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ORIGINAL ARTICLE

# Effect of *Debaryomyces hansenii* combined with Qiweibaizhu powder extract on the gut microbiota of antibiotic-treated mice with diarrhea

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## Abstract

The aim of this study was to investigate the effects of an extract of Qiweibaizhu powder combined with *Debaryomyces hansenii* on the gut microbiota of antibiotic-treated mice with diarrhea. Mice were gavaged with a mixture of gentamycin sulfate and cefradine to induce diarrhea. After diarrhea was observed, 25% dose of ultra-micro Qiweibaizhu powder extract combined with 25% dose of *Debaryomyces hansenii* (QCD) was gavaged to mice with diarrhea. DNA of intestinal contents in mice was extracted for 16S rRNA gene sequence analysis by high-throughput sequencing following treatment finished. The results showed that the QCD increased the species richness and diversity, but did not recover the diversity to the original level. Antibiotics and QCD significantly altered the composition of gut microbiota at different taxonomic levels. At the genus level, the relative abundance of Bacteroidales S24-7 group_unidentified and *Bacteroides* returned to baseline after QCD treatment. Additionally, QCD suppressed the growth of *Oscillospira* and *Ruminococcus*, and promoted the proliferation of Erysipelotrichaceae_norank and *Blautia* compared with the healthy and diarrheal mice. Our results indicated that QCD modulated the diversity and composition of the gut microbiota in antibiotic-treated mice with diarrhea**.** The synergistic effect between Qiweibaizhu powder extract and *Debaryomyces hansenii* may be related to *Bifidobacterium* and Bacteroidales S24-7 group_unidentified.

**Keywords** Qiweibaizhu powder · *Debaryomyces hansenii* · Gut microbiota · Antibiotic-associated diarrhea · Diversity

## Introduction

Antibiotic-associated diarrhea (AAD) is a drug-induced disease commonly regarded as an adverse effect of antibiotics. It has been reported that 5–25% of patients who use antibiotics experience diarrhea (Lichtman et al. 2016). Gut microbiota dysbiosis is responsible for the development of AAD (Hogenauer et al. 1998; Anand et al. 2017). Therefore, the key aspect of AAD treatment is re-establishing the balance of gut microbiota.

Qiweibaizhu powder (QWBZP), a traditional Chinese herbal formula in "Xiaoer Yaozheng Zhijue", has been used to treat infantile diarrhea in China for thousands of years. Our studies have supported that QWBZP is a valuable therapeutic for AAD, and cures diarrhea by adjusting the intestinal microecology and re-establishing the gut microbiota balance (Tan et al. 2012; Zhang et al. 2014). Superfine pulverization technology can enhance the dissolution of effective components in traditional Chinese medicine (TCM) and notably decrease the dosage of TCM (Deng et al. 2011). Our previous studies confirmed that 50% dose of ultra-micro QWBZP extract is equivalent to the 100% dose of traditional QWBZP extract when treating AAD. Additionally, 50% dose of ultra-micro QWBZP extract is superior to the 100% dose of traditional QWBZP extract in regulating the metabolic diversity of gut microbiota, repairing intestinal mucosa, and promoting the proliferation of *yeast* and *Bifidobacterium* (Zhang et al. 2014; Deng et al. 2011).

Probiotics are live microorganisms that confer a health benefit on the host when administered in adequate amounts (Silverman et al. 2017). Usually, *Lactobacillus*, *Bifidobacterium*, *yeast*, *Enterococcus* and *Bacillus* are used to treat AAD (Goldenberg et al. 2015; Szajewska et al. 2016). In addition to *Saccharomyces boulardii*, several y*east* strains, such as *Debaryomyces hansenii* (*D. hansenii*), *Torulaspora delbrueckii*, and *Kluyveromyces lactis*, also possess antibacterial and probiotic effects (Hatoum et al. 2012). *D. hansenii*,

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isolated from natural habitats, food and animal intestines, has been found to have anti-inflammatory effects on human dendritic cells (Ochangco et al. 2016). Furthermore, *D. hansenii* can stimulate innate immunity, antioxidant parameters, and immune-related gene expression in newborn goats (Angulo et al. 2019). We isolated *D. hansenii* from the gut of experimental mice and revealed its potency in modulating the diversity of gut bacteria and stimulating the density of *Lactobacillus* and *Bifidobacterium* (Guo et al. 2015; He et al. 2017).

Clinically, the combination of TCM and probiotics effectively alleviates diarrhea and shortens the clinical treatment time (Lei et al. 2018). Importantly, the dosages of TCM and probiotics were notably reduced, which is cost saving. Based on both QWBZP extract and *D. hansenii* can modulate the gut microbiota and cure AAD, we wanted to know whether the combination of the two has synergistic effect in AAD treatment. Our previous studies showed that 25% dose of ultra-micro QWBZP extract combined with 25% dose of *D. hansenii* (QCD) exhibited synergistic effects in recovering the density of total intestinal bacteria and *Escherichia coli*, and in increasing the diversity of *Lactobacillus.* The therapeutic effect of QCD is equivalent to the 50% dose of ultra-micro QWBZP extract and 100% dose of traditional QWBZP extract (Guo et al. 2015; Long et al. 2018; Liu et al. 2016). In this study, we further discussed the synergistic mechanism of QCD by high-throughput sequencing and provided support for the clinical application of QCD.

