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WJGS mainly publishes articles reporting research results and findings obtained in the field of gastrointestinal surgery and covering a wide range of topics including biliary tract surgical procedures, biliopancreatic diversion, colectomy, esophagectomy, esophagostomy, pancreas transplantation, and pancreatectomy, *etc*.

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ORIGINAL ARTICLE

Case Control Study

Alteration of ascending colon mucosal microbiota in patients after cholecystectomy

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Abstract

BACKGROUND

Cholecystectomy is a successful treatment option for gallstones, although the incidence of colorectal cancer (CRC) has notably increased in post-cholecystectomy (PC) patients. However, it remains uncertain whether the altered mucosal microbiota in the ascending colon is related.

AIM

To investigate the potential correlation between gut microbiota and the surgical procedure of cholecystectomy.

METHODS

In total, 30 PC patients and 28 healthy controls underwent colonoscopies to collect mucosal biopsy samples. PC patients were divided based on their clinical features. Then, 16S-rRNA gene sequencing was used to analyze the amplicon, alpha diversity, beta diversity, and composition of the bacterial communities. Additionally, the Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt) database, sourced from the Kyoto Encyclopedia of Genes and Genomes, was used to predict the functional capabilities of the bacteria.

RESULTS
PC patients were comparable with healthy controls. However, PC patients older than 60 years had a distinct composition compared to those under 60 years old. Bacteroidetes richness was considerably higher at the phylum level in PC patients. *Bacteroides*, *Parabacteroides*, and *Bilophila* were more abundant in the PC group than in the control group. Furthermore, PC patients exhibited greater enrichment in metabolic pathways, specifically those related to lipopolysaccharide biosynthesis and vancomycin group antibiotic production, than controls.

CONCLUSION
This study indicated that the mucosal microbiota in PC patients was altered, perhaps offering new perspectives on the treatment possibilities for CRC and diarrhea following cholecystectomy.

Key Words: Post-cholecystectomy; Ascending colon; Mucosal Microbiota; Colorectal cancer; Diarrhea

Core Tip: Cholecystectomy is an effective treatment for gallstones, which may change the gut microbiota and relate to colorectal cancer (CRC). To investigate the potential correlation between gut microbiota and cholecystectomy, we performed *16S rRNA* sequencing in ascending colon mucosa in post-cholecystectomy patients and healthy controls. We found some altered species that may affect the bile acid metabolism and have a relationship with CRC. Therefore, intestinal flora altered after cholecystectomy, which may be a new target to attenuate related diseases after cholecystectomy.

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INTRODUCTION

Cholecystectomy, a gallbladder removal surgery, is considered the most effective therapy for symptomatic cholelithiasis and is appropriate for over 90% of patients[1]. Approximately 8000000 cholecystectomies are performed in the United States annually, with an increasing trend observed in China[2,3]. However, some studies have suggested a relationship between cholecystectomy and colorectal cancer (CRC). An analysis combining data from 10 cohort studies conducted in Europe, America, and China revealed that post-cholecystectomy (PC) patients are at an increased risk of CRC, particularly in the ascending colon[4]. Similar results were found when the subjects were stratified by age and sex[5]. Nevertheless, the precise mechanism connecting the spread of the gut microbiota with bile acid metabolism remains unknown.

The gut microbiota, with its vast quantity and intricate functions, is closely linked to human health[6]. Some species are thought to increase the incidence of CRC. Fusobacterium nucleatum, a bacterium prevalent in the human oral cavity, is abundant in CRC tissues. Bacteria can affect the growth of CRC cells and modify the tumor microenvironment by activating Wnt target genes and the NF-κB pathway. It also triggers pro-inflammatory cytokines and suppresses anticancer immune responses, as demonstrated in cell and animal studies[7].

Similarly, *Bacteroides fragilis* (*B. fragilis*) initiates a pro-carcinogenic multistep inflammatory process in colonic epithelial cells by secreting *B. fragilis* toxins, which activate IL-17R, NF-κB, and STAT3 signaling. This process may influence the development of polyp-adenoma-CRC[8]. Furthermore, when patients with PC are categorized based on colonoscopic mucosal pathology, individuals with precancerous lesions and/or CRC have the same species within the same genus as those with sporadic CRC[9].

Nonetheless, research has indicated that cholecystectomy usually results in a smaller bile acid pool and higher rates of enterohepatic bile acid recirculation[10]. Simultaneously, studies have shown that cholecystectomy typically reduces the size of the bile acid pool and increases the enterohepatic recirculation rates of bile acids. Consequently, the connection between the bile acid reservoir and gut bacteria increases, leading to elevated levels of secondary bile acids (particularly deoxycholic and lithocholic acids) in the feces of patients[11]. Notably, secondary bile acids are carcinogenic in CRC[12]. In addition, several studies have examined the gut microbiota of patients with PC and found that cholecystectomy can greatly alter the balance of intestinal bacteria[9,13-15], which may contribute to post-operative complications such as diarrhea[14], CRC[9], and metabolic-related diseases[16-19]. Therefore, cholecystectomy can disturb bile acid metabolism balance and cause gut microbiota disturbance, particularly in the right colon[4,9,20]. However, specific changes in flora are inconsistent and may be caused by different research populations, geographical factors, or observation times. In addition, most previous studies focused on assessing fecal samples, with few examining the association between bacteria in the ascending colon mucosa and cholecystectomy.

In this study, we collected ascending mucosal tissue from 30 PC patients and 28 healthy controls. We performed bacterial *16S rRNA* gene amplicon sequencing to investigate the relationship between gut microbiota and cholecystectomy in PC patients. Additionally, we assessed the impact of age, sex, and duration on the intestinal microbiota in these patients. Furthermore, we utilized molecular bioinformatics to predict metabolic pathways related to cholecystectomy, potentially offering new perspectives on treatment options for CRC and PC diarrhea.

MATERIALS AND METHODS

Study design and sample collection

The study included 58 patients who received colonoscopy at the Digestive Endoscopy Center at Jiading Branch of Shanghai General Hospital in Shanghai, China. Thirty patients undergoing laparoscopic cholecystectomy for gallstones were allocated to the test group and were subjected to subgroup analysis based on age, diarrhea, and duration. Twenty-eight healthy controls (HC, control group) were chosen based on a similar age range and sex ratio as the test group following a normal physical examination. The selection criteria of the test group were as follows: Undergoing cholecystectomy for gallstones, aging between 30 and 75 years old, having no enrollment in other research projects and having signed informed consent. We confirm that patients who had used antibiotics or probiotics in the past 2 months or had a personal history of colon cancer, inflammatory bowel disease, or CRC were not included in the study. Besides, all the patients without diabetes, body mass index ≥30 kg/m^2 or other systemic chronic diseases. This research was conducted with the agreement of the research ethics boards of Shanghai General Hospital under the reference number [No. (2023)252].

We followed the split-dose regiment of bowel preparation as Horton *et al*[21]. Samples from the ascending colon were obtained utilizing biopsy instruments in both the test and control groups during colonoscopy and were promptly frozen and stored at -80 °C following sampling.

