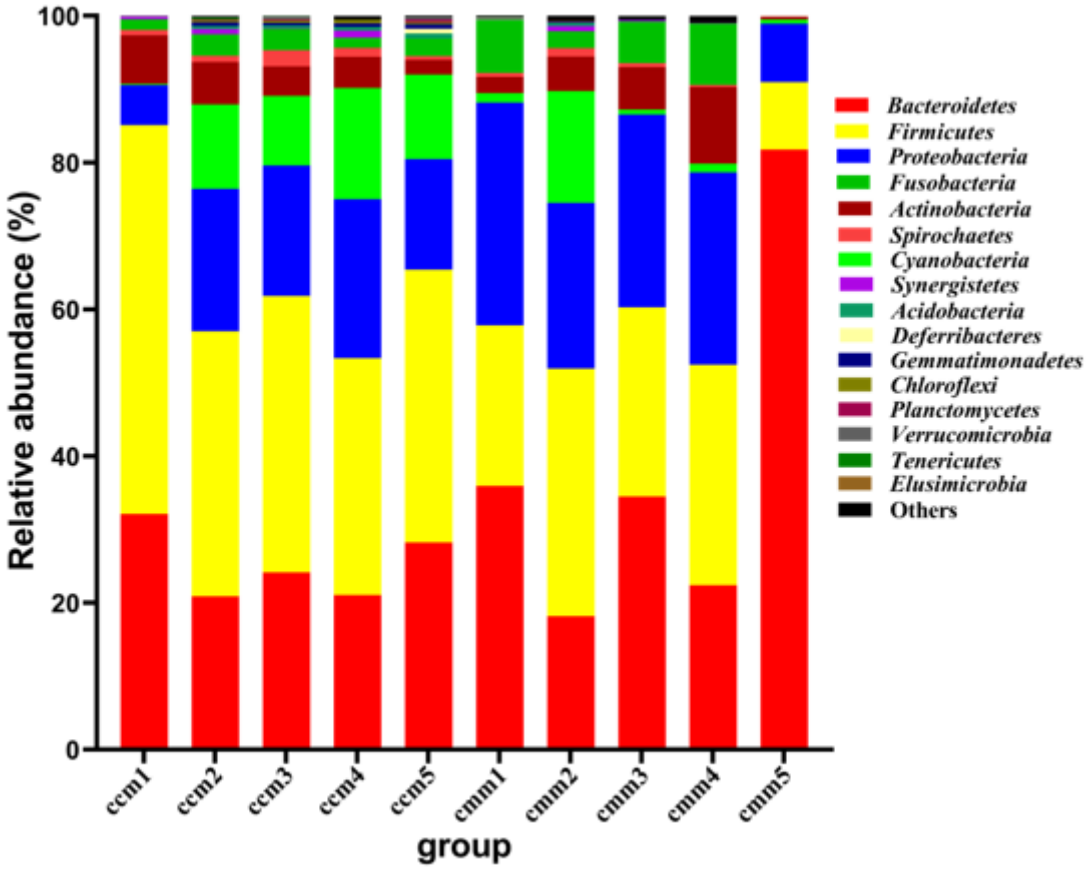

**Figure 2**

Analysis of alpha diversity of intestinal mucosal flora in the ccm group and cmm group. (A) Chao index. (B) Observed species index. (C) Shannon index. (D) Simpson index. Data were mean ± standard deviation, n = 5, $p>0.05$. ccm group: control group; cmm group: model group.