## Materials and methods

### Preparation of 100% dose of ultra-micro QWBZP extract

QWBZP is composed of the slices of seven Chinese herbs: Ginseng Radix et Rhizoma (6 g), Aucklandiae Radix (6 g), Poria (10 g), Atractylodis Macrocephalae Rhizoma Tostum (10 g), Pogostemonis Herba (10 g), Puerariae Lobatae Radix (10 g), and Glycyrrhizae Radix et Rhizoma (3 g). All Chinese herb slices were purchased from The First Hospital of Hunan University of Chinese Medicine. The slices of the seven Chinese herbs were processed into ultra-micro powder, and subsequently brewed in boiling water. The solution was centrifuged at 4000 × g, and the supernatant was collected. The concentration of the supernatant was 2 g $mL^{-1}$, and was subsequently diluted to 25% dose. The diluted supernatant was stored at 4 °C and reheated to 25–30 °C before use.

### Preparation of 100% dose of *D. hansenii*

*D. hansenii* was activated according to our previous study (Guo et al. 2015). In short, *D. hansenii* was inoculated into liquid potato sucrose medium and shaken at 28 °C for 36 h. The suspension was centrifuged at 2000 × g for 4 min. The sediment was washed 1–2 times using sterile stroke-physiological saline solution and then transferred to a centrifuge tube. After counting with a hemocytometer, the number of *D. hansenii* was $10^{10}$ cells $mL^{-1}$, and was subsequently diluted to 25% dose for further use.

### Reagents

The antibiotic mixture was composed of gentamicin sulfate (No. 5120106, Yichang Renfu Pharmaceutical Co., Ltd.) and cefradine (No. 110804, Suzhou Zhonghua Pharmaceutical Industry Co., Ltd.). The concentration of the antibiotic mixture was 62.5 g $L^{-1}$ (Zhang et al. 2014). Tris-saturated phenol–chloroform–isoamyl alcohol (25:24:1), protease K, TE buffer, lysozyme, and acetone were purchased from Beijing Dingguo Changsheng Biotechnology Co., Ltd. and were used to extract DNA.

### Animal ethics statement

Eighteen specific-pathogen-free (SPF) Kunming (KM) mice (nine males and nine females, weight 18–22 g, license number [SCXK (Xiang) 2013-0004]) were purchased from Hunan Slaccas Jingda Laboratory Animal Co., Ltd. Fodder was provided by the Laboratory Animal Center of Hunan University of Chinese Medicine. The study was approved by the Animal Care and Use Committee of Hunan University of Chinese Medicine.

### Experimental design

After adaptive feeding (3 days) under controlled conditions (23–25 °C, 50–70% humidity and a 12 h light/12 h dark cycle), the mice were randomly divided into three groups with three males and three females in each group: healthy group (qck), AAD group (qm), and QCD treatment group (qjq). The mice in the AAD group and QCD treatment group were first gavaged with a mixture of gentamycin sulfate and cefradine (0.35 mL) twice per day for 5 days, while the mice in the healthy group were gavaged with the same amount of sterile water. After diarrhea was observed, the mice in the healthy group and AAD group were gavaged with sterile water, and the mice in the QCD treatment group were administered QCD (0.35 mL), twice per day for 4 days. After 4 days of treatment, all mice were euthanized by cervical

dislocation, and the intestinal contents were collected immediately. The intestinal contents of one male and one female in the same group were mixed and then stored at 4 °C for further use.

### 16S rRNA gene sequence analysis

Microbial genomic DNA was extracted from each sample according to the protocol in our previous report (Wu et al. 2012). The variable region of bacterial 16S rRNA V4 was amplified using the primers 520F (5′-AYTGGG YDTAAAGNG-3′) and 802R (5′-TACNVGGGTATCTA ATCC-3′). The PCR amplification system included 2.0 μL of dNTPs (2.5 mmol $L^{-1}$), 5.0 μL of 5 × Q5 reaction buffer, 5.0 μL of 5 × Q5 high enhancer, 1.0 μL of forward primer (10 μmol $L^{-1}$), 1.0 μL of reverse primer (10 μmol $L^{-1}$), 0.25 μL of Q5 polymerase (5 U $\mu L^{-1}$), 2.0 μL of template DNA (0.2 ng $\mu L^{-1}$), and 8.75 μL of sterilized $ddH_2O$. The PCR conditions were as follows: 98 °C for 30 s; 25 cycles of 98 °C for 30 s, 50 °C for 30 s and 72 °C for 30 s; and 72 °C for 5 min. The PCR products were examined by 2% agarose gel electrophoresis. Sequencing was performed by Shanghai Personal Biotechnology Co., Ltd.

### Bioinformatic analyses

Quantitative insights into microbial ecology (QIIME, version 1.7.0, https://qiime.org/) was used to analyze raw DNA sequences, operational taxonomic units (OTUs), rank abundance curves, community structures, and beta diversity (Caporaso et al. 2010; Edgar 2010; Oberauner et al. 2013). Alpha diversity and species abundance were analyzed by MOTHUR (version 1.35.1, https://www.mothur.org/) (White et al. 2009). Information on species evolution and abundance was provided by MEGAN (https://ab.inf.uni-tuebingen.de/software/megan/) (Huson et al. 2011). The Greengene (Release 13.8, https://greengenes.secondgenome.com/) database was used to annotate taxonomic information. SPSS 21.0 (IBM Corp., Armonk, NY, USA) was used to compare the differences among the three groups. Analyses of significant differences were performed by an independent one-way analysis of variance (ANOVA) in terms of the least significant difference (LSD) multiple comparison test.

## Results

### DNA sequences of the intestinal bacteria in AAD mice

All the raw data were submitted to NCBI (Accession number: SRP245727). A total of 1,221,890 high-quality sequences were detected in all samples. The average proportions of high-quality sequences in the healthy group, AAD group and QCD treatment group were 92.63%, 92.23%, and 93.84%, respectively (Fig. 1). The QCD treatment group possessed more DNA sequences than the other two groups. The results indicated that QCD treatment increased the number of gut bacteria species in mice with AAD.