DNA extraction, PCR amplification, and sequencing

The ascending colon samples were immersed in 790 μL of sterile lysis buffer, consisting of 4.0 M guanidine thiocyanate, 10% N-lauroyl sarcosine, and 5% N-lauroyl sarcosine combined with 0.1 M phosphate buffer adjusted to pH 8.0. This mixture was placed in a 2 mL screw-cap test tube along with 1g of glass beads (0.1 mm, sourced from BioSpec Products, Inc., United States). The combination was vigorously agitated and heated to 70 °C for an hour, with intermittent high-speed bead beating for 10 minutes to ensure efficient lysis. Subsequently, genomic DNA from the microbial biopsies of the ascending colon was extracted using the E.Z.N.A.® Stool DNA Kit from Omega Bio-tek, Inc., GA. The extracted DNA was then stored at -20 °C for future analysis. This DNA served as a template for amplifying the V3-V4 region of the *16S rRNA* gene. The amplification process employed primers F1 and R2 (5′-CCTACGGGNGGCWGCAG-3′ and 5′-GACTACHVGGGTATCTAATCC-3′), targeting the specified region in each sample *via* PCR. These primers align with positions 341 to 805 in the Escherichia coli *16S rRNA* gene sequence. PCR reactions were then carried out using a Thermal Cycler (Analytik Jena Corp., AG), adhering to a precise program: an initial denaturation step at 95 °C for 3 minutes, followed by 21 cycles of denaturation at 94 °C for 30 seconds, annealing at 58 °C for 30 seconds, and extension at 72 °C for 30 seconds. This was concluded with a final extension period at 72 °C for 5 minutes. Shanghai Mobio Biometric Co. Ltd. then combined equal amounts of samples from various sources for sequencing on the Miseq platform by Illumina Inc., United States, strictly adhering to the manufacturer's guidelines.

Data availability

The 16S sequence data generated in this study were now available in the Sequence Read Archive database under accession number PRJNA938946.

Bioinformatics and statistical analysis

Using the USEARCH capability (version 11.0.667), the clean data were extracted from the raw data, and the corresponding models were as follows: (1) Setting each instance is canceled using each file with zero confusion; (2) Sequences that overlapped by fewer than sixteen basis points were excluded; (3) Error rates greater than 0.1 overlaps were eliminated; and (4) Sequences with fewer than 400 bp were eliminated after connecting. To identify representative sequences using UPARSE, in line with the UPARSE operational taxonomic unit (OTU) analysis pipeline, the mass filter sequences were segmented into unique sequences and organized in descending order of abundance. At this point, individual sequences were disregarded. The OTU was annotated using the SILVA reference database (SSU138) and classified based on 97% similarity, following the removal of chimeric sequences using UPARSE (version 7.1). Mothur v1.42.1 was employed to assess alpha diversity metrics, including the abundance-based coverage estimator (ACE), Chao 1 estimator, Shannon-Wiener diversity index, and Simpson diversity index. Significant differences between the two groups were examined using the non-parametric Mann-Whitney *U* test. We compared multiple groups using a non-parametric Kruskal-Wallis test, and we computed the difference between the weighted and unweighted UniFrac, as well as the Bray-Curtis, in QIIME. Principal coordinate analysis (PCoA) plots and PERMANOVA were provided by R (version 3.6.0) within the vegan 2.5-7.0 package, facilitating the examination of statistical significance across groups through 10,000 permutations. To differentiate between species with fluctuating numbers in groups, the linear discriminatory analysis (LDA) effect (LEfSe) was also utilized (lefse 1.1, https://github.com/SegataLab/Lefse). Additionally, Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt2) v2.4.1 (https://github.com/picrust/

picrust2/wiki) was utilized to predict beneficial overflow based on *16S rRNA* quality successions.

RESULTS

Clinical characteristics summary

In this study, 30 PC patients and 28 healthy controls were included. In addition, subgroup analysis was carried out in PC patients according to age (YMA: age ≤ 60, n = 17; SNR: age > 60, n = 13), diarrhea (DG: diarrhea, n = 14; NG: no diarrhea, n = 16) and time after operation (Lon: ≥ 5 years, n = 16; Sht: < 5 years, n = 14). Clinical characteristics of patients (test group, n = 28) and healthy controls (control group, n = 28) were shown in Table 1.

Table 1 Clinical characteristics summary

Variable	Test group (n = 28)	Control group (n = 28)	P value
Age (mean ± SD)	56.39 ± 10.01	53.43 ± 10.41	0.282
Gender, n (%)			0.591
Female	14 (50.00)	17 (60.71)	
Male	14 (50.00)	11 (39.29)	
BMI (mean ± SD)	23.79 ± 1.39	23.74 ± 1.16	0.888
History of smoking, yes, n (%)	9 (32.14)	3 (10.71)	0.101
History of diarrhea, yes, n (%)	14 (50.00)	4 (14.26)	0.009
Years after cholecystectomy (mean ± SD)	7.08 ± 6.67	-	-

BMI: Body mass index.

Comparison of diversity and richness in the test and control groups

We generated 747 OTUs with a similarity cutoff of 97%. The Venn diagram illustrated that 569 out of the 747 OTUs were common to both groups, whereas 111 OTUs were exclusive to the test group, and 67 were distinct to the control group (Figure 1A). The mean observed OTUs in the single sample from the test group and control group were 201 and 212 respectively, showing that the control group had a tendency for higher OTUs, but without significant differences (Figure 1B). The alpha diversity of the mucosal microflora in the ascending colon of the test group was comparable to that of the control group. There were no notable distinctions between the two groups based on the ACE, Chao, Shannon, and Simpson indices (Figure 1C-F).

To assess the similarities of all the samples, ecological distances were visualized using the PCoA plot, which was calculated based on Weighted UniFrac distances. PCoA, using the relative abundance of OTUs, revealed that the sample points of the test group and the control group were intermingled, suggesting an overall similarity between the sample sites of the two groups. Adonis' study revealed no statistically significant differences between the two groups (P > 0.05, Figure 2A). In addition, a nonmetric multidimensional scaling analysis using Weighted UniFrac distances indicated that the bacterial microbiota architectures of both groups were comparable (Figure 2B).

Differences of composition in the test and control groups

Twenty phyla were identified by categorizing the species of all OTUs in the ascending colon mucosa. The gut microbiota of both study groups was predominantly composed of Firmicutes, followed by Bacteroidetes and Proteobacteria at the phylum level. In the test group, the dominating flora proportions were 33.06%, 26.96%, and 25.71%, while those of the control group were 36.03%, 18.56%, and 30.80%, respectively (Figure 3A). At the genus level, the gut microbiota of the control group was primarily dominated by Bacteroides, with subsequent contributions from *Acinetobacter*, *Dietzia*, *Escherichia-Shigella*, and the *Ruminococcus* torques group, accounting for 11.56%, 10.58%, 8.90%, 7.50%, and 6.18% of the total microbiota, respectively. Similarly, in the test group, Bacteroides remained the most abundant genus, followed by *Acinetobacter*, *Escherichia-Shigella*, *Dietzia*, and *Faecalibacterium*, with proportions of 19.08%, 8.11%, and 7.42%, 5.79% and 5.00%, respectively (Figure 3B).