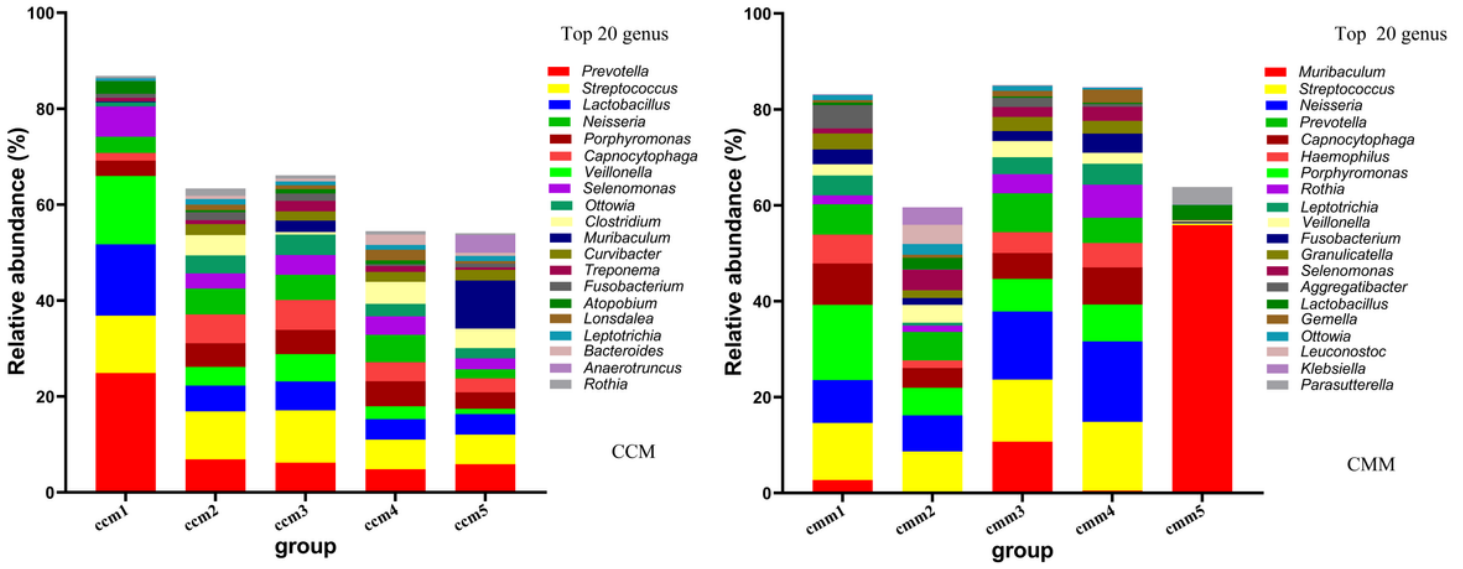

## Figure 3

PCoA analysis of intestinal mucosal flora in the ccm group and cmm group. PCoA based on weighted UniFrac distance represented the differences of the intestinal microbiota between two groups. Shapes of different colors represented different grouped samples. Data were mean ± standard deviation, n = 5, p>0.05. ccm1-5: control group sample 1-5; cmm1-5: model group sample 1-5.




## Figure 4

Bacterial community composition of each sample at the phylum level. The abscissa represented groups and the ordinate represented relative abundance. Different colors represented different phyla of bacteria. ccm group: control group; cmm group: model group.




## Figure 5

Bacterial community composition of each sample at the genus level. The figure on the left showed the genus relative abundance of intestinal flora of mice in the ccm group and the figure on the right showed the genus relative abundance of intestinal flora of mice in the cmm group. The abscissa represented groups and the ordinate represented relative abundance. Different colors represented different genus of bacteria. ccm group: control group; cmm group: model group.