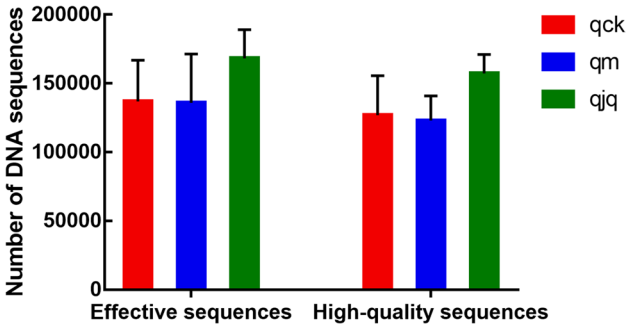


**Fig. 1** DNA sequences of the intestinal bacteria in different groups. qck, healthy group; qm, antibiotic-associated diarrhea (AAD) group; qjq, Qiweibaizhu powder extract combined with *Debaryomyces hansenii* (QCD) treatment group

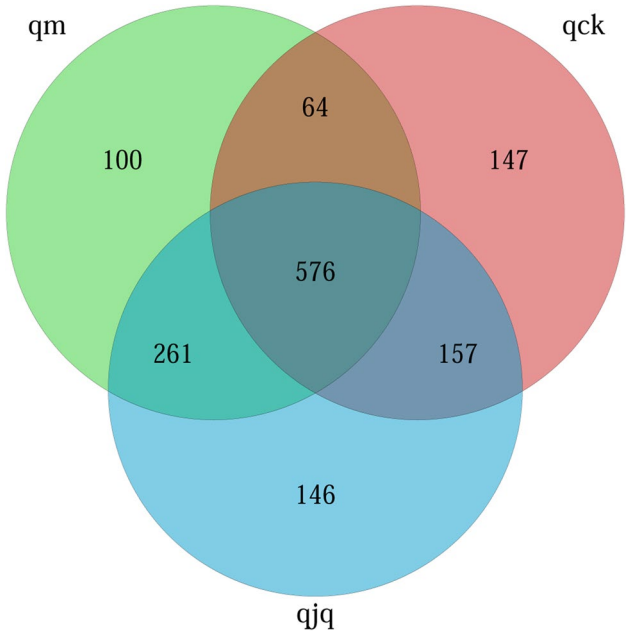


**Fig. 2** Shared operational taxonomic unit (OTU) analysis of the different groups. Venn diagram of OTUs based on the sequences with a threshold similarity of 97%. qck, healthy group; qm, AAD group; qjq, QCD treatment group

### OTU number of the intestinal bacteria in AAD mice

High-quality sequences were clustered at 97% similarity by QIIME. The unique and shared OTUs in different groups are presented in Fig. 2. The Venn diagram shows that

1451 OTUs were identified in total, and 576 of these OTUs were shared by the three groups. In addition, the numbers of OTUs identified in the healthy group, AAD group, and QCD treatment group were 944, 1001, and 1140, respectively. Briefly, QCD treatment increased the OTU number, which implied that QCD treatment increased the number of intestinal bacteria.

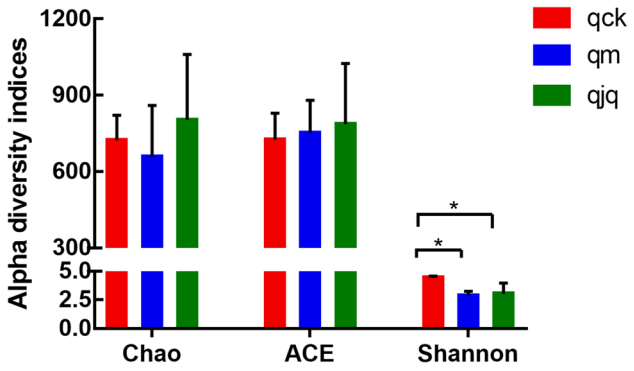


**Fig. 3** Alpha diversity analysis of the gut microbiota in different groups based on the Chao, ACE, and Shannon indices. qck, healthy group; qm, AAD group; qjq, QCD treatment group. Data are the mean ± SE, $n = 3$. *$p < 0.05$ vs. the healthy group

## Alpha diversity of the intestinal bacteria in AAD mice

Alpha diversity indices (Chao, ACE, Shannon and Simpson) reflect community richness and diversity. Commonly, Chao and ACE are related to community richness, while Shannon and Simpson are associated with the diversity of species. In this study, the highest community richness was observed in the QCD treatment group, but the differences among the three groups were not significant. On the other hand, significant differences in the Shannon index were found between the control group and the other two groups. QCD treatment increased the species diversity, which was reduced by antibiotics. However, the species diversity did not fully recover to normal levels (Fig. 3). These results were also confirmed by the rank abundance curve (Fig. 4).