The Wilcoxon rank-sum test revealed substantial variations in the microbial composition between the test and control groups. At the phylum level, a remarkable increase in the abundance of Bacteroidetes was observed in the test group compared to the control group. When examining the genus level, *Bacteroides*, *Parabacteroides*, *Lachnoclostridium*, and *Tyzzerella* were found to be significantly enriched in the test group relative to the control group. Conversely, the abundances of *Enterobacteriaceae_unclassified*, *Erysipelotrichaceae UCG-003*, *Elizabethkingia*, *Clostridia UCG-014*, *Cloacibacterium* and *Howardella* were notably reduced in the test group compared to the control group (Figure 4A and B).

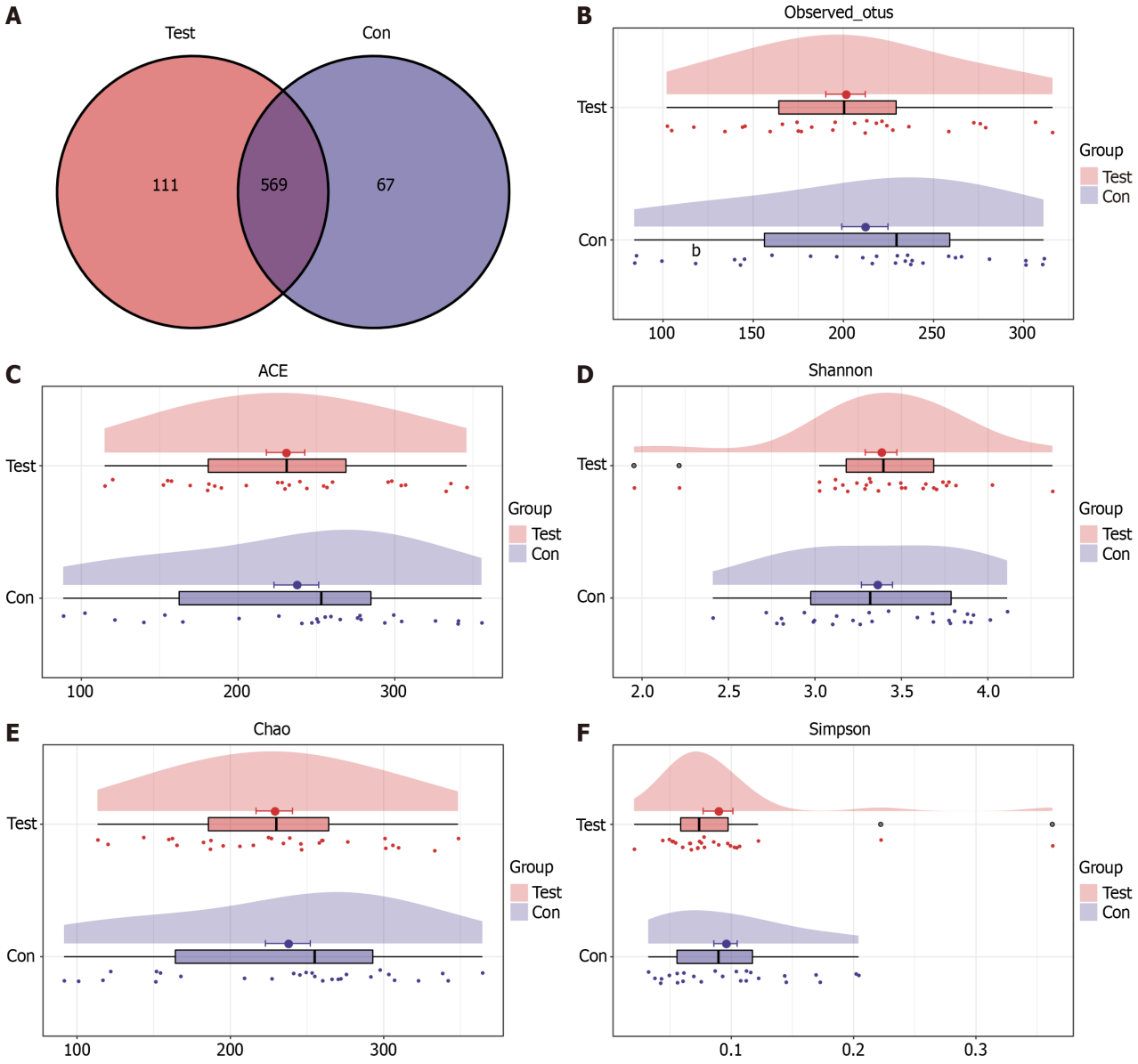


Figure 1 Clustering of operational taxonomic units and alpha diversity study of mucosal intestinal microflora in the test group and the control group. A: A Venn image showing the common and unique operational taxonomic units (OTUs) between the test and control groups; B: The OTUs that were found in a single sample from each group; C: Abundance-based coverage estimator of the test group and the control group; D: Chao index of the test group and the control group; E: Shannon-Wiener diversity index of the test group and the control group; F: Simpson diversity index of the test group and the control group.

A LEfSe analysis revealed considerably greater abundances of numerous taxa, including *Clostridia UCG-014*, *Enterobacteriaceae unclassified*, *Erysipelotrichaceae UCG-003*, *Cutibacterium*, *Elizabethkingia*, and *Adlercreutzia*, were significantly higher in the control group than in the test group. In contrast, the levels of *Bacteroides*, *Parabacteroides*, *Lachnoclostridium*, *Tyzzerell*, and *Bilophila* were elevated in the test group (Figure 4C).

As shown by the heatmap, a total of 14 OTUs were distinct amongst the sample groups, including *Clostridia_UCG-014*, *Tyzzerella*, *Oscillibacter*, *Cutibacterium*, *Bilophila*, *Parabacteroides*, *Bifidobacterium*, *Erysipelotrichaceae UCG-003*, *Achromobacter*, *Bacteroides*, *Dietzia*, *Butyricicoccaceae*, *Peptostreptococcaceae*, and *Enterobacteriaceae unclassified* (Figure 4D).

Subgroup analysis of PC patients

To further determine the effect of age, diarrhea, and duration on gut microbiota, we subdivide the PC patients into three pairs of groups. According to ACE, Chao, Shannon, and Simpson indices, the alpha diversity of intestinal microflora showed no significant variations across the three pairings of groups. Comparing the composition of the flora of different subgroups, a significant difference was observed in the composition of intestinal mucosa flora between patients over and under 60 years of age (P = 0.0314). However, no significant differences were found in the flora composition between patients with and without diarrhea, or between patients more than 5 years and less than 5 years post-surgery (P > 0.05, Figure 5).

