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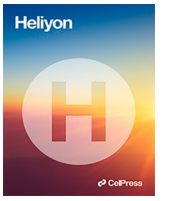

Research article

Comprehensive analysis of the gut microbiota in patients with chronic obstructive pulmonary disease of varying severity-A prospective, observational study

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ABSTRACT

Accumulating evidence has highlighted the influence of the gut microbiota on lung immunity. We examined the effects of changes in intestinal microecology on the development of Chronic Obstructive Pulmonary Disease (COPD) and identified microbial biomarkers for acute exacerbations of COPD (AECOPD). Fecal samples were collected from 30 patients with stable COPD, 30 patients with AECOPD, and 10 healthy individuals. Fecal microbiological profiles were analyzed using 16S rRNA gene sequencing. The results showed a distinct difference in the bacterial community composition between the AECOPD, COPD, and healthy control groups. The COPD and AECOPD groups had higher levels of Firmicutes but lower levels of Bacteroidetes compared to the healthy control group at the phylum level. At the genus level, there was an increased abundance of Lachnoclostridium, Alistipes, Streptococcus, and Prevotella in COPD and AECOPD patients. Increasing levels of Lachnoclostridium and Prevotella may indicate an acute exacerbation of COPD. This study identified specific microbial biomarkers associated with AECOPD and characterized the composition of gut microbiota in patients with AECOPD.

1. Introduction

Chronic obstructive pulmonary disease (COPD) is an inflammatory lung disease characterized by progressive airflow obstruction that causes symptoms such as shortness of breath, cough, and increased sputum. COPD is projected to rank fifth worldwide in terms of burden of disease and third in terms of mortality [1]. The acute exacerbation of COPD (AECOPD) refers to a sudden worsening of symptoms in individuals with chronic obstructive pulmonary disease, characterized by a rapid onset of increased breathlessness, cough, and heightened sputum production [2]. AECOPD often triggers higher mortality and morbidity rates, a rapid decline in lung function, and increased healthcare expenses [3].

Accumulating evidence has highlighted the influence of the gut microbiota on lung immunity [4], referred to as the gut–lung axis,

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Abbreviations

AECOPD	Acute Exacerbation of Chronic Obstructive Pulmonary Disease
ALB	Albumin
CCA	Canonical Correlation Analysis
COPD	Chronic Obstructive Pulmonary Disease
CRP	C-reactive protein
DNA	Deoxyribonucleic acid
ESR	Erythrocyte sedimentation rate
FEV1/FVC	Forced Expiratory Volume in 1 s/Forced Vital Capacity
GOLD	Global Initiative for Chronic Obstructive Lung Disease
IL-6	Interleukin-6
OTU	Operational Taxonomic Unit
PA	Pulmonary Artery
RNA	Ribonucleic acid
SAA	Serum amyloid A

though the underlying mechanisms are still areas of intensive research. There are reports indicating that *Streptococcus* sp*000187445* was enriched in patients with COPD and was correlated with reduced lung function [5]. Multiple *Haemophilus* spp. were negatively associated with incident COPD [6]. Li et al. demonstrated a negative correlation between Actinobacteria and the frequency of AECOPD [7]. Despite the strong correlation between gut microbiota, inflammation, and COPD, the relationship between variations in gut microbiota profiles and the severity of COPD remains unclear. Here, we hypothesized that the frequent aggravating phenotype of COPD may be driven by multiple gut microbiota. In this study, we used 16S sequencing to analyze the gut microbes, elucidate the relationship between the gut microbiota and COPD, and explored potential markers for AECOPD.