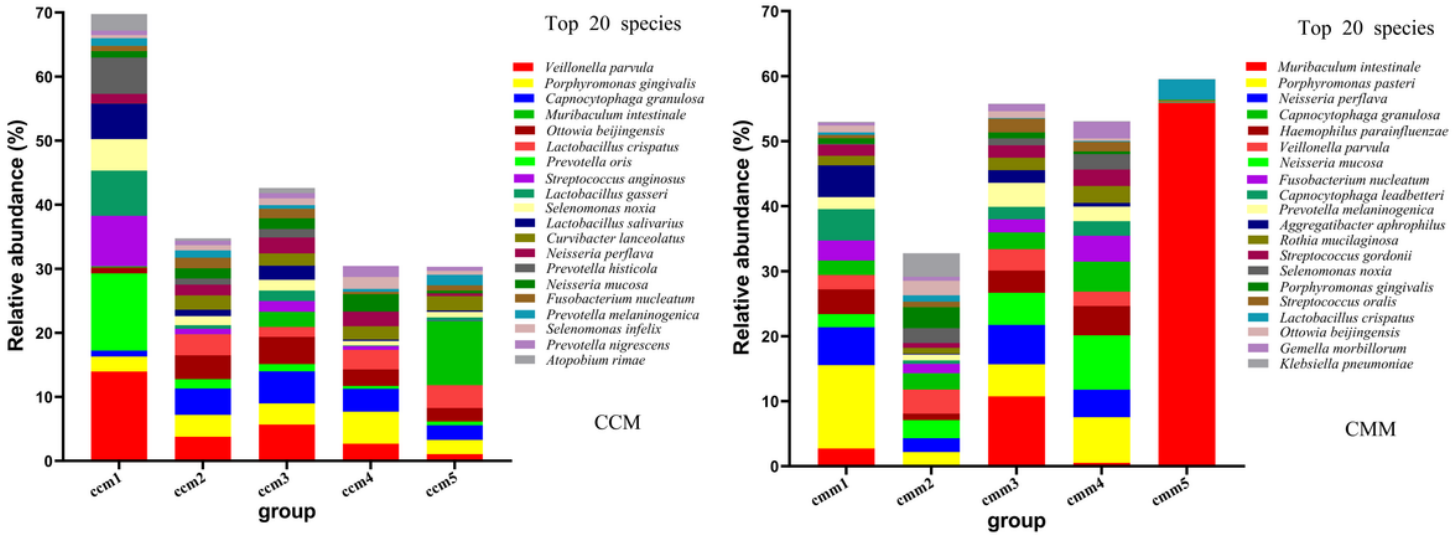



**Figure 6**

Bacterial community composition of each sample at the species level. The figure on the left showed the species relative abundance of intestinal flora of mice in the ccm group and the figure on the right showed the species relative abundance of intestinal flora of mice in the cmm group. The abscissa represented groups and the ordinate represented relative abundance. Different colors represented different species of bacteria. ccm group: control group; cmm group: model group.

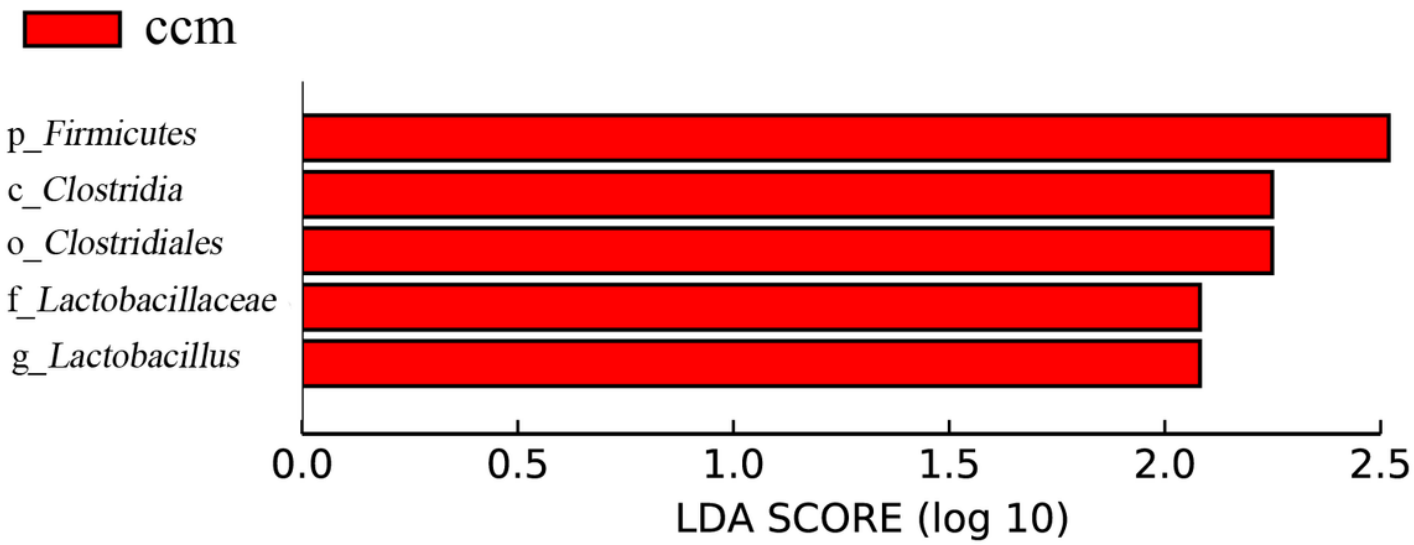



**Figure 7**

Effect of diarrhea with IDHS on the characteristic flora of intestinal mucosal flora in mice. The ordinate was the classification unit with significant difference among groups, while the abscissa was a bar chart to intuitively show the LDA analysis logarithmic score value of each classification unit. ccm group: control group.