The Wilcoxon rank-sum test was performed to identify specific bacteria in subgroups. No significant differences in phylum were detected among the three pairs of groups. However, at the genus level, some bacteria differed between the

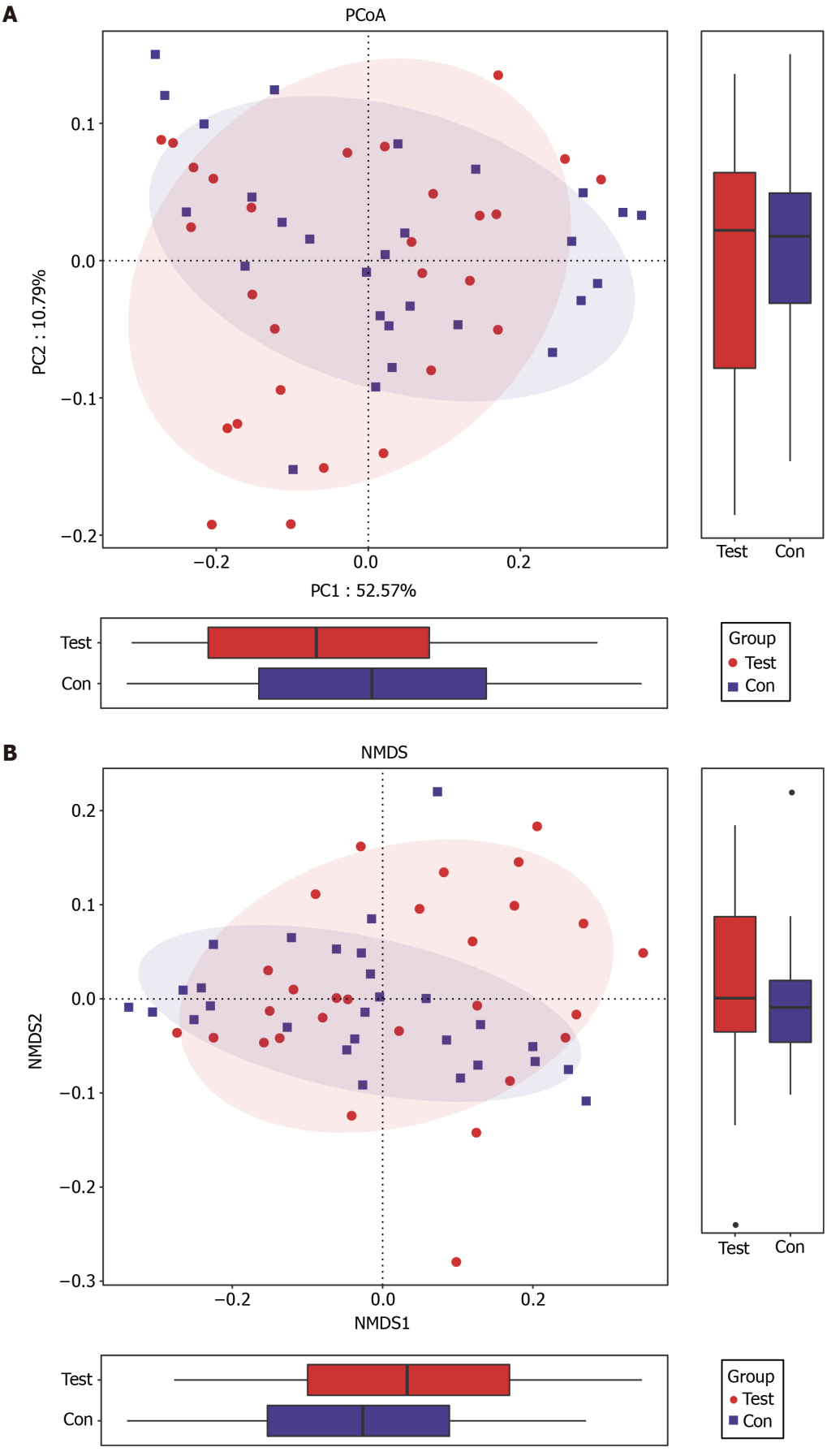


Figure 2 Examination of beta diversity using operational taxonomic units levels within the test and control groups. A: Analysis of principal coordinates analysis plots using Weighted UniFrac distances; B: Analysis of nonmetric multidimensional scaling using Weighted UniFrac distances. One sample is

represented by each symbol. PCoA: Principal coordinate analysis.

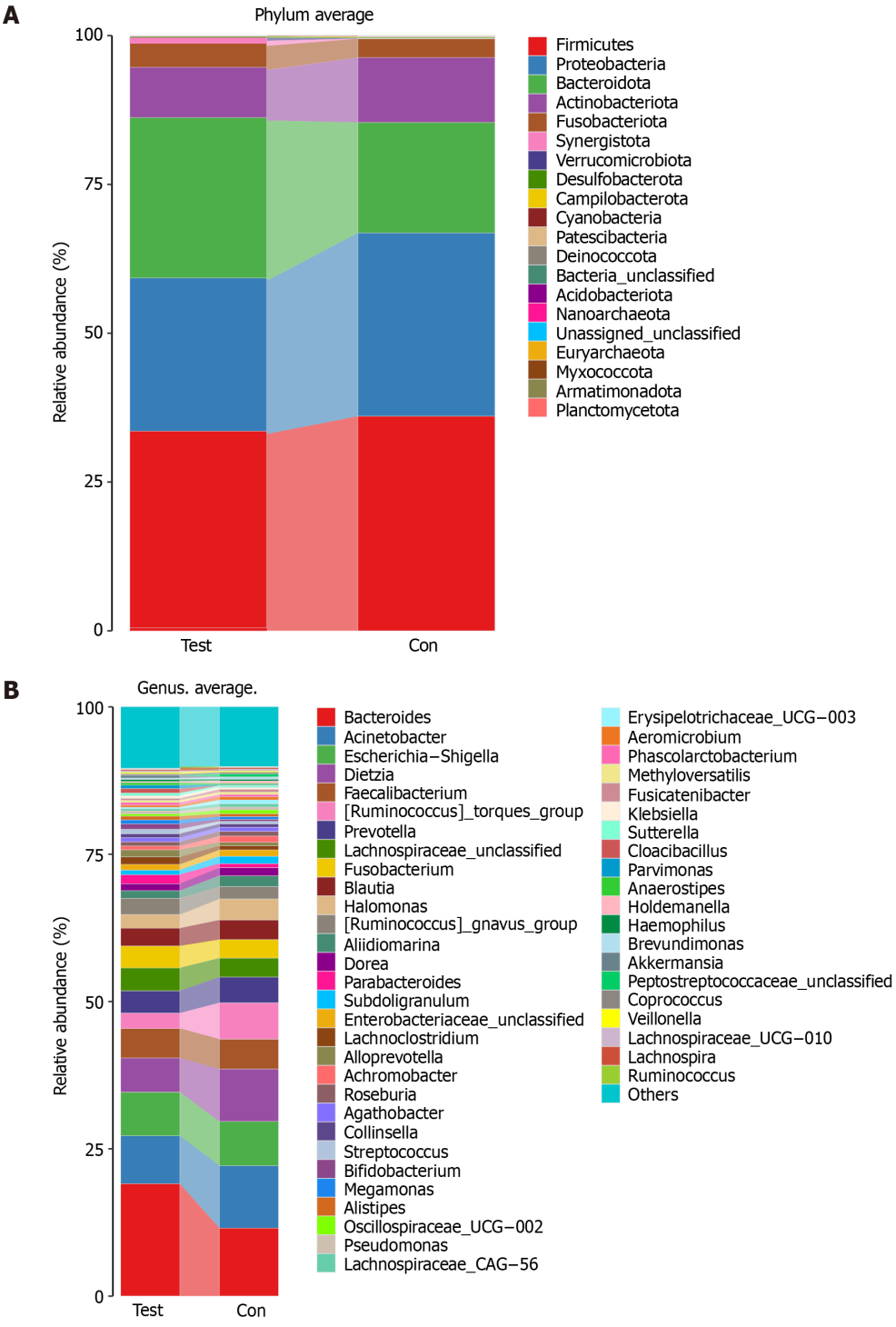


Figure 3 Microbiome floral composition in the test and control groups. A: The composition of the flora at the phylum level; B: The composition of the flora at the genus level.