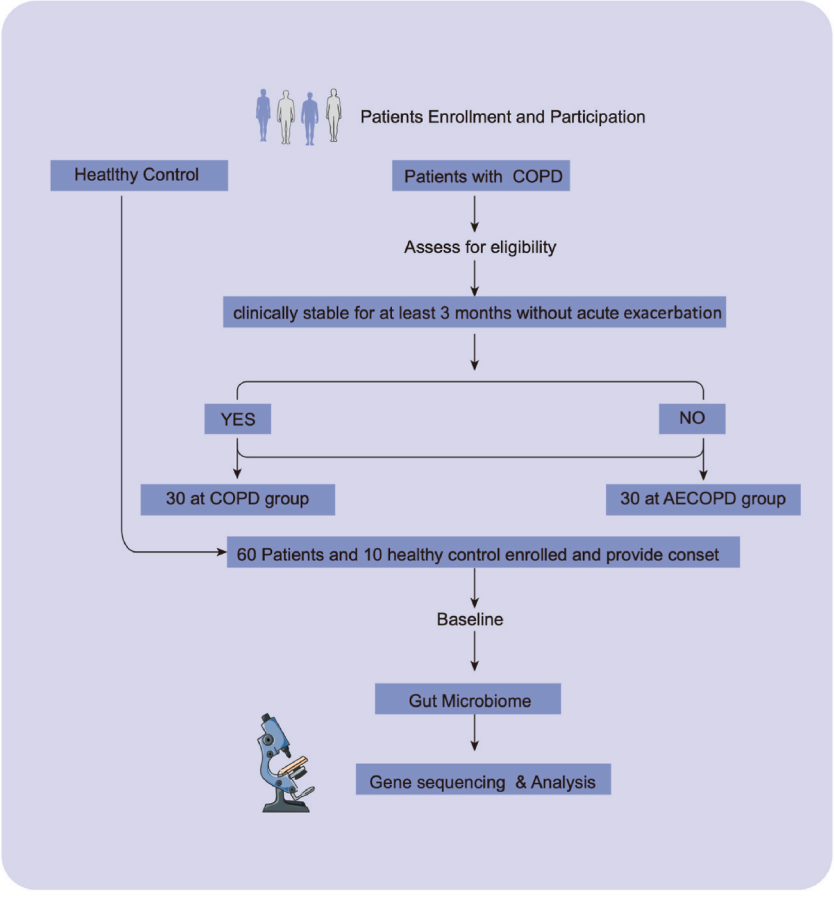

Fig. 1. Flow chart of the study.

2. Materials and methods

2.1. Study settings and patient and public involvement

This study was completed in Guangdong Provincial Hospital of Traditional Chinese Medicine. Patients and the public were not involved in the design, conduct and reporting of the research.

Ethical approval

This study has been registered in www.chictr.org.cn (registration number ChiCTR2000032870) and was approved by the ethics committee of Guangdong Provincial Hospital of Traditional Chinese Medicine (Approval number ZF2019-219-03). Patients who met the inclusion criteria were enrolled after obtaining signed informed consent.

2.2. Patient population and eligibility criteria

Seventy patients were recruited in the study, including 10 healthy controls, 30 stable COPD patients and 30 AECOPD patients in Guangdong Provincial Hospital of Traditional Chinese Medicine, from September 2020 to January 2021. The stage of the COPD was severe and very severe (Stage III and IV). The enrollment and participation flow chart of the study are show in Fig. 1. The program followed the guidelines of 2020 Global Strategy for Prevention, Diagnosis and Management of COPD (2020 GOLD reports). The diagnostic criteria for COPD and AECOPD were based on the 2020 GOLD reports. All subjects were written informed consent after understanding the purpose, potential risks, and benefits of the study. The study inclusion and exclusion criteria were shown in Table 1.

2.3. Diagnostic criteria and grouping

The COPD and AECOPD were defined under the GOLD criteria, the recruited patients were divided into COPD and AECOPD groups, the eligibility criteria were as follows.

(1) C group: Healthy people.
(2) M group: (a) diagnosed with COPD (FEV1/FVC ratio <70 %) based on the GOLD criteria; (b) chronic cough or sputum, shortness of breath or dyspnea, wheezing and chest tightness, repeated lower respiratory tract infection; visual barrel chest, bilateral intercostal space widening, weakened palpation voice tremor, percussion showed overcleaning, prolonged expiratory breath weakening, some can smell and dry and wet rales; (c) clinically stable for at least 3 months without acute exacerbation; (d) not complicated with lung cancer, asthma, or other relevant lung diseases; (e) no history of severe infection; and (f) no history of tumors, autoimmune diseases, or malignant hematologic diseases.
(3) AE group: (a) diagnosed with COPD according to the GOLD criteria; (b) exhibiting at least two major symptoms (increased dyspnea, increased sputum purulence, and increased sputum volume or one major and one minor symptom nasal discharge/congestion, wheezing, sore throat, and cough for at least 2 consecutive days according to the definitions of the GOLD criteria and (c) for the same exclusion criteria as described for COPD.