## Beta diversity of the intestinal bacteria in AAD mice

Principal component analysis (PCA) was used to compare the structure of the intestinal bacteria among different groups. The variation in PC1 and PC2 represented 70.98% of the total difference among the samples in this study (Fig. 5). After weighted UniFrac analysis, the nonmetric multidimensional scaling (NMDS) results showed that the samples in

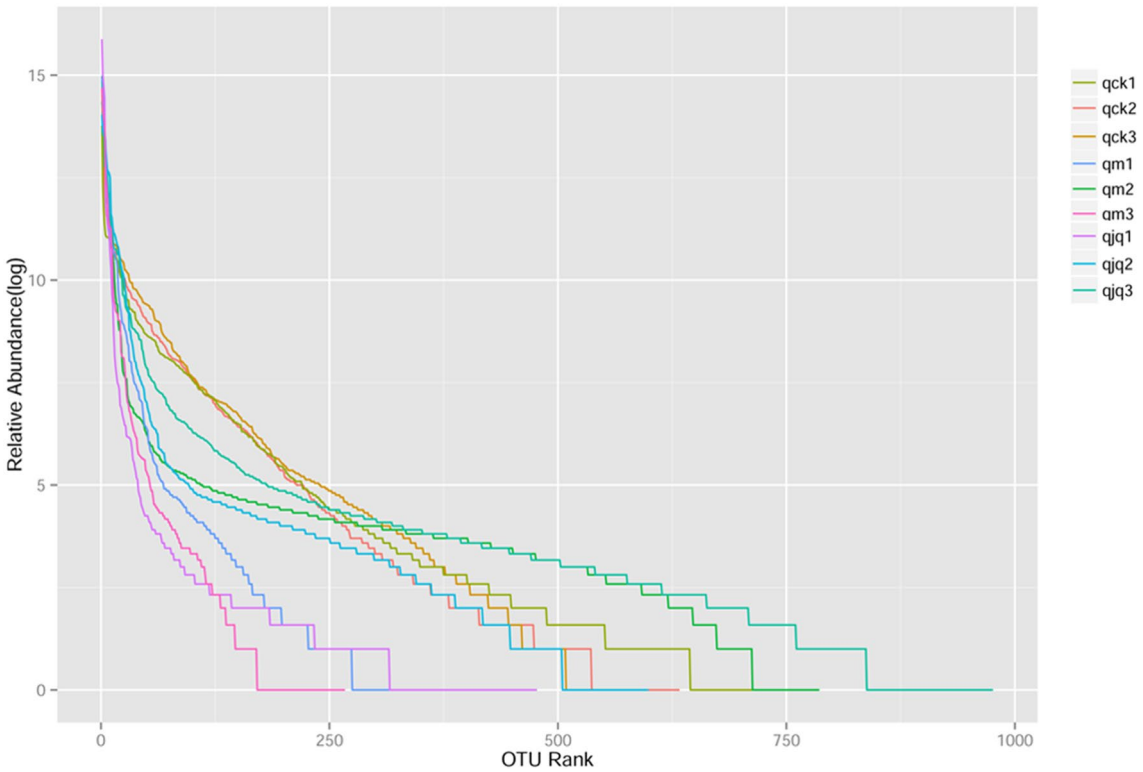


**Fig. 4** The OTU abundance of each sample in different groups. Each polyline represents one sample. The larger the curve span, the richer the species. The flatter the curve, the more homogeneous the species. qck 1–3, healthy group; qm1-3, AAD group; qjq1-3, QCD treatment group

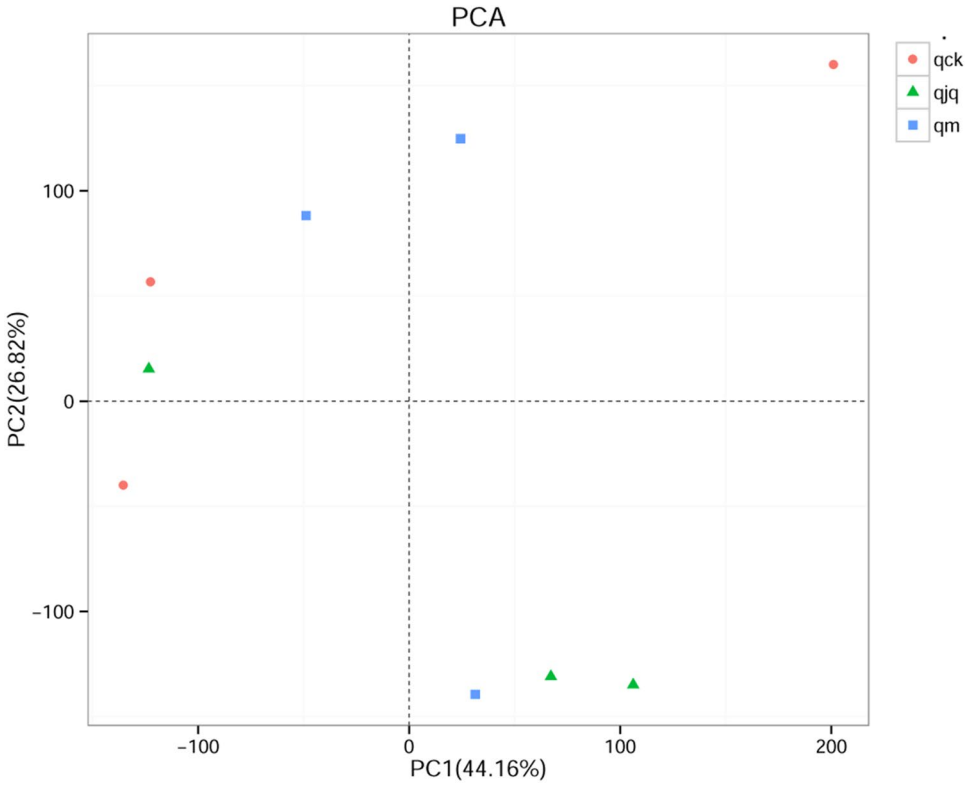


**Fig. 5** Bacterial similarity among different groups as represented by principal component analysis (PCA). Each point in the figure represents a sample. qck, healthy group; qm, AAD group; qjq, QCD treatment group

the healthy group and QCD treatment group were relatively concentrated. However, the distance between the healthy group and the other two groups was large (Fig. 6). This means that the microbial composition of the AAD group and QCD treatment group was dramatically different from that of the healthy group.