groups. In the YMA and SNR groups, *Subdoligranulum*, *Cloacibacillus*, *Megamonas*, *Ruminococcus*, *Holdemanella*, *Christensenellaceae* R-7 group, and *Eubacterium siraeum* group were significantly higher in the YMA group than those in the SNR group. In the DG and NG groups, the abundance of *Corynebacterium* was substantially greater in the NG group than in the DG group. Conversely, the DG group had significantly larger abundances of *Ruminococcus*, *Christensenellaceae R-7* group and *Tyzzerella*. *Christensenellaceae R-7* group, *Tyzzerella* and *Eubacterium siraeum* group were more abundant in the Sht group compared to the Lon group (Figure 6).

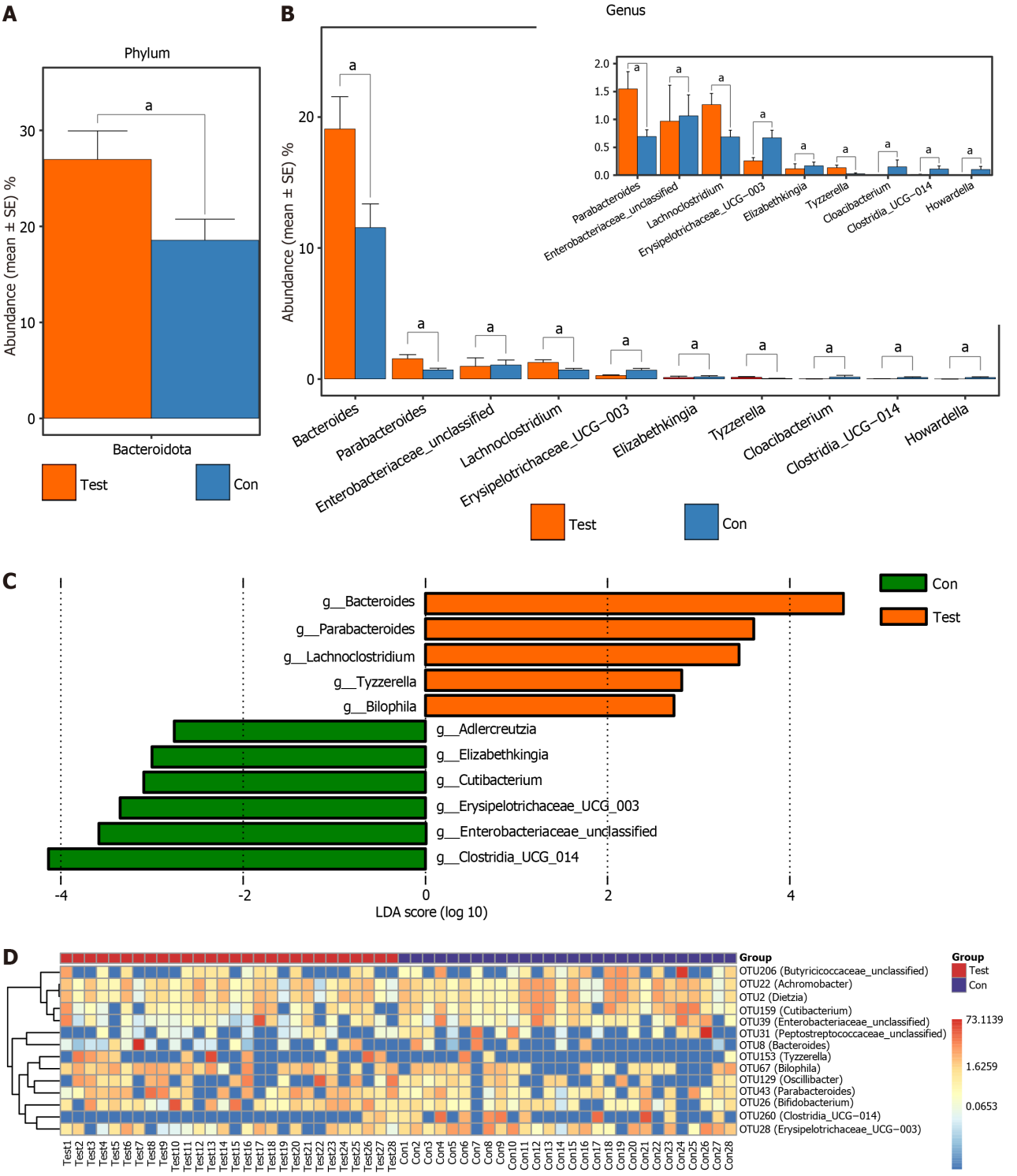
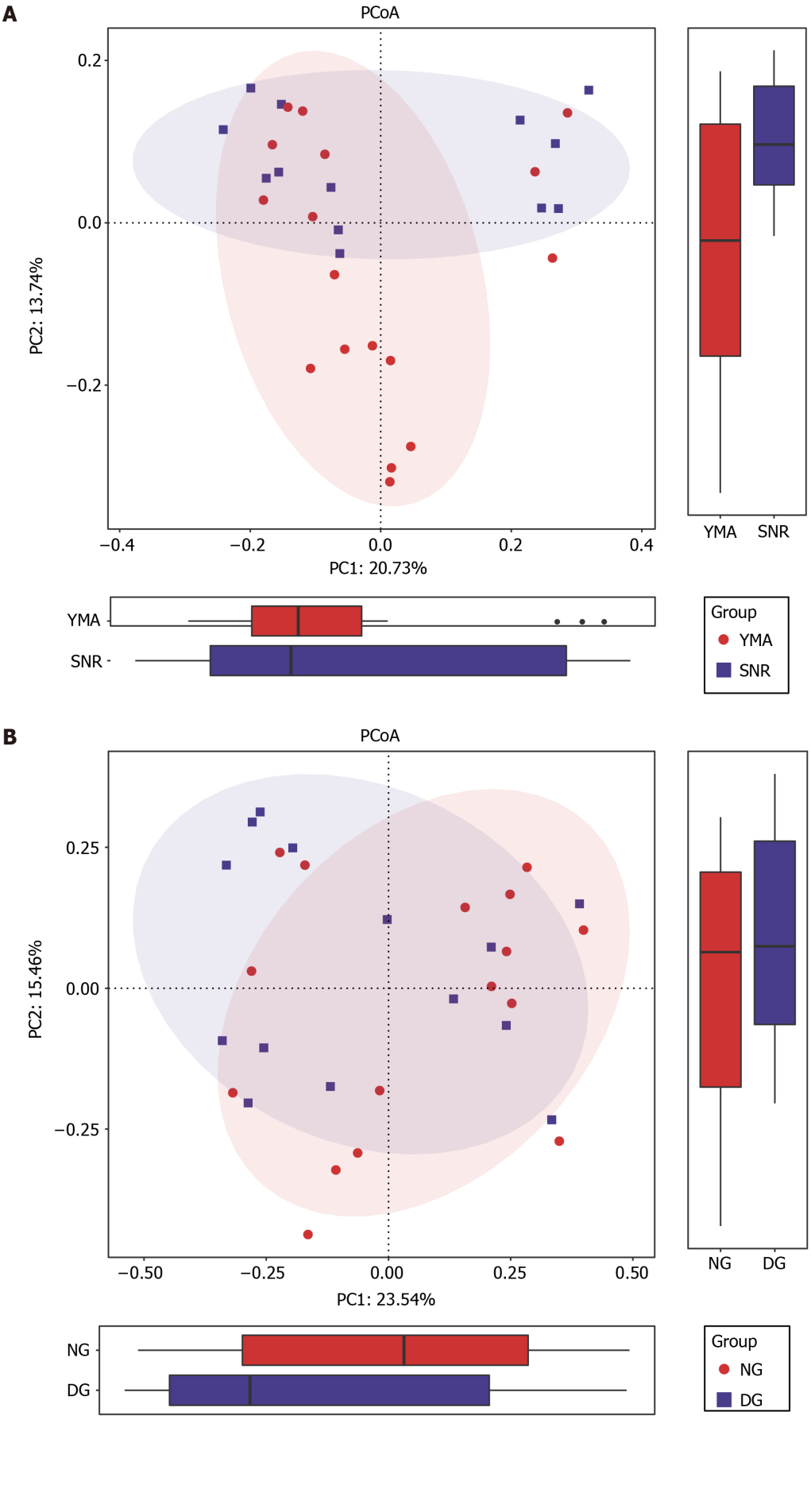