2.4. Sample collection

We collected fresh fecal samples from all patients and healthy controls before therapy. The patients were instructed to avoid picking the part that is in contact with the bottom and edge of the bedpan, placing it in the stool collection tube, and flicking the stool to the bottom of the stool collection tube. The tube of stool collection was stored in the refrigerator at −80 °C until analyses.

2.5. 16S ribosomal RNA gene sequencing

2.5.1. Bacterial DNA isolation

Stool specimens were obtained by E.Z.N.A.® Soil DNA Kit extraction kit for DNA extraction, DNA concentration and purity were

Table 1
Eligibility criteria.

Inclusion criteria	Exclusion criteria
Hospitalised patients with COPD[a]	Chronic consumable diseases such as tumors and tuberculosis
40~85 years of age	Ulcerative colitis, Crohn's disease, or other intestinal diseases
Male or female	
Not smoking or quitting smoking for more than 1 year	Patients with oral hormones or immunosuppressive agents within 3 months
No antimicrobial drugs were used in the last 1 month	Previous regular oral probiotics, prebiotics or other microbial agents
Able to understand and communicate to ensure the completion of the trial	Poor compliance

[a] The diagnostic criteria for COPD are based on the recommendation in Global Initiative for Chronic Obstructive Pulmonary Disease guideline.

detected using the NanoDrop 2000, DNA template amplified by PCR, PCR product recovered using AxyPrep DNA gel recovery kit (Axygen Biosciences), the preparation tube with destination DNA was washed in a clean centrifuge tube with 25–30 μL of water in the preparation film by centrifugation again. The PCR products were detected and quantified by QuantusTM Fluorometer, and the bacterial DNA was isolated and purified.

2.5.2. Library construction and Illumina sequencing

The library construction process was carried out utilizing the NEXTFLEX RapidDNA-SeqKit. The ultimate library was generated through a combination of joint linking, enrichment of library templates via PCR amplification, and recovery of PCR products using magnetic beads. Sequencing was conducted on the Illumina Corporation's Miseq PE300 platform. Genomic DNA is extracted to complement one end of the DNA fragment, linking it to the primer on the chip, while the other end randomly complements an adjacent primer, forming a bridge structure. The DNA clusters were generated after PCR amplification, and the amplicons were linearized into single-stranded DNA. In the reaction system, DNA polymerase, adapter primers, and four dNTPs labeled with base-specific fluorescent tags are simultaneously added. The 3′-OH of these dNTPs is protected by a chemical method, allowing the addition of only one dNTP at a time. This ensures that during the sequencing process, only one base is added at a time. After the dNTPs are added to the synthesis chain, all unused free dNTPs and DNA polymerase are washed away. Subsequently, a buffer for fluorescence excitation is added, fluorescence signals are excited by laser, and optical equipment records the fluorescence signals. Finally, the optical signals are converted into sequencing bases. After completing the fluorescence signal recording, a chemical reagent is added to quench the fluorescence signals and remove the 3′-OH protective groups of dNTPs, allowing for the next round of sequencing reactions. Finally, the fluorescence signal results of each round were counted to obtain the overall sequence of the sample DNA fragments.

2.5.3. Data optimization and OTU clustering

Quality control of the raw sequencing sequences was performed using fastp software (https://github.com/OpenGene/fastp, version 0.20.0), and the splicing was done by FLASH software (http://www.cbcb.umd.edu/soft-ware/flash). UPARSE software (http://drive5.com/uparse) was used to remove nonrepetitive single sequences, and OTU clustering of non-repetitive sequences (excluding single sequences) was performed according to the similarity of 97 %. In the process, the chimeras were removed to obtain the OTU representative sequence. Species classification annotation was performed using RDP classifier, aligned to the Silva database (Release132 http://www.arb-silva.de), and the alignment threshold was set to 70 %.

2.6. LEfSe multilevel discriminant analysis of species differences

Linear discriminant analysis Effect Size (LEfSe) combines linear discriminant analysis (LDA) with non-parametric statistical testing to identify significant differences between groups. First, non-parametric Kruskal-Wallis tests was used to compare the abundance or frequency of features across different groups (AE and M group). Then, features that show statistically significant differences via Kruskal-Wallis tests are further tested using Wilcoxon rank-sum tests to identify features with significant differences between groups. Next, LDA was employed to score these differentiating features, determining their ability to discriminate between groups. Features with the highest LDA scores are considered the most discriminative biomarkers for distinguishing between groups. Finally, LEfSe ranks these biomarkers based on both statistical significance and biological relevance (as indicated by LDA scores) and provides graphical representation and interpretation.

2.7. Statistical analysis

Differences between AE group and the M group were compared using independent samples *t*-test or Mann-Whitney *U* test for quantitative data and $\chi 2$ test or Fisher test for qualitative data, and differences were considered statistically significant when $P < 0.05$. One-way analysis of variance (ANOVA) was used to compare differences among groups (When comparing the three groups (AE, M, C)), followed by Student-Newman-Keuls tests. Bioinformatics analysis was done by R language, and the diversity, commonality and differences of subjects' intestinal flora were compared using mixOmics package, stats package, and LefSe package, and the correlation between different flora and immune and nutritional indicators was analyzed using vegan package, pheatmap package, and MaAsLin package, and the differences were considered statistically significant when $^{P} < 0.05$.

Table 2
Baseline of participants. ($\overline{x}$ ±s/M).

Project	AE	M	*t/Z*	*P*
Age	71.90 ± 6.89	72.50 ± 8.07	−0.310	0.758
Height	165(161.75,170.00)	165(161.75,170.00)	−0.974	0.330
Weight	54.14 ± 11.82	56.32 ± 11.57	−0.721	0.474
BMI	18.00(16.92,23.21)	20.07(17.99,24.21)	−1.560	0.119
CAT	26.50(21.75,29.00)	22.50(18.75,27.25)	−1.934	0.053

3. Results

3.1. Basic clinical characteristics

In total, 30 stable COPD patients, 30 patients with AECOPD, and 10 healthy people were recruited. The demographics and clinical features of the patients are summarized in Table 2. No statistical differences in age, gender, BMI, CAT were observed among the groups. Risk factor exposure, home treatment, inflammatory indicators, immune indicators, and nutritional indicators were also collected, as shown in Supplementary Tables S1–S8.

3.2. The gut microbiota diversity levels (alpha diversity) in patients with COPD and AECOPD

The differences in Sobs, Shannon, Simpson, Shannoneven and Simpsoneven indices between AECOPD and COPD were not statistically significant ($P > 0.05$), indicating that there was no difference in community richness, diversity and homogeneity between the two groups, as shown in Table 3. This indicates that the differences between the two groups were not significant.

3.3. Gut microbiota composition differs among the healthy, stable COPD patients, and AECOPD patients

In the AECOPD group, dominant genera were primarily from the *Bacteroides*, *Streptococcus* and *Prevotella*, while in the COPD group, the dominant genera were *Bacteroides*, *Escherichia*, and *Enterococcus* (Table 4). As shown in Fig. 2A, most of the dominant groups in the AECOPD Group (AE) were *Bacteroides* and *Prevotella*. Most of the dominant groups of COPD Group (M) were *Bacteroides* and *Enterococcus*. And the Species of different group was shown in Fig. 2B, all three groups had a predominance of *Bacteroides dorei* at the species level. Community heatmap analysis at the genus and species levels is presented in Fig. 2C and D, respectively.

3.4. Gut microbiota alterations in the healthy control, stable COPD patients and AECOPD patients

By comparing the species diversity of different disease states, we explored the similarities or differences in overall community structure between the AECOPD and COPD groups. We used PCoA analysis to identify important elements and structures in gut microbiome sequencing data. There were certain differences between the three groups, indicating that the gut microbiomes of AECOPD and COPD patients are significantly different (Fig. 3A and B). We used PLS-DA for the comparative analysis of the three groups. As shown in Fig. 3C, the fecal samples from AECOPD, COPD, and C group clustered into three clusters, and the gut flora of the three groups of subjects varies greatly between the groups. Additionally, we performed further sample flora typing analysis between different groups, excluding external influences like environmental factors. We explored dominant flora structure typing using cluster analysis and inter-sample distances with Bray-Curtis matrix algorithm, obtaining species-level intestinal flora typing for the three groups (Fig. 3D). The three sets of samples could be clustered into a total of seven enterotypes, namely *Bacteroides plebeius, Subdoligranulum uncultured bacterium, Prevotella*, *Strepto-coccus unclassfiecd, Faecalibacterium prausnitzii, Bacteroides dorei, and Enterococcus faecium*).

3.5. Specific microbial signatures of stable COPD patients and AECOPD patients

(1) Inter-group difference significance test

The bacterial species data in the three sample groups did not adhere to a normal distribution or exhibit equal variances. Therefore, the Kruskal-Wallis rank-sum test was employed for the multi-group comparison of differences, and Bonferroni correction was applied for multiple testing. Statistical significance was considered when $P < 0.05$. According to the statistical analysis, the distribution differences of *Roseburia uncultured organism, Escherichia coli, Prevotella macrogenus*, and *Bacteroides fragilis* in the three groups of samples were statistically significant ($P < 0.05$), among which the differences of *Roseburia uncultured organism* ($P = 0.002$) and *Escherichia coli* ($P = 0.009$) were more significant (Fig. 4A).

(2) Lefse multilevel species difference analysis

According to the Lefse analysis, the AECOPD group had a higher LDA value (>3.5) for *Prevotella*, *Faecalibacterium unclassified*, and

Table 3
Alpha diversity analysis of AECOPD (AE) group versus COPD (M) group.

Project	AE	M	*P*	*Q*
Sobs	163.00±64.89	127. 75±40.57	0.120	0. 597
Shannon	2.57±0.99	2. 51±0.55	0.554	0.776
Simpson	0.24±0.26	0.19±0.10	0.776	0.776
Shannoneven	0.50±0.18	0.52±0.10	0.776	0.776
Simpsoneven	0.05±0.03	0.05±0.02	0.369	0.776

Table 4
The distribution of the major fecal bacterial genera.

Item	C	M	AE
Bacteroides	36.80 %	18.65 %	12.33 %
Roseburia	8.60 %	2.13 %	4.20 %
Faecalibacterium	7.17 %	4.02 %	4.39 %
Lachnospira	2.96 %	5.40 %	0.54 %
Subdoligranulum	2.37 %	3.13 %	5.18 %
Blautia	2.32 %	6.41 %	1.42 %
Lachnoclostridium	1.57 %	2.38 %	9.41 %
Escherichia-Shigella	0.98 %	6.99 %	2.12 %
Alistipes	0.39 %	0.44 %	4.60 %
Streptococcus	0.15 %	1.44 %	7.49 %
Prevotella	0.02 %	0.87 %	10.91 %
Enterococcus		7.44 %	1.21 %
Clostridia	0.00 %	4.46 %	0.06 %

Lactobacillus salivarius. The COPD group had a higher LDA value (>3.5) for *Enterococcus faecium*, *Lachnospira uncultured bacterium*, and *Lactobacillus fermentum*. The relative abundances of *Prevotella* and *Lactobacillus* were higher in the AECOPD group than in the other two groups, while the relative abundances of *Enterococcus* and *Lachnospira* were higher in the non-frequent acute exacerbation group. The relative abundances of *Bacteroides* and *Roseburia* were higher in the control group (Fig. 4B).

3.6. Relationship between differences in Gut Microbiota and Clinical Factors by group

We used CCA analysis and db-RDA (distance-based redundancy analysis) to identify important clinical drivers and species influencing the differences in gut microbiota between AECOPD and COPD. *Blautia unclassified*, a genus of unclassified bacteria, showed a positive correlation with MNA. *Lachnospira uncultured bacterium*, an uncultured species of the *Lachnospira* genus, showed a negative correlation with IgA. *Lachnospiraceae unclassified*, an unclassified genus of the Lachnospiraceae family, showed a positive correlation with ALB. *Faecalibacterium prausnitziii* showed a positive correlation with MNA. *Bacteroides dorei* showed a positive correlation with MNA > ALB. *Roseburia uncultured organism*, an uncultured species of the Roseburia genus, showed a positive correlation with MNA. *Escherichia coli* showed a positive correlation with CD4+/CD8+ and a negative correlation with $CD3^{+}CD8^{+}$. *Enterococcus faecium* showed a positive correlation with CD4+/CD8+ and a significant negative correlation with MNA and PA, as well as a negative correlation with ALB. *Streptococcus unclassified*, an unclassified genus of the Streptococcus family, showed a significant negative correlation with ALB and PA (Fig. 5A–C).

3.7. MaAsLin analysis to find flora associated with clinical indicators

Using the MaAsLin package in R software, a linear model was established to calculate the correlation coefficient (