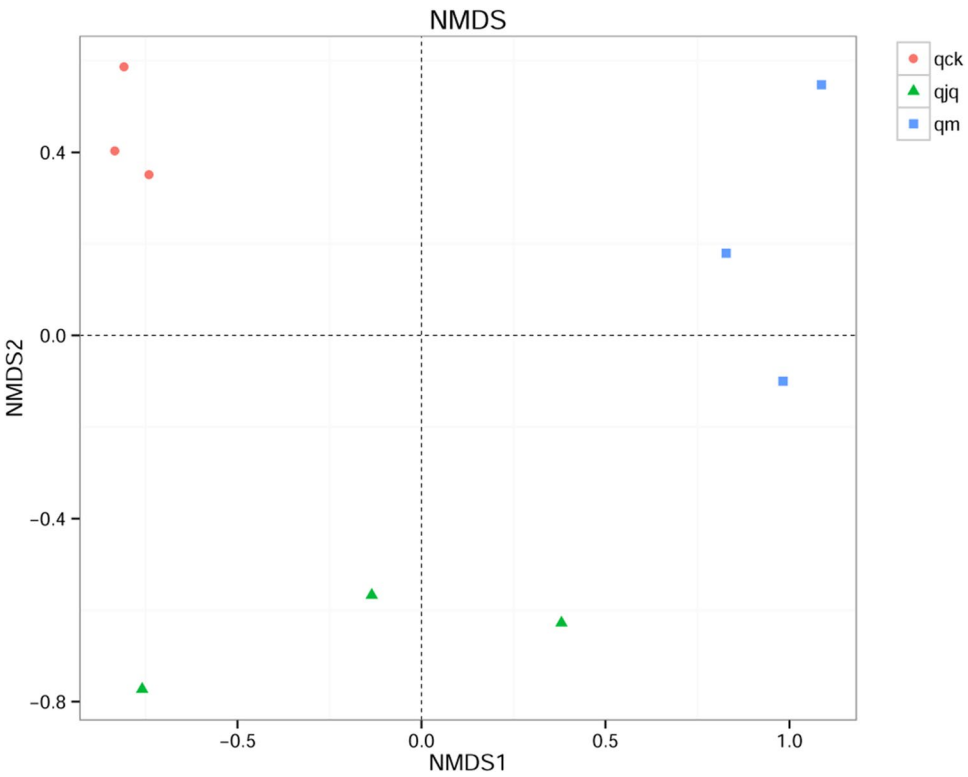


**Fig. 6** Bacterial similarity among different groups as represented by nonmetric multidimensional scaling (NMDS). The NMDS score plot is based on weighted UniFrac analysis. Each point in the figure represents a sample. qck, healthy group; qm, AAD group; qjq, QCD treatment group

## The composition of gut bacteria at different taxonomic levels

Figure 7 shows the number of taxa at different taxonomic levels. Compared with the healthy group and AAD group, the QCD treatment group possessed the largest number of species, but no significant differences were detected among the three groups at any taxonomic level.

Thirty-one phyla were identified in this study. Firmicutes and Bacteroidetes were the two predominant phyla in the three groups. The relative abundance of Firmicutes in the healthy group, AAD group, and QCD treatment group was 49.6%, 57.5%, and 59.1%, respectively. Bacteroidetes accounted for 44.9%, 37.0%, and 26.9% in the healthy group, AAD group and QCD treatment group, respectively (Fig. 8). Antibiotics use increased the relative abundance of Firmicutes while reducing the relative abundance of Bacteroidetes. Although QCD treatment enhanced the increasing and decreasing tendencies of Firmicutes and Bacteroidetes, respectively, the relative abundances of Firmicutes and Bacteroidetes among the three groups were not statistically significant ($p > 0.05$). Euryarchaeota was found in the healthy group and QCD treatment group but not in the AAD group. TM7 and Tenericutes were detected in only the healthy group. BRC1, OD1, and Synergistetes were found in only the AAD group, whereas Chlamydiae was found in only the QCD treatment group.

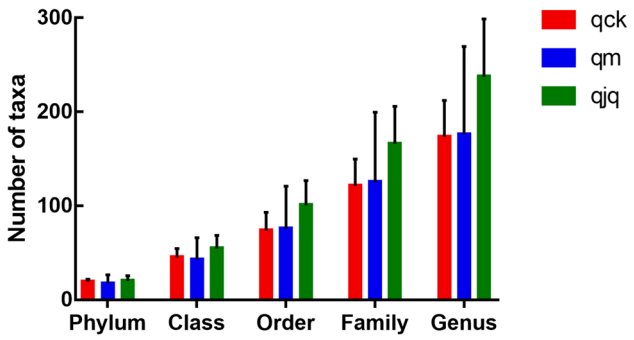


**Fig. 7** Number of taxa at different taxonomic levels in different groups. qck, healthy group; qm. AAD group; qjq, QCD treatment group. Data are the mean ± SE, $n = 3$

Obvious changes in the microbial composition at the genus level were observed. Figure 9 shows the abundance and evolutionary relationship of the bacterial species. The healthy group was predominantly composed of Bacteroidales S24-7 group_unidentified, Clostridiales_norank, and *Lactobacillus*, which accounted for 30.9%, 20.8%, and 8.6% of the total microbial population, respectively. In contrast, antibiotics use resulted in *Bacteroides* (22.3%), *Eubacterium* (12.9%), and *Parabacteroides* (9.5%) becoming the predominant genera. Moreover, Bacteroidales S24-7 group_unidentified, *Blautia*, and Erysipelotrichaceae_norank were the predominant genera in the QCD treatment group, accounting for 23.9%, 15.1%, and 13.1%, respectively. In summary, antibiotics and QCD treatment significantly altered the microbial composition.