Figure 4 Differences in microbiome composition between the test and control groups. A: The test group showed a significantly higher abundance of *Bacteroidetes* at the phylum level, determined by a Wilcoxon rank-sum test; B: Differences in the microbiota at the genus level between the groups, determined by a Wilcoxon rank-sum test; C: Microbiota variances at the genus level, assessed by linear discriminatory analysis effect size; D: Analysis of microbiome heatmap through a random forest model. LDA: Linear discriminatory analysis.

Functional alterations of gut microbiomes in the test and control groups

The 16S sequencing data were analyzed using the Kyoto Encyclopedia of Genes and Genomes pathway database for functional prediction. Subsequently, substantial changes in metabolic pathways (L3 level) between the two groups were identified by LEfSe analysis. The selected metabolic pathways in the figure had significant *P* value and LDA ≥ 3.0. The results demonstrated that the test group showed considerably increased levels of glycan degradation, Biosynthesis of vancomycin group antibiotics, Glycosaminoglycan degradation, lipopolysaccharide (LPS) biosynthesis, Selenocompound metabolism, Protein digestion and absorption compared to the control group. However, Pyruvate metabolism, Dioxin degradation, Sulfur relay system, and Xylene degradation were all notably higher in the control group (Figure 7).


A
PCoA
0.2
0.0
−0.2
PC2: 13.74%
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−0.2
0.0
0.2
0.4
PC1: 20.73%
YMA
SNR
YMA
SNR
Group
YMA
SNR
B
PCoA
0.25
0.00
−0.25
PC2: 15.46%
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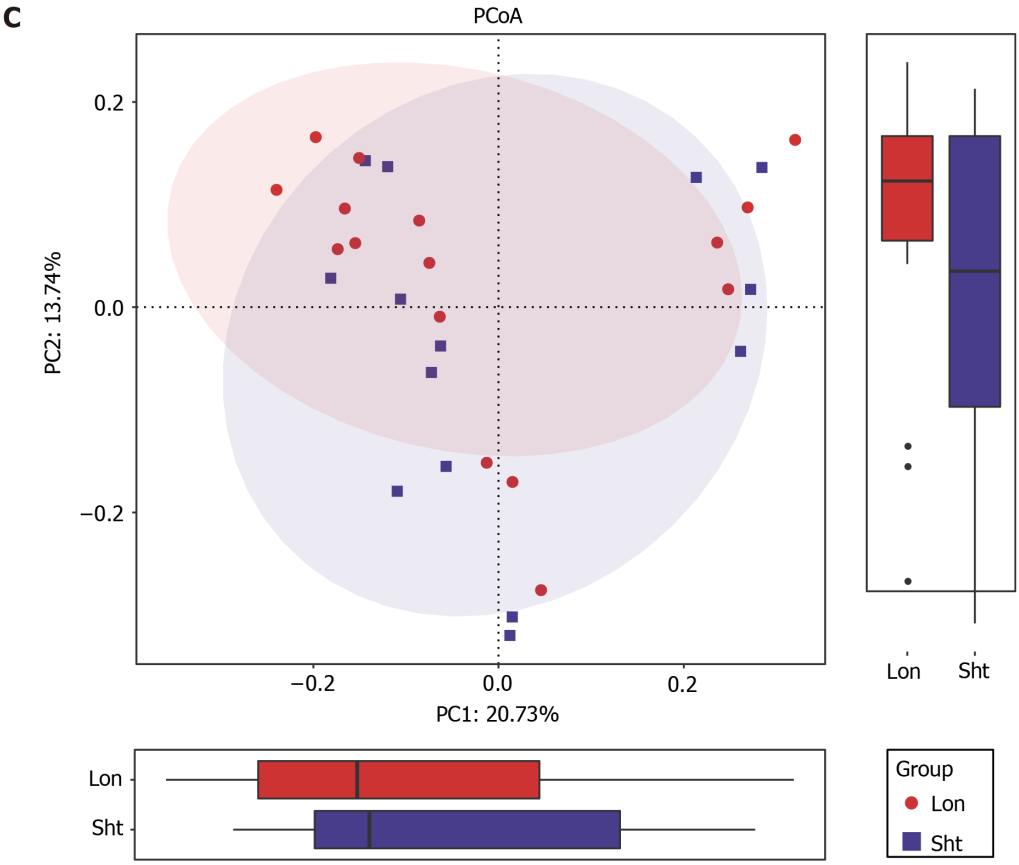

Figure 5 Examining beta diversity applying operational taxonomic units levels in subgroups. A: Principal coordinate analysis (PCoA) plots based on Unweighted UniFrac distances of patients over 60 years and patients under 60 years groups; B: PCoA plots based on Bray-Curtis distances of patients had diarrhea and patients without diarrhea groups; C: PCoA plots based on Unweighted Unifrac distances of post-operative patients over 5 years and post-operative patients less than 5 years groups. YMA: Patients over 60 years; SNR: Patients under 60 years; DG: Patients had diarrhea; NG: Patients without diarrhea; Lon: Post-operative patients over 5 years; Sht: Post-operative patients less than 5 years; PCoA: Principal coordinate analysis.

DISCUSSION

A cholecystectomy is a common surgical procedure. The risk of colon cancer increases with the duration of cholecystectomy[9,22], which is associated with altered gut microbiota and disturbed bile acid metabolism[12]. However, most studies have focused on detection in stool samples. However, fecal samples may contain transient organisms that may change because of bowel preparation; therefore, the samples may not reflect the mucosa-associated microbiota. In contrast, the bacterial community in the colonic mucosa is relatively stable and may accurately reflect the composition of the intestinal bacteria[23]. Adherent bacteria may be more prone to affect gene expression in colon mucosal cells than transient bacteria expelled from feces[24]. In addition, because all patients prepare their intestines in the same manner, the influence of intestinal preparation on the intestinal mucosal flora is basically equal. However, the specific changes in the flora are inconsistent and their impact on human health remains unclear.

Deoxycholic acid and some pathogenic microorganisms are believed to have tumorigenic properties[12,25]. Research has indicated higher levels of secondary bile acids in the right colon[26], and a higher incidence of right-sided CRC following cholecystectomy has been found[4,9,20]. In this study, there were no differences in the diversity or overall composition of the flora between the two groups. *Bacteroidetes*, *Parabacteroides*, and *Bilophila* were significantly higher in the test group. In addition, the metabolic pathways involved in LPS biosynthesis and biosynthesis of vancomycin antibiotics were enriched in the test group compared to the control group.

Our study found that the number of OTUs was lower in the test group than in the control group; however, the difference was not significant. The alpha diversity was not significantly different between the two groups. Beta diversity, assessed using several indices, revealed that the general flora composition of the two groups was comparable, which is consistent with data from Korea and Russia[15,27]. However, alternative studies have yielded contradictory results. Li *et al*[14] found reduced beta diversity in the PC group, and microbiota abundance differed between the PC and HC groups.

In contrast, Ren *et al*[9] and Wang *et al*[22] concluded that cholecystectomy altered the microbiota of patients because they showed significant differences in flora composition between groups by the Adonis test. A study that included 580 pairs of samples discovered that cholecystectomy decreased microbial richness and altered flora composition in the PC group[28]. However, these investigations used fecal samples, whereas we obtained mucosal materials from the ascending colon, perhaps resulting in divergent findings.

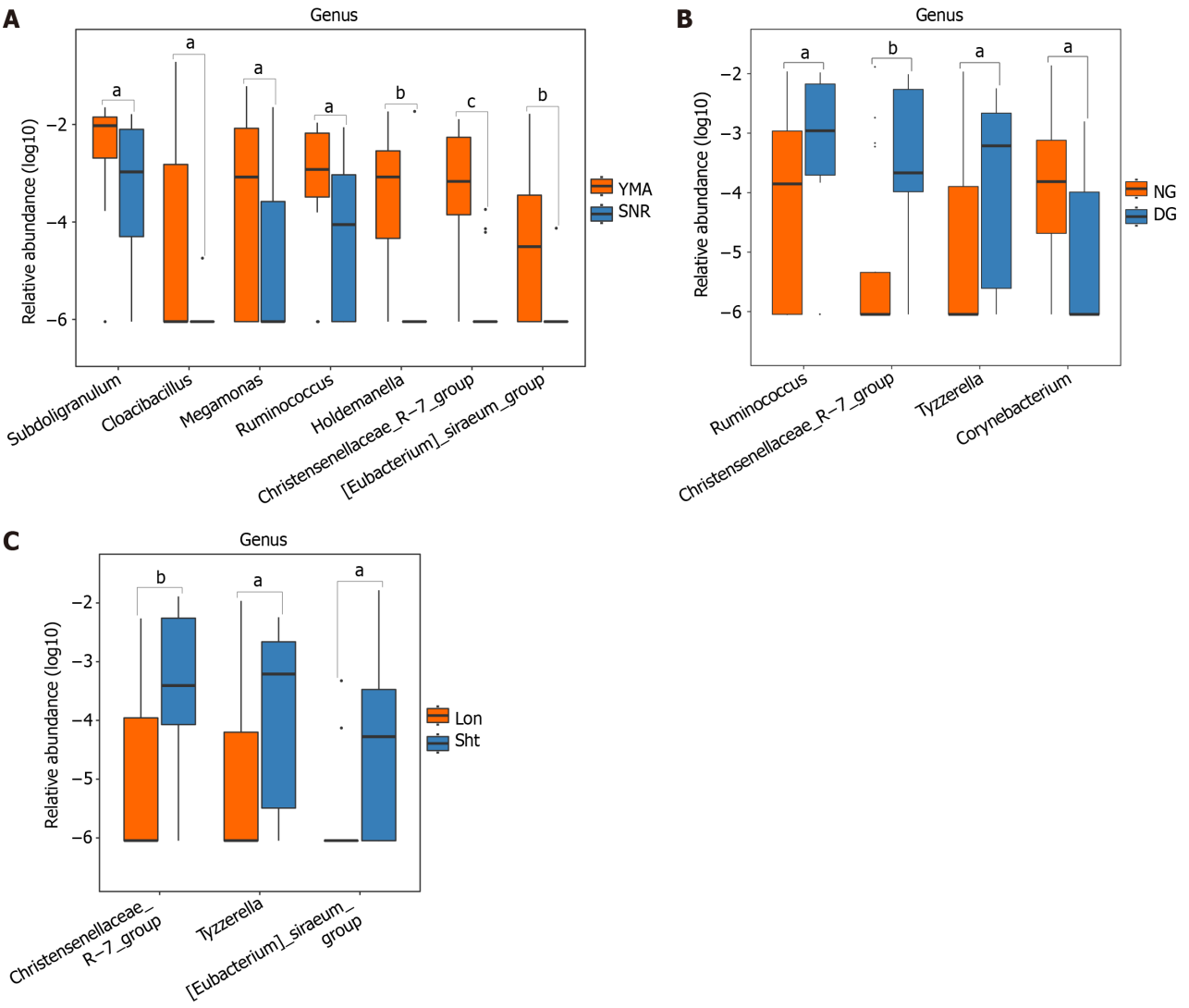

Figure 6 Microbiome composition variations in microflora among subgroups were evaluated at the genus level using the Wilcoxon rank-sum test. A: Variations in genus-level microflora between the patients over 60 years and patients under 60 years populations; B: Variations in genus-level microflora between the patients had diarrhea (DG) and patients without diarrhea (NG) groups; C: Variations in genus-level microflora between the DG and NG groups. YMA: Patients over 60 years; SNR: Patients under 60 years; DG: Patient had diarrhea; NG: Patients without diarrhea; Lon: Post-operative patients over 5 years; Sht: Post-operative patients less than 5 years.

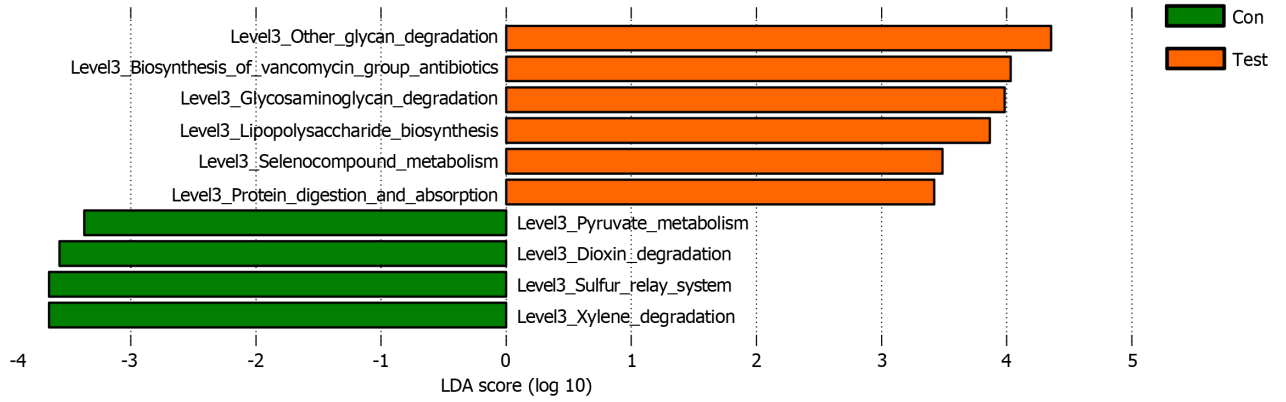

Figure 7 Changes in metabolic pathways between the test and control groups, as illustrated by the histogram of linear discriminatory analysis scores. LDA: Linear discriminatory analysis.

Since the occurrence of CRC is age-related, Kim *et al*[29] observed that individuals over the age of 60 years are at a higher risk of developing gastrointestinal cancer after undergoing cholecystectomy due to the age-related nature of CRC. Therefore, we separated the PC group into two age groups: ≤ 60 years and > 60 years. We observed a substantial difference in flora composition, indicating that a more noticeable shift in bacterial composition occurred as time after cholecystectomy increased. To investigate the relationship between postoperative diarrhea and intestinal flora, we further divided the test group into the diarrhea group (DG) and the no-DG (NG). Similar flora richness and structure were observed in both groups. However, Xu *et al*[30] found that the abundance and homogeneity of the intestinal flora were significantly lower in the DG group than in the NG group, as well as a significant difference in the composition of the intestinal flora, which could be linked to increased levels of secondary bile acids following cholecystectomy. The accumulation of secondary bile acids in the colon stimulates colonic 5-HT levels and increases colonic motility, leading to diarrhea[31]. Li *et al*[14] and Kang *et al*[32] reported similar results. This differs from our findings, and factors like different sampling sites and observation times may explain these differences.

In our study, the control group was dominated by Firmicutes, followed by Proteobacteria and Bacteroidetes; these results are consistent with those of other studies[33]. To further determine the different flora, we performed the Mann-Whitney *U* test, LEfSe analysis, and random forest model. We found that the PC group had significantly higher *Bacteroidetes*, *Bacteroides*, *Parabacteroides*, and *Bilophila*.

Within the test group, the *Bacteroidetes* phylum had a notably greater percentage, consistent with a previous study's findings[13]. Members of Bacteroidetes are believed to play a role in immunological and metabolic processes[34] and are promoters of CRC[14]. At the genus level, we found an accumulation of *Bacteroides* and *Parabacteroides* in the PC group, which agrees with Ren *et al*[9]. Some species of *Bacteroides*, such as *B. fragilis* and *B. vulgatus*, detoxify taurine-conjugated bile acids using bile salt hydrolases to detoxify the bile acids. However, free taurine can be metabolized to hydrogen sulfide (H2S), which enhances the turnover of colonocytes and may be linked to the development of CRC[35]. In particular, *B. fragilis* was found to be more abundant in the mucosa of late-stage CRC than in the adjacent healthy tissue [36]. Moreover, enterotoxigenic *B. fragilis* (ETBF) produces *B. fragilis* toxin (BFT), which triggers the breakdown of the tumor-suppressor protein E-cadherin and enhances the permeability of epithelial cells by attaching to colonic epithelial cells[37]. *Parabacteroides distasonis* (*P. distasonis*) is one of the major Parabacteroides[38]. *P. distasonis* is reported to deconjugate bile acid salts and transform primary bile acids into secondary bile acids[39]. Although some types of secondary bile acids have been confirmed to have carcinogenic effects on CRC[12], Koh *et al*[40] showed that *P. distasonis* has anti-inflammatory and anticancer properties by suppressing (Toll-like receptor 4) and Akt signaling and promoting apoptosis. Thus, further studies are needed to determine the relationship between Parabacteroides and tumors[40].

Based on LEfSe analysis, we found that Bilophila, a representative of Proteobacteria and Desulfovibrionaceae, accumulated remarkably in patients with PC. Known as sulfate-reducing bacteria[41], Bilophila can produce H2S primarily through cysteine degradation. As H2S is a genotoxic compound that damages DNA, leading to genomic or chromosomal instability, H2S-producing bacteria have an increased relative abundance in CRC[42].

Metabolic pathways, such as LPS biosynthesis, were enriched in the test group, which was consistent with the results of Wang *et al*[22]. LPS, often known as an endotoxin, is a constituent of the outer membrane of gram-negative bacteria. Once bound to the LPS-binding protein, LPS engages with CD14 and toll-like receptor 4 on cell membranes, such as those of monocytes and macrophages, triggering intracellular signaling pathways that lead to the production of inflammatory molecules, such as TNF, IL-1, and IL-6, causing an inflammatory response[43]. Overexpression of this pathway causes inflammation of the intestinal epithelial cells and accelerates the progression of inflammatory bowel disease[44]. Furthermore, we observed a substantial increase in the production of vancomycin in patients with PC. The metabolites involved in antibiotic biosynthesis have been observed to be elevated in CRC tissues, indicating a potential influence of microbiota makeup and arrangement on the development of CRC[45]. Lipid metabolism is enhanced in NG patients, potentially explaining why individuals with DG who consume excessive fat develop diarrhea frequently[14].

However, this study had some limitations. First, the sample size was not sufficiently large, and this was a retrospective, single-center study. Second, each patient may have had differences in the microbiome before cholecystectomy. Although we lacked data on the patients' flora before cholecystectomy, we selected two groups of patients with similar baseline data for comparison to reduce statistically constructed differences. Third, we only analyzed the differences in the microbiota between patients with or without cholecystectomy and did not further analyze causality or related mechanisms. In addition, we performed analyses at the genus level, ignoring discrepancies between different species within the same genus. Therefore, a prospective large-scale study is needed to determine the potential correlation between microbiota alterations and PC syndrome, especially CRC. Future studies should use omics data to study the intestinal microbiome, which may provide more opportunities to elucidate the mechanisms underlying the increasing incidence of colon cancer after cholecystectomy.

CONCLUSION

We report mucosal bacterial dysbiosis in patients after cholecystectomy due to alterations in flora composition based on the Wilcoxon rank-sum test and LEfSe. In particular, we found that age notably affected bacterial composition in patients with PC. Subsequently, we noticed that some specific bacteria changed between the groups, possibly related to CRC after cholecystectomy. Moreover, we used PICRUSTs to predict metabolic pathways and discovered that some of these pathways were remarkably altered in patients with PC. Thus, our study provides new insights into the mechanisms and therapeutics that could target intestinal flora to attenuate related diseases after cholecystectomy.

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FOOTNOTES

Author contributions: Fan MY and Jiang QL drafted the manuscript and collected patient samples and data; Cui MY and Zhao MQ performed the *16S rRNA* sequencing data analysis; Wang JJ provided guidance for sample processing methodology; Lu YY was involved in the study design and critical revision of the manuscript; and all authors have read and approved the final manuscript.

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