Escherichia coli O_{101}-induced diarrhea develops gut microbial dysbiosis in rats

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Abstract. Enterotoxigenic *Escherichia coli* (ETEC)-induced diarrhea is a devastating disease and one of the third leading causes of infectious disease-associated mortalities worldwide. Despite recent advances in the identification of the association between gut microbiota and diarrhea, a lack of understanding exists on the status of gut microbiota in rats treated with ETEC. In the present study, a rat model of *Escherichia* (*E.*) *coli* O_{101}-induced diarrhea was established. The diarrhea incidence and index, as well as histological changes, were assessed. In addition, Illumina MiSeq sequencing of V3-V4 hypervariable regions of 16S ribosomal RNA was employed to investigate the changes in the gut microbiota profiles in the feces of the diarrhea rats. The results indicated that *E. coli* O_{101} increased the diarrhea index and injury in the intestinal tissues, whereas it decreased the bacterial richness and shifted the distribution pattern of the bacterial communities in the phylum, order and genus levels in the fecal samples. Notably, the proportion of bacteria *Prevotella, Enterococcus* and *Akkermansia* was significantly decreased, while the pathogenic bacteria *Escherichia/Shigella* were significantly increased in diarrhea rats. Taken together, the gut microbiota is closely associated with *E. coli* O_{101}-induced diarrhea in lower microbial diversity and dysbiosis of gut microbiota at different taxonomical levels.

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Introduction

Diarrhea is a common clinical symptom and is the third leading cause of infectious disease-associated mortalities worldwide, mainly affecting children (1). Approximately 1.87 million children succumb to diarrhea annually worldwide (2), and children with an age of <5 years in developing countries are reported to experience an average of three diarrheal episodes per year (3). The most common cause of diarrhea is an infection of the gastrointestinal tract due to viruses (4), bacteria (5), or parasites (6). Enterotoxigenic *Escherichia coli* (ETEC) is considered to be the most common cause of bacterial diarrhea, also known as traveler's diarrhea (7). The major serotypes of ETEC are O_6, O_{27}, O_{148}, O_{159}, O_{149} and O_{101} (8,9). O_{101} is commonly associated with diarrhea and poses a significant threat worldwide (10). Thus, the present study attempted to establish a rat model of *Escherichia* (*E.*) *coli* O_{101}-induced diarrhea.

Gut microbiota, the complex microbial communities harbored in the digestive tracts of animals, serve a major role in the host's metabolism (11,12), nutrient absorption or production (13), and immune system (14), greatly contributing to the overall health status of the host (15,16). Accumulating evidence indicated that gut microbiota is closely associated with the incurrence and development of a variety of diseases, including obesity (17,18), diabetes (19) and diarrhea (20). Previous studies have suggested that diarrhea can cause changes in intestinal microbiota (21,22), and altered intestinal microbial composition and function may result in an increased risk of bacterial diarrhea (20). However, the specific changes in intestinal microbiota in individuals suffering from *E. coli*-associated diarrhea are poorly understood. Therefore, it would be of great interest to identify the systemic microbiome alterations and the specific microorganisms involved in *E. coli*-associated diarrhea.

The next-generation sequencing technique facilitates the investigation of the taxonomic composition of intestinal microbiota and provides a new perspective for studying *E. coli*-induced diarrhea (23). In the present study, the effects of *E. coli* O_{101} on the intestinal tissues of rats were investigated, the fecal microbiota from diarrhea rats was compared with

that in the control rats, and the characteristic bacterial diversity and compositions were identified. In addition, the current study provided an insight into the pathology of *E. coli* O_{101} and provided evidence for identifying bacteria for the diagnosis and treatment of diarrhea.

Materials and methods

Animals and ethics statement. Specific pathogen-free male Sprague-Dawley rats (n=22; 190-210 g; 6 weeks old) were obtained from Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China). The animals were maintained at a temperature of 22˚C and a 12-h light/dark cycle environment for at least one week prior to use in the experiments. The animals were fed the same batch of standard laboratory diet to minimize the variation of environmental factors. The present study was approved by the Institutional Animal Care and Use Committee of the Academy of Military Medical Sciences (Beijing, China). All animal care and experimental procedures were conducted according to the Chinese Laboratory Animals' Welfare and Ethics guidelines (24).

E. coli O_{101} treatment. The *E. coli* O_{101} strain was purchased from the China Institute of Veterinary Drug Control (Beijing, China) and used to establish a diarrhea model in the rats. A total of 22 rats were divided into two groups, including the diarrhea (n=11) and control (n=11) groups. The diarrhea group received intraperitoneal (ip) injections with *E. coli* O_{101} ($1x10^{11}$ colony-forming units/kg) for three consecutive days. The normal group received ip injection with an equivalent volume of sterile physiological saline for three consecutive days. The animals were sacrifices after 3 days.

Fecal sample collection. The fecal score was recorded two times per day using a four-grade system, with a score of 0 indicating firm, dry and normal consistency of feces, 1 indicating pasty feces, 2 indicating thick and fluid feces, and 3 indicating watery feces (25). Diarrhea was defined as the daily sum score of ≥2. The diarrhea incidence and diarrhea index (diarrhea index=rate of loose stools per day * the degree of diarrhea) were used to assess the establishment of an *E. coli* O_{101}-induced diarrhea rat model (26). Fresh fecal samples of rats were collected individually on the third day, immediately frozen in liquid nitrogen and stored at -80˚C for further analysis.

Histopathological analysis. Partial intestinal tissues were dissected, fixed in 4% paraformaldehyde for 24 h, dehydrated and embedded in paraffin. Next, 4-μm sections were cut and stained with hematoxylin and eosin. Histopathological changes were observed and scored under an Olympus microscope (Olympus Corporation, Tokyo, Japan). The criteria for grading the intestinal histopathological changes were as follows (27): Score 0, no evident pathological changes; score 1-3, mild injury characterized by slight edema and a decrease in the number of mucous epithelial cells; score 4-5, moderate injury characterized by inflammatory cell infiltration, congestion, cell apoptosis and necrosis; score 6-10, severe injury characterized by massive inflammatory cell infiltration, severe hemorrhage and congestion, evident edema, coagulation necrosis and focal necrosis.

DNA extraction and pyrosequencing. Microbial DNA was extracted from fecal samples using the E.Z.N.A.® Soil DNA kit (Omega Bio-tek, Inc., Norcross, GA, USA) according to the manufacturer's protocol. The V3-V4 region of the bacterial 16S ribosomal RNA (rRNA) was amplified by polymerase chain reaction (PCR), conducted under the following conditions: 95˚C for 3 min, followed by 25 cycles at 95˚C for 30 sec, 55˚C for 30 sec and 72˚C for 45 sec, a final extension at 72˚C for 10 min, and maintained at 10˚C. The primers used in PCR were as follows: 338F, 5'-ACTCCTACGGGAGGCAGCAG-3', and 806R, 5'-GGACTACHVGGGTWTCTAAT-3'. The PCR reactions were performed in triplicate in a mixture with a total volume of 20 μl, which contained 0.4 μl FastPfu Polymerase, 4 μl 5X FastPfu buffer (both Beijing Transgen Biotech Co., Ltd., Beijing, China), 2 μl of 2.5 mM dNTPs (Vazyme, Piscataway, NJ, USA), 0.8 μl of each primer (5 μM) and 10 ng template DNA. PCR products were purified on agarose gels using an AxyPrep DNA Gel Extraction kit (Axygen; Corning Incorporated, Corning, NY, USA) according to the manufacturer's protocol. Equimolar concentrations of purified PCR products were pooled and paired-end sequenced (2x300 bp) on an Illumina MiSeq platform (Illumina, Inc., San Diego, CA, USA) according to the manufacturer's recommendations.

Sequencing analysis. Raw Fastq files were demultiplexed and quality-filtered using the QIIME bioinformatics pipeline (version 1.17; http://qiime.org/). The criteria used were as follows: i) 300 bp reads were truncated at any site receiving an average quality score of <20 over a 50 bp sliding window, discarding any truncated reads that were <50 bp; ii) exact barcode matching, mismatch of 2 nucleotides in primer matching and reads containing ambiguous characters were removed; and iii) only sequences that overlapped by >10 bp were assembled according to their overlap sequence. Reads were discarded if they could not be assembled.

Bioinformatics analysis. The operational taxonomic unit (OTU) is a classified operation unit that is set up for a specific unit (such as strain, species, genus and grouping) for the convenience of analysis in phylogenetic or population genetics research (28). In the analysis of microbial diversity, OTU is divided for all sequences based on different similarity levels (29). Thus, an OTU is defined by a similarity of >97% (taxonomic rank) between sequences, and each OTU represents a species (30). In the present study, the OTUs were clustered with a similarity cutoff value of 97% using UPARSE software (version 7.1; http://drive5.com/uparse/) with a novel 'greedy' algorithm that performs chimera filtering and OTU clustering simultaneously, as previously described (31). The taxonomy of each 16S rRNA gene sequence was analyzed using the Ribosomal Database Program (RDP) classifier (http://rdp.cme.msu.edu/) against the SILVA (SSU 115) 16S rRNA database (https://www.arb-silva.de/), with a confidence threshold of 70%. Subsequently, the sequences were classified taxonomically to different levels (including phylum, class, order, family, genus and species) using the RDP classifier. The α-diversity indices, including Ace, Chao1, Shannon and Simpson, were then calculated using QIIME from rarefied samples for richness and diversity indices of the bacterial community.

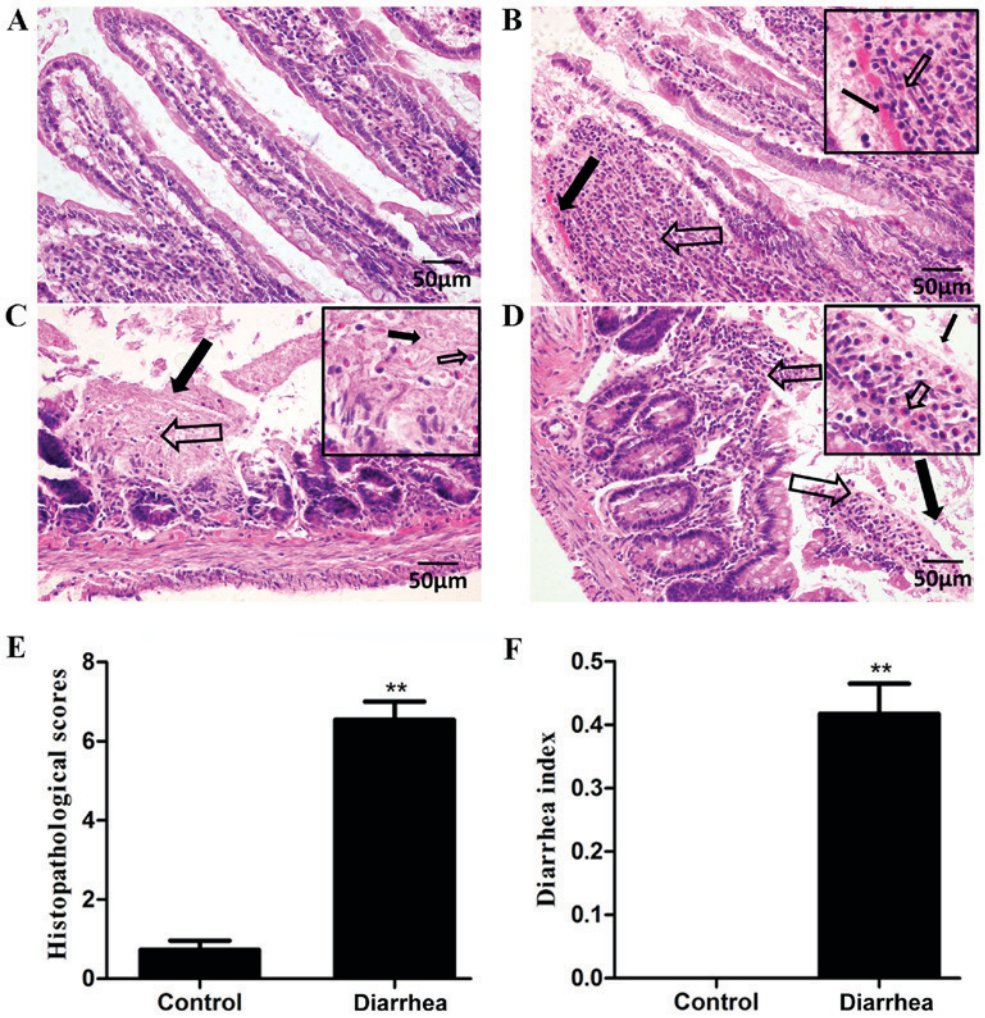

Figure 1. Histological analysis of rat intestinal tissues. Rat intestinal tissues were fixed, embedded in paraffin and stained with hematoxylin and eosin. (A) Normal intestinal tissue, demonstrating a normal mucosal structure and intact epithelium. (B) Mildly infected intestinal tissue, demonstrating mucosal lamina propria with congestion (black arrow) and inflammatory cellular infiltrates (empty arrow). (C) Moderately infected intestinal tissue, exhibiting mucosal lamina propria with coagulation necrosis (black arrow) and focal necrosis with inflammatory cell infiltrates (empty arrow). (D) Severely infected intestinal tissue, demonstrating the mucosa with disrupted surface epithelium (black arrow) and villous lamina propria with inflammatory cellular infiltrates (empty arrow). (E) Pathological scores of the intestine, indicating the severity of pathological intestine lesions. (F) Diarrhea index of the control and diarrhea rats. Statistical comparisons were performed by a Student's t-test. **P<0.01 vs. the control group.

OTUs that reached 97% similarity were used for diversity (Shannon and Simpson), richness (Chao1 and Ace), Good's coverage, rarefaction curve and Shannon-Wiener curve analyses (32). The community structure was compared using principal component analysis (PCA) based on the weighted UniFrac distance. A hierarchical cluster, rank-abundance and heatmap were constructed and analyzed using R software package (http://www.r-project.org) (33).

Statistical analysis. Data are represented as the mean ± standard deviation. Statistical analyses were performed with Student's t-test using GraphPad Prism software (version 6.0; GraphPad Software Inc., La Jolla, CA, USA). P<0.05 was considered to indicate a statistically significant difference.

Results

Following an intraperitoneal injection of *E. coli* O_{101}, the histopathological changes in the intestinal tissues of the rats that received bacteria injection were investigated. The intestinal tissues of healthy rats exhibited a normal mucosal structure and intact epithelium (Fig. 1A). By contrast, the jejunal tissues obtained from rats injected with *E. coli* O_{101} demonstrated congestion and inflammatory cellular infiltrates in the mucosal lamina propria (Fig. 1B). The mucosal lamina propria did not exhibit a normal tissue structure, but coagulation necrosis and focal necrosis with inflammatory cell infiltration were observed (Fig. 1C). In addition, a disrupted surface epithelium and inflammatory cellular infiltrate in the villous lamina propria were observed in the intestinal mucosa (Fig. 1D). The severity scores for the intestinal lesions are listed in Fig. 1E, which indicates that the histological scores of the diarrhea group were significantly increased as compared with those of the control group (P<0.01). In addition, the incidence rate of liquid stools in rats was 100%, while the control group did not produce any liquid stools. In addition, the diarrhea rats had a higher diarrhea index as compared with that of the control group (P<0.01) (Fig. 1F).

To characterize the bacterial diversity and abundance in the fecal microbiota of the control and diarrhea rats, high throughput sequencing was performed on the V3-V4 hypervariable region of bacterial 16S rRNA gene using the Illumina MiSeq system. Following denoising and filtering steps, a total of 712,814 valid reads were obtained from the 22 samples, with a mean of 32,400 reads/sample. A dataset consisting of 32,810±1,315 reads from the control group (n=11) and 31,990±1,372 from the diarrhea group (n=11), with an average length of 442 bp, was used in the final analysis. The Good's coverage of all samples was 0.9977±0.0007%, indicating that the 16S rRNA sequences represented the majority of the bacteria in the samples. Based on a sequence similarity of >97%, an average of 376 and 267 OTUs were defined for the control and diarrhea groups, respectively. The rarefaction curves indicated that the diversity of the bacteria was addressed, while the rank abundance curves presented the abundance and evenness of the two groups (Fig. 2A). The levels of the indicators of community abundance (Ace and Chao1) in the diarrhea group were significantly decreased as compared with those in the control group (P<0.05) (Fig. 2B). Furthermore, the Simpson and Shannon indices indicated that the diversity of the microbial community of the control group was higher than that of the diarrhea group (Fig. 2C).

The differences and similarity between the microbial communities in the diarrhea and control groups were revealed by determination of the weighted Unifrac PCA and hierarchical cluster analysis, respectively (Fig. 2D). Weighted UniFrac PCA demonstrated a high degree of variation between individual rats. Nevertheless, the first principal component (PC1), which explained 63.51% of the variance in the data, distinctly separated the diarrhea group from the control group. Further hierarchical cluster analysis revealed that the control and diarrhea groups were distinguished into two major clusters, indicating robust differences in the microbial communities of the two groups.

The phylum-level distribution patterns of the control and diarrhea groups are shown in Fig. 3A. In the control group, the major bacterial communities included Firmicutes (70.05±4.59%), Bacteroidetes (24.41±3.39%), Proteobacteria (3.25±3.02%), Verrucomicrobia (0.73±0.26%) and Actinobacteria (0.19±0.04%). In the diarrhea group, the order of the major bacterial communities was Firmicutes (39.91±7.02%), Proteobacteria (35.82±5.70%), Bacteroidetes (23.27±3.79%), Actinobacteria (0.50±0.12%) and Verrucomicrobia (0.17±0.08%). Thus, the results suggested that the most abundant communities in the two groups are the three most populated bacterial phyla, namely Firmicutes, Bacteroidetes and Proteobacteria, followed by the low abundance phyla of Verrucomicrobia, Actinobacteria, Candidate division TM7, Cyanobacteria, Deferribacteres, Elusimicrobia and Spirochaetes. As shown in Fig. 3B, statistical analysis revealed that the relative abundance of Firmicutes and Verrucomicrobia in the diarrhea group was significantly lower in comparison with that in the control group (P<0.05). By contrast, the relative abundance of Proteobacteria and Actinobacteria in the diarrhea group was significantly higher compared with that in the control group (P<0.05). Bacteroidetes, one of the most dominant phyla, was not significantly altered in the diarrhea group.

The order-level distribution patterns of the two groups are shown in Fig. 4A. In the control group, the orders of the most dominant bacterial communities included Lactobacillales (42.95±7.47%), Clostridiales (24.62±5.27%), Bacteroidales (24.39±3.39%), Enterobacteriales (3.06±3.04%) and Verrucomicrobiales (0.76±0.25%), accounting for a total of 95.77% of the overall bacteria presented in this group. In the diarrhea group, the orders of the most dominant bacterial communities were Enterobacteriales (35.39±5.70%), Bacteroidales (23.26±3.79%), Lactobacillales (19.75±4.92%), Clostridia (18.15±3.30%) and Verrucomicrobiales (0.17±0.08%), accounting for a total of 96.72% of the overall bacteria presented in this group. Furthermore, statistical analysis demonstrated that the proportion of Lactobacillales belonging to the phylum Firmicutes and the proportion of Verrucomicrobiales of the phylum Verrucomicrobia were significantly decreased in the diarrhea group as compared with that in the control group (P<0.05). By contrast, the proportion of Enterobacteriales belonging to the phylum Proteobacteria was significantly increased in the diarrhea group (P<0.05). However, the levels of Bacteroidales and Clostridiales did not differ significantly between the two groups (P>0.05) (Fig. 4B).

Furthermore, the difference of the microbiota distribution at the genus level was compared between the control and diarrhea rats. The microbial distribution was significantly different at the genus level, suggesting that the composition of microbiota in the intestine of the rats was severely altered due to *E. coli* O_{101} infection. Among the 100 genera that are displayed in the heatmap in Fig. 5A, certain of these exhibited a significant difference between the control and diarrhea groups, including the genera *Enterococcus*, *Prevotella*, *Akkermansia* and *Escherichia/Shigella* (P<0.05) (Fig. 5B). In addition, the specific phylotype at the OTUs level was identified in response to *E. coli* O_{101} infection. A total of 18 OTUs of relative abundance presented in the two groups were selected for comparison. The proportion of *Enterococcus* (2 OTUs) belonging to the order Lactobacillales was significantly lower in the diarrhea group (mean, 1.74%) as compared with that in the control group (mean, 32.88%; P<0.001). The proportion of *Prevotella* (14 OTUs) belonging to the order Bacteroidales was also significantly lower in the diarrhea group (mean, 1.51%) in comparison with that in the control group (mean, 10.62%; P<0.01). Furthermore, the proportion of *Akkermansia* (1 OTU) belonging to the order Verrucomicrobia was significantly lower in the diarrhea group (mean, 0.17%) as compared with the control group (mean, 0.73%; P<0.001). Finally, the proportion of *Escherichia/Shigella* (1 OTU) belonging to the order *Enterobacteriales* was significantly higher in the diarrhea group (mean, 34.16%) as compared with that in the control group (mean, 3.05%; P<0.001) (Fig. 5B).

Discussion

ETEC strains that produce multiple enterotoxins are major causes of severe dehydrating diarrhea in humans and animals (34,35). Several studies have reported that the diarrhea-associated diseases, such as cholera (36), diarrhea-predominant irritable bowel syndrome (37) and

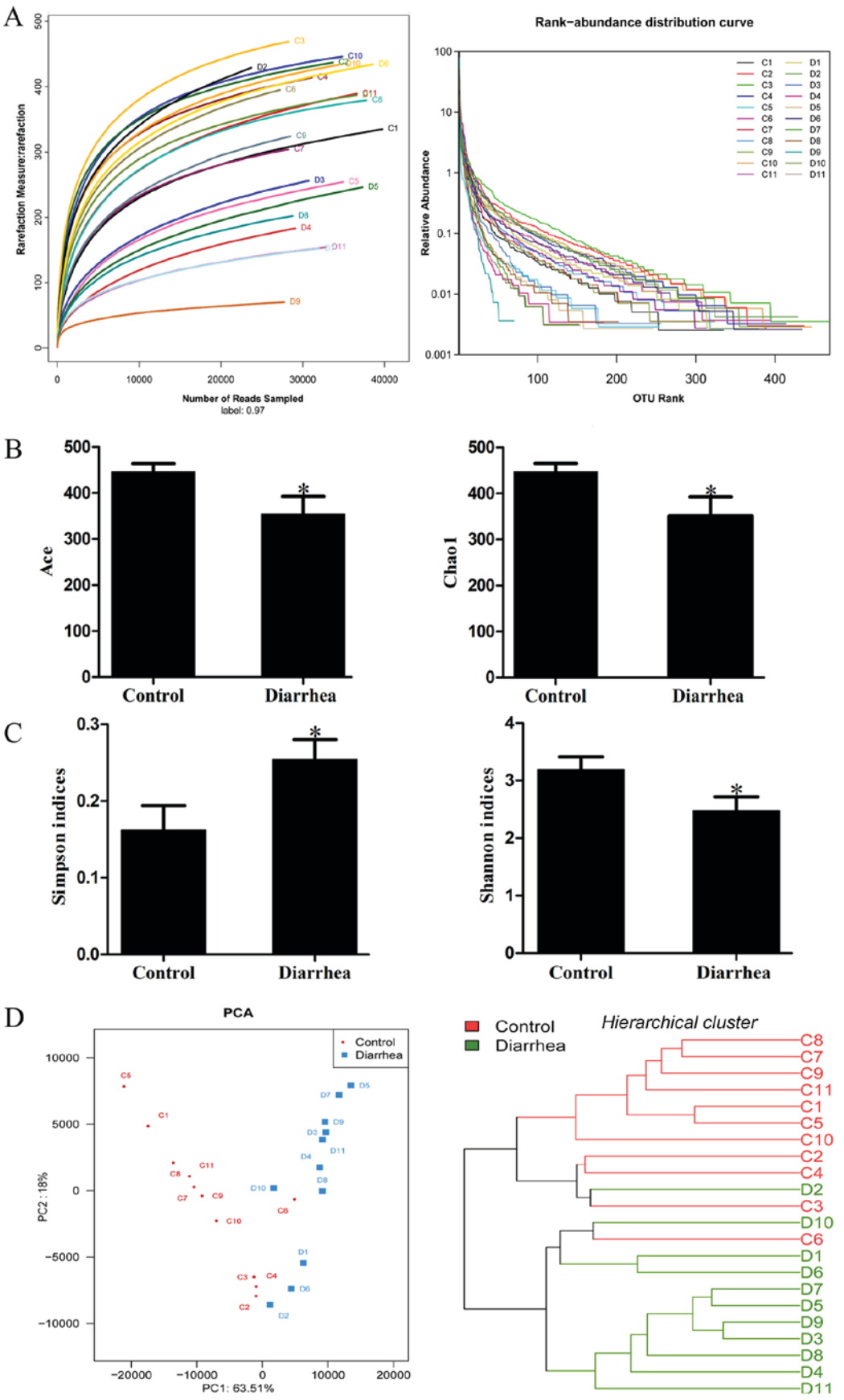

Figure 2. Structural comparison of fecal microbiota between the control and *Escherichia coli* O_{101}-induced diarrhea group. (A) Richness and diversity of the rat fecal microbiota between the two groups. Rarefaction curves were used to estimate the abundance (at a 97% similarity level) of the fecal microbiota, while the Rank abundance curve was used to estimate the abundance and evenness between the two groups. (B) Abundance of the two groups evaluated by the Ace and Chao1 indices, and (C) diversity of the two groups evaluated by the Shannon and Simpson indices, based on 16S rRNA gene sequences. (D) Difference and similarity of microbial communities between the diarrhea and control groups revealed by weighted Unifrac PCA and hierarchical cluster analysis. Statistical comparisons were performed by Student's t-test. *P<0.05 vs. the control group. PCA, principal component analysis.

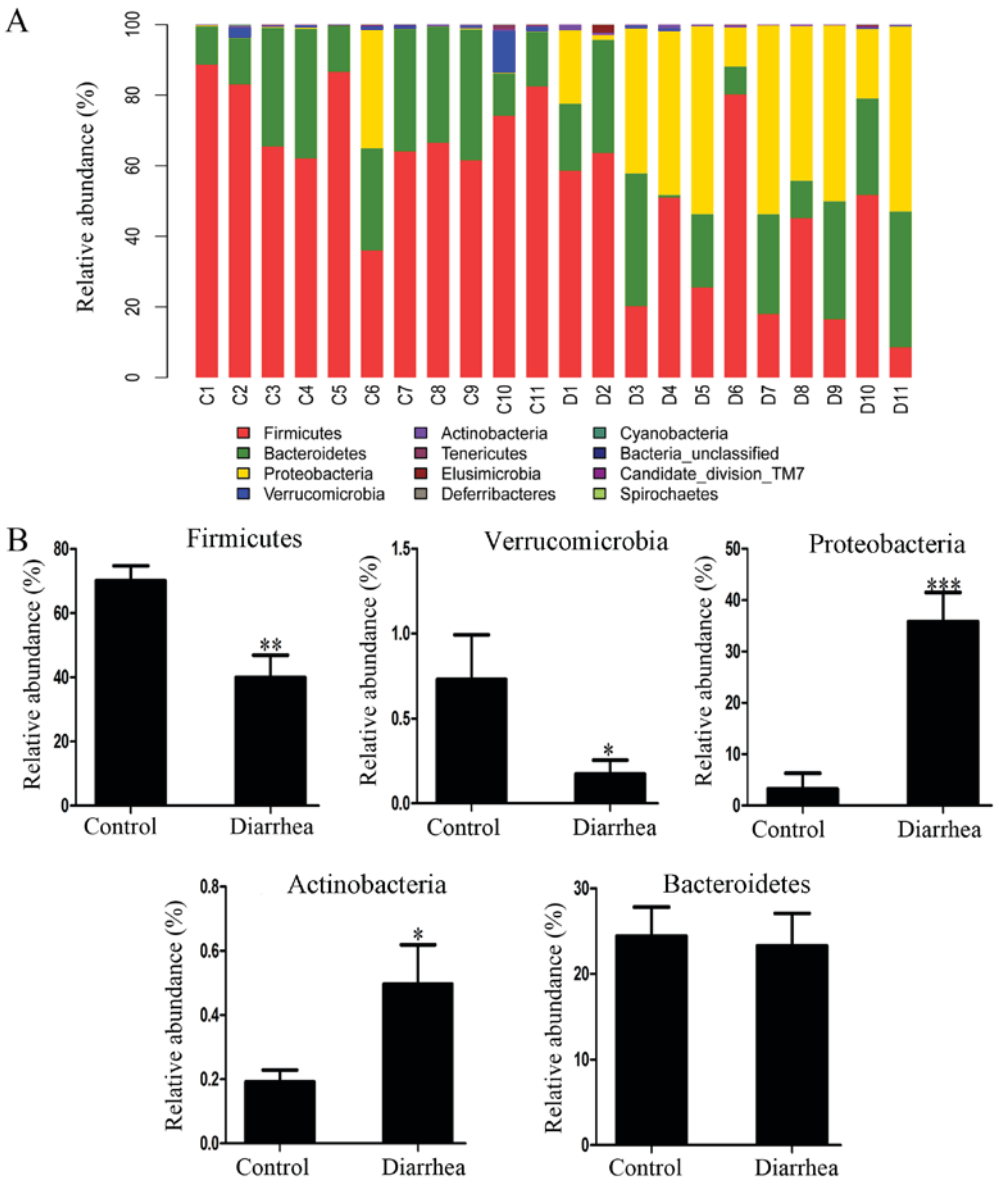

Figure 3. Microbial composition of the control and diarrhea rats at the phylum level. (A) Distribution of bacterial taxa in the fecal samples and (B) comparison of the main phylum in the control and diarrhea groups. Sequences that could not be classified into any known group were designated as 'unclassified'. Statistical comparisons were performed by Student's t-test. *P<0.05, **P<0.01 and ***P<0.001 vs. the control group.

porcine epidemic diarrhea (38), can change the composition of the gut microbiota. This evidence indicated a potential association between gut microbiota and diarrhea. However, little is known regarding the status of gut microbiota and the histopathological changes in intestinal tissues following ETEC infection in animals. Therefore, elucidating the role of ETEC in altering the composition of gut microbiota and the intestinal tissue in a model of ETEC-induced diarrhea is essential.

Notably, the intestinal mucosa is a vital barrier for protecting the body against infection by pathogenic microorganisms (39,40). Histological assessment is commonly used in the diagnosis of gastrointestinal diseases (41,42). In a previous study, the investigation of histological sections of intestinal tissue from diarrhea mice revealed damaged surface epithelium with inflammatory infiltrates in the lamina propria (43). Similarly, the results of the present study also revealed that the intestinal mucosa of diarrhea rats was damaged, and the surface epithelium and villous lamina propria were disrupted by the inflammatory cellular infiltrates. According to the histopathological scores, the diarrhea rats exhibited severe injury in the intestinal tissues.

The gut microbiota in the intestinal mucosa serves a crucial role in the development and integrity of the mucosal epithelium (44-46). As fecal microbial communities represented the highest bacterial diversity in the gut (47), fecal sample were used in the present study. Barcoded Illumina MiSeq sequencing of the V3-V4 hypervariable region of 16S rRNA was employed, in order to compare the composition of the fecal microbiota between the normal and *E. coli* O_{101}-treated rats. Chao1 and Ace analysis revealed greater microbial diversity in the control group compared with that in the diarrhea group, while the Shannon and Simpson indices also indicated that the bacterial community diversity of the control group was higher than that of the diarrhea group. Therefore, it is concluded that *E. coli* O_{101} infection reduced the diversity of the gut microbiota in rats.

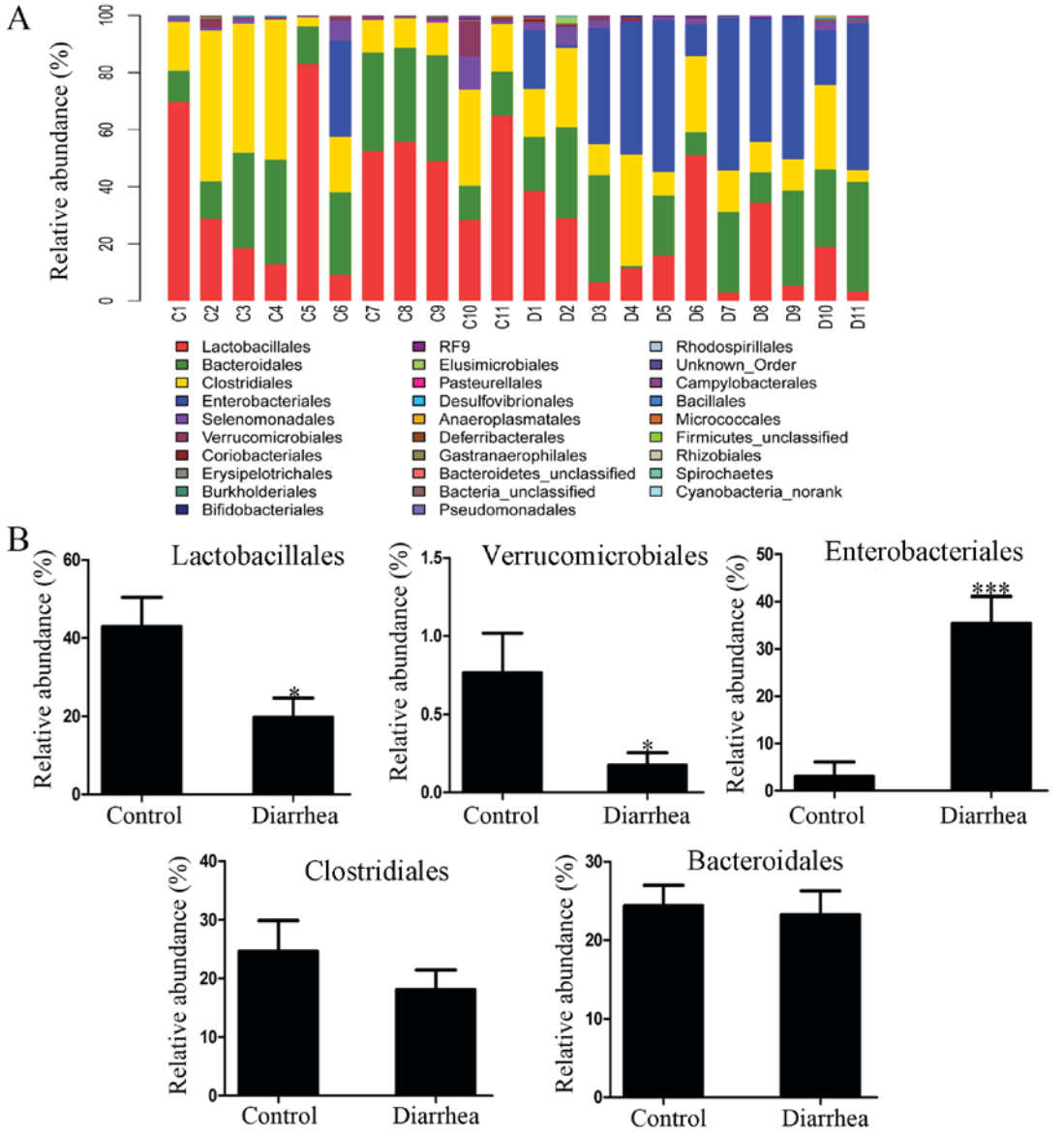

Figure 4. Microbial composition of control and diarrhea rats at the order level. (A) Distribution of bacterial taxa in the fecal samples and (B) comparison of main order in the control and diarrhea groups. Sequences that could not be classified into any known group were designated as 'unclassified'. Statistical comparisons were performed by Student's t-test. *P<0.05 and ***P<0.001 vs. the control group.

The phylum-level distribution of the bacterial communities in the control and diarrhea groups of the present study revealed that the two prevalent phyla, namely Bacteroidetes and Firmicutes, are dominant irrespective of *E. coli* O_{101} infection. This observation was in agreement with the findings of a previous study, which demonstrated that Bacteroidetes and Firmicutes are the main phyla in rats regardless of the age (48). In the present study, Bacteroidetes, Firmicutes, Proteobacteria, Verrucomicrobia and Actinobacteria were dominant in the groups, which was in agreement with previous findings (49). However, these dominant bacteria phyla displayed a different tendency subsequent to *E. coli* O_{101} infection. The proportion of Firmicutes and Verrucomicrobia was decreased, while that of Proteobacteria and Actinobacteria was significantly increased. As reported previously, the phylum Verrucomicrobia was absent in mice treated with cyclophosphamide, a potent immunosuppressive agent (50,51). Thus, it can be speculated that *E. coli* O_{101} infection decreased the abundance of Verrucomicrobia and that it may suppress the immune function of the host. Furthermore, Proteobacteria is the main pathogenic bacterial phylum that is closely associated with the presence of diarrhea symptoms (52,53). As a consequence of *E. coli* O_{101} infection, the abundance of Proteobacteria was increased significantly.

The comparison at the order level was in agreement with that at the phylum level in the current study. For instance, the orders Lactobacillales and Verrucomicrobiales were significantly decreased in the diarrhea group, while the order Enterobacteriales was significantly increased in the diarrhea group. The phyla of these microbes, namely Firmicutes, Verrucomicrobia and Proteobacteria, respectively, exhibited the same tendency of decrease or increase. Thus, the alteration at the order level contributed to that at the phylum level.

Among the fully classified genera in the present study, several genera of specific interest were identified. For instance, *Escherichia/Shigella*, belonging to the order Enterobacteriales and the phylum Proteobacteria, exhibited a higher abundance

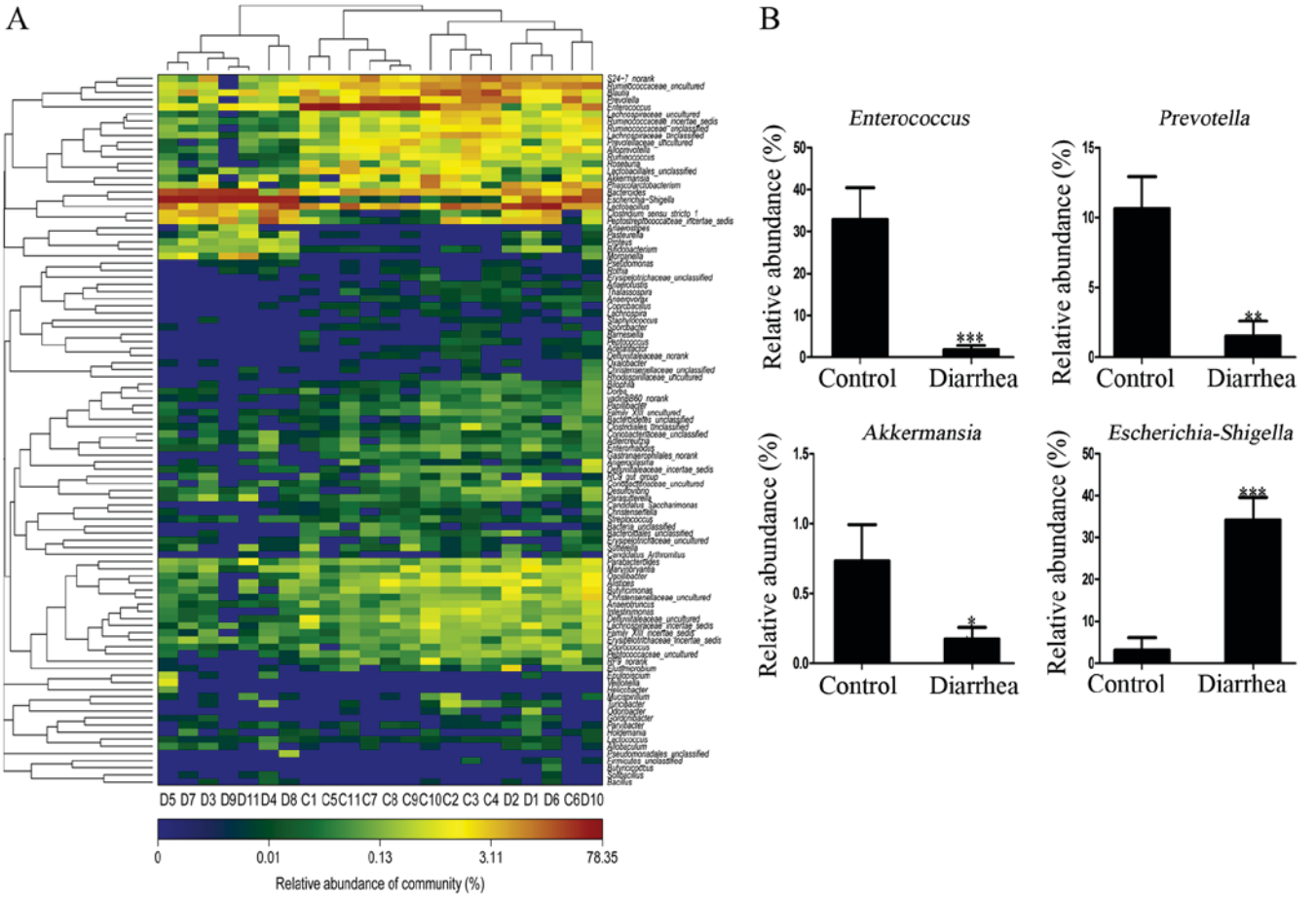

Figure 5. Microbial composition of control and diarrhea rats at the genus level. (A) Heatmap of hierarchy cluster, indicating the abundance of the genus in the feces. Colors indicate the relative abundance from low (blue) to high (red). (B) Comparison of main genera in the control and diarrhea groups. Sequences that could not be classified into any known group were designated as 'unclassified'. Statistical comparisons were performed by Student's t-test. *P<0.05, **P<0.01 and ***P<0.001 vs. the control group.

(11.2-fold) in the diarrhea group as compared with that in the control group. Thus, it can be inferred that the increase in the abundance of *Escherichia/Shigella* caused the increase of the order Enterobacteriales and the phylum Proteobacteria in the diarrhea rats. In addition, the genus *Prevotella* exhibited the most significant difference in relative abundance between the diarrhea and control groups. The abundance of *Prevotella* was significantly lower (7.0-fold) in the diarrhea group in comparison with the control group. Similar to the current observations, Pop *et al* (54) reported that diarrhea in young children from low-income countries led to a low abundance of *Prevotella*. Kang *et al* (55) also found a significantly lower abundance of *Prevotella* in autistic children. De Filippo *et al* (56) further demonstrated that *Prevotella* suppresses the pathogenic *Escherichia/Shigella*, resulting in a low incidence of gastrointestinal disorders among African children. These observations suggested that *Prevotella* may exert a beneficial physiological role in human health, particularly in children. Furthermore, the proportion of *Enterococcus*, a large genus of lactic acid bacteria of the order Lactobacillales and the phylum Firmicutes, was markedly reduced (18.9-fold) in diarrhea rats as compared with normal rats. The most common species of *Enterococcus spp.* include *Enterococcus faecium* and *Enterococcus faecalis*, which can be isolated from the gastrointestinal tract, the oral cavity and the vagina of animals as normal commensals (57). *Enterococcus faecium* strains are frequently used in pig production to decrease the incidence of diarrhea and the count of *E. coli* in pigs, as well as improve the animals' performance and feed conversion. Hu *et al* (58) reported that *Enterococcus faecalis* LAB31 effectively reduced the incidence of diarrhea in weaned piglets by increasing the relative number of *Lactobacilli*. The abundance of *Akkermansia*, belonging to the order Verrucomicrobiales and phylum Verrucomicrobia, was also evidently reduced in the diarrhea group. Similarly, Liu *et al* (38) revealed that *Akkermansia* was highly abundant in the control group than in the porcine epidemic diarrhea group. Diarrhea patients with microscopic colitis also had a significantly lower amount of *Akkermansia* (59). Furthermore, several studies suggested that *Akkermansia* may be used as a beneficial bacterium to regulate the host immunity, as well as an indicator for its evaluation. Shin *et al* (60) previously reported that *Akkermansia* attenuated tissue inflammation by activating the Foxp3 regulatory T-cells. *Akkermansia muciniphila*, belonging to the genus *Akkermansia*, altered the mucosal gene expression profiles toward altering immune responses (61). Taken together, the present study revealed the *E. coli* O_{101} infection decreased the proportion of the beneficial bacteria *Prevotella*, *Enterococcus* and *Akkermansia*, while increasing the proportion of the pathogenic bacteria *Escherichia/Shigella*. Therefore, the beneficial bacteria *Prevotella*, *Enterococcus* and

Akkermansia protected rats against the pathogenic bacteria *Escherichia/Shigella*, while *E. coli* O_{101} infection altered the balance.

In an attempt to distinguish *Escherichia/Shigella* in feces from rats injected with *E. coli* O_{101}, the current study also conducted a sequence comparison and found a 99% similarity (data not shown), which suggested that *Escherichia/Shigella* in feces were putatively of the same genus of *E. coli* O_{101} that were injected into the peritoneum of the rats. Thus, the present study hypothesized that injected *E. coli* O_{101} may colonize in the intestinal tract of rats and compete with the beneficial bacteria, leading to the decreased abundance of the beneficial bacteria and consequently resulting in an imbalance of gut microbiota.

The gut microbiome is hypothesized to serve a critical role in gastrointestinal diseases, such as diarrhea. By regulating the balance of intestinal flora, increasing the beneficial bacteria and reducing the harmful bacteria, the symptoms of diarrhea can be alleviated and diseases can be treated. Historically, antibiotics were primarily used to treat individuals with diarrhea. However, the blind use of antibiotics may eliminate the sensitive beneficial bacteria and aggravate the microbiota imbalance. Therefore, in order to prevent further aggravation of the microbiota disorder as identified in the current study, appropriate use of drugs should be considered for adjuvant therapy. The efficiency of specific probiotics for the treatment of infection-associated diarrhea in adults has been supported by clinical studies (62). Dietary fiber benefits human health and can also modulate gut microbiota for treating diarrhea infections (63). Furthermore, the Chinese herbal formula SLBZS has been demonstrated to have an effect in shifting the gut microbiome structure during the treatment of rats with antibiotic-associated diarrhea (64). Taken together, the current study may provide a theoretical basis for gut microbiota and potential novel targets for the control of the disease.

In conclusion, given the crucial role of gut microbiota in maintaining intestinal health, identifying the changes in systemic gut microbiota and specific microbes is essential. As a first step to achieve this long-term goal, the present study established an *E. coli* O_{101}-induced diarrheal rat model with increasing diarrhea index and injury in the intestinal tissues. Next, several key changes in fecal microbiota subsequent to treatment with *E. coli* O_{101} were identified. It was revealed that the diarrhea rats tended to have a less diverse gut microbiome, while a shifted distribution pattern of the bacterial communities was demonstrated at the phylum and order levels in diarrhea rats. Finally, several individual genera, primarily the beneficial *Prevotella, Enterococcus* and *Akkermansia*, exhibited significantly lower abundance, while the pathogenic *Escherichia/Shigella* had significantly higher abundance in diarrhea rats as compared with the control group. Taken together, the data of the present study provided crucial insights into *E. coli* O_{101}-induced dysbiosis in gut microbiota in the fecal samples. Thus, further genomic studies are necessary to better characterize the indicative bacteria and assess the potential development of a microbiological intervention for treating diarrhea.

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Availability of data and materials

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

Authors' contributions

XS and XW performed the DNA extraction and pyrosequencing, statistical analysis and drafted of manuscript. YH, YW and BF participated in the design of the study and performed the statistical analysis. YG participated in drafted of manuscript. YG, GH and XM revised the manuscript and interpreted the data. HD and YZ conceived of the study, participated in the study design and coordination, and helped to draft the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The current study was approved by the Institutional Animal Care and Use Committee of the AMMS. All animal care and experimental procedures were conducted according to the institutional ethical guidelines.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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