Antibiotics use diminished the relative abundance of Bacteroidales S24-7 group_unidentified ($p = 0.01$), Clostridiales_ norank ($p = 0.003$), *Lactobacillus*, *Oscillospira* ($p = 0.014$), *Ruminococcus* ($p = 0.045$), Rikenellaceae_norank ($p = 0.003$), *Bifidobacterium*, and *Sutterella*,

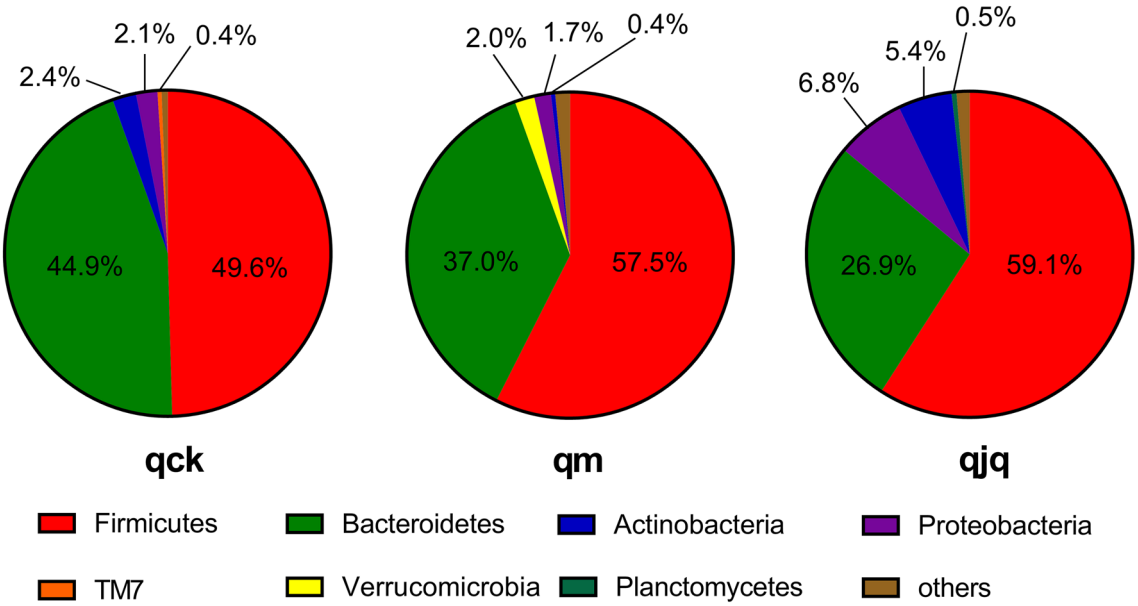


**Fig. 8** Histogram of microbiota community structures at the phylum level. qck, healthy group; qm, AAD group; qjq, QCD treatment group

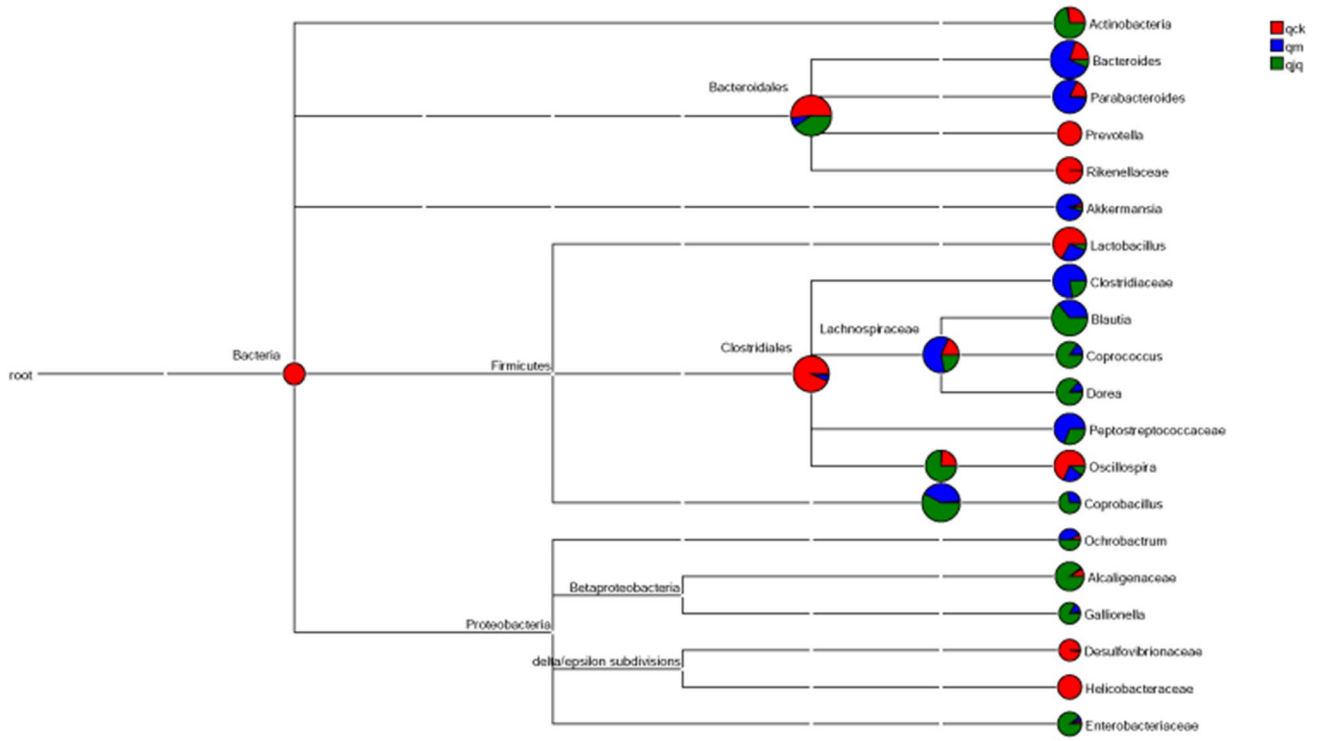


**Fig. 9** The abundances and evolutionary relationships of intestinal bacteria in different groups. qck, healthy group; qm, AAD group; qjq, QCD treatment group

while increasing the relative abundance of *Bacteroides* ($p = 0.00$), *Parabacteroides*, Erysipelotrichaceae_norank, *Eubacterium* ($p = 0.037$), *Blautia*, *Akkermansia*, and Peptostreptococcaceae_norank ($p = 0.031$) (Table 1). Notably, the relative abundances of Bacteroidales S24-7 group_unidentified and *Bacteroides* recovered to baseline after QCD treatment. *Blautia* ($p = 0.04$) and Erysipelotrichaceae_norank ($p = 0.004$) increased notably after QCD treatment.

## Discussion

It is well known that antibiotics alter the diversity and composition of gut microbiota, a condition known as dysbiosis, which allows for infection, diarrhea, and inflammation. Probiotics play a vital role in maintaining the balance of intestinal microecology. However, probiotics did not shift the overall diversity of the gut microbiota (Grazul et al. 2016). In fact, probiotics remodel the intestinal microecology of an individual recovering from antibiotic treatment by changing the composition of intestinal bacteria (Grazul et al. 2016). In accordance with Grazul et al., we found that although QWBZP extract and *D. hansenii* did cause an increase in microbial diversity when used alone or in combination, the diversity of gut microbiota was not restored to its original levels. This result indicated that QCD treatment helped to restore microbial diversity during the recovery period, but a long time was still required for the gut microbiota become rebalanced after antibiotics use.

Notably, antibiotics use and QCD treatment led to a drastic change in the gut microbiota composition. The *Bacteroides* population is commonly believed to be beneficial to the host's nutrition, mucosa, and immunity. On the other hand, *Bacteroides* can escape into the sterile peritoneum when colonic integrity is disrupted and act as opportunistic pathogens (Wick and Sears 2010). Enterotoxigenic *Bacteroides fragilis* (ETBF) is the only strain of *Bacteroides* associated with diarrhea (Wick and Sears 2010). In this study, antibiotics induced the growth of *Bacteroides*. In contrast, QCD treatment efficiently eliminated *Bacteroides* to the original level. This result indicated that the diarrhea induced by gentamicin sulfate and cefradine may be due to ETBF infection, and that QCD treatment can relieve ETBF infection.

Hernández et al. (2019) evaluated the association of the gut microbiota in patients with *Clostridioides difficile* (*C. difficile*) infection (CDI) and CDI risk factors. It was found that Peptostreptococcaceae was positively correlated with *Akkermansia*, which may predict the presence of CDI (Hernández et al. 2019). Yutin and Galperin (2013), suggested that *C. difficile* should be reclassified within Peptostreptococcaceae in the order Clostridiales. Hence, this

**Table1** Relative abundance of the intestinal bacteria in different groups at the genus level

| Genus | qck | qm | qjq |
|---|---|---|---|
| Bacteroidales S24-7 group_unidentified | 0.3090 ± 0.0355 | 0.0467 ± 0.0201** | 0.2389 ± 0.1441$^{\#}$ |
| Clostridiales_norank | 0.2080 ± 0.8933 | 0.0079 ± 0.0112** | 0.0037 ± 0.0034** |
| *Lactobacillus* | 0.0862 ± 0.0485 | 0.0335 ± 0.0533 | 0.0088 ± 0.0097 |
| *Bacteroides* | 0.0605 ± 0.0238 | 0.2228 ± 0.0275** | 0.0235 ± 0.0227$^{\#\#}$ |
| *Oscillospira* | 0.0527 ± 0.0235 | 0.0120 ± 0.0061* | 0.0064 ± 0.0060** |
| *Parabacteroides* | 0.0221 ± 0.0082 | 0.0953 ± 0.0942 | 0.0024 ± 0.0030 |
| *Ruminococcus* | 0.0142 ± 0.0083 | 0.0039 ± 0.0024* | 0.0000 ± 0.0000* |
| Rikenellaceae_norank | 0.0106 ± 0.0031 | 0.0013 ± 0.0020** | 0.0013 ± 0.0017** |
| Erysipelotrichaceae_norank | 0.0055 ± 0.0009 | 0.0431 ± 0.0238 | 0.1309 ± 0.0549**$^{\#}$ |
| *Eubacterium* | 0.0032 ± 0.0023 | 0.1293 ± 0.0349* | 0.1009 ± 0.0936 |
| *Blautia* | 0.0028 ± 0.0006 | 0.0828 ± 0.0512 | 0.1505 ± 0.1090* |
| *Akkermansia* | 0.0008 ± 0.0007 | 0.0187 ± 0.0203 | 0.0010 ± 0.0008 |
| *Bifidobacterium* | 0.0011 ± 0.0012 | 0.0007 ± 0.0007 | 0.0476 ± 0.0750 |
| *Sutterella* | 0.0032 ± 0.0010 | 0.0006 ± 0.0006 | 0.0345 ± 0.0304 |
| Peptostreptococcaceae_norank | 0.0000 ± 0.0000 | 0.0134 ± 0.0086* | 0.0045 ± 0.0053 |

Data are the mean ± SD, $n = 3$

qck, healthy group; qm, AAD group; qjq, QCD treatment group

Compared with the healthy group, $^{*}p < 0.05$, $^{**}p < 0.01$. Compared with the AAD group, $^{\#}p < 0.05$

phylogenetic relationship could explain why Peptostreptococcaceae is of prime importance in CDI. In our study, the relative abundance of Peptostreptococcaceae was increased by antibiotics, which indicated that the diarrhea induced by gentamicin sulfate and cefradine may also be due to *C. difficile* infection.

Sangster et al. (2016) also observed an increase in *Akkermansia* in CDI patients. Over the excessive growth of *Akkermansia* was also found in the gut of individuals who received special antibiotic therapy (Derrien et al. 2017). *Akkermansia* is a mucin-degrading bacterium that was isolated from human fecal samples. There has been a growing interest in *Akkermansia* because of its correlation to health and because of its potential as a biomarker for disease (Geerlings et al. 2018). Furthermore, *Akkermansia* was found to increase the expression of immune response genes and strengthen the mucosal barrier in mice (Everard et al. 2013; Derrien et al. 2011; Fujio-Vejar et al. 2017). This study revealed that the number of *Akkermansia* was increased by antibiotics. The immune responses triggered by *Akkermansia* may be regarded as a self-recovery mechanism and be responsible for this change. Furthermore, QCD treatment decreased the relative abundance of *Akkermansia.*

In this study, the relative abundance of *Ruminococcus*, *Oscillospira*, and *Blautia* also changed notably after QCD treatment. The dysbiosis of feedlot cattle with hemorrhagic diarrhea was characterized by increasing *Blautia* and decreasing *Ruminococcus* and *Oscillospira*, while dogs with acute hemorrhagic diarrhea were characterized by decreasing *Ruminococcus* and *Blautia* (Zeineldin et al. 2018; Suchodolski et al. 2012). Although the changes in *Ruminococcus*, *Oscillospira*, and *Blautia* in different animals and diarrhea were debatable, our study was in line with the observations of Zeineldin. However, QCD treatment enhanced the tendency of the change in the three genera.

Our previous study revealed that a low concentration of QWBZP extract promoted the proliferation of *D. hansenii* in vitro (Guo et al. 2013). A recent study confirmed that *yeast* promoted the growth and survival rate of other coexisting probiotics by increasing the biofilm formation capacity and hydrophobicity of probiotics, increasing lactic acid levels, reducing pH values, and decreasing the reducing sugars and free amino nitrogen levels of probiotics (Zoumpourtikoudi et al. 2018). In this study, QCD treatment accelerated the growth of *Bifidobacterium*. *Bifidobacterium* can regulate intestinal immune homeostasis by inhibiting the infection of pathogenic bacteria, improving the function of the mucosal barrier, suppressing proinflammatory cytokines, and altering the function of dendritic cells (Azad et al. 2018). Therefore, the synergistic effect of QWBZP extract and *D. hansenii* may be associated with increased *Bifidobacterium*. According to previous studies, QWBZP extract and *D. hansenii*, when used alone or in combination, promoted the proliferation of *Lactobacillus* in vitro. In contrast, QCD treatment reduced the relative abundance of *Lactobacillus* in vivo. QCD treatment showed different effects on *Lactobacillus* in vitro and in vivo.

Bacteroidales family S24-7 is highly localized to the intestinal tracts of homeothermic animals and is recognized to degrade fiber and polysaccharide (Ormerod et al. 2016; Garcia-Mazcorro et al. 2018). Considering the function of S24-7 family, it seemed that S24-7 family members

promoted the efficiency of carbohydrate metabolism in the QCD treatment group. In particular, S24-7 was found to be notably higher in mice that efficiently metabolized *Panax ginseng* (Dong et al. 2017). Interestingly, Bacteroidales S24-7 group_unidentified increased in the gut of mice with AAD, but did not recover to baseline after *D. hansenii* treatment (He et al. 2017). However, the abundance of Bacteroidales S24-7 group_unidentified returned to normal levels after treatment with QCD. The results implied that Bacteroidales S24-7 group_unidentified may be the key genus that responded to QWBZP extract. Moreover, Bacteroidales S24-7 group_unidentified may be regarded as evidence to suggest the synergistic effect between QWBZP extract and *D. hansenii*. In summary, *D. hansenii* increased the relative abundance of Bacteroidales S24-7 group_unidentified, which was drastically reduced by antibiotics. Moreover, increasing Bacteroidales S24-7 group_unidentified helped to metabolize *Panax ginseng*, which is an important ingredient of QWBZP, and promoted the efficacy of QWBZP.

## Conclusions

Taken together, QCD treatment dramatically changed the structure of gut microbiota, and these changes were benefit to cure AAD. *Bifidobacterium* and Bacteroidales S24-7 group_unidentified may be regarded as the key genera to show the synergistic effect between QWBZP extract and *D. hansenii*. The exact synergistic mechanism of QCD in treating AAD requires further researches.

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**Author contributions** ZT designed the study. Material preparation, data collection, and analysis were performed by GX, YW, TZ, and KS. The first draft of the manuscript was written by GX. The decision to submit the manuscript for publication was made by all the authors.

### Compliance with ethical standards

**Conflict of interest** The authors declare that there is no conflict of interests regarding the publication of this paper.

**Institutional animal care and use committee statement** The study was approved by the Animal Ethics and Welfare Committee of Hunan University of Chinese Medicine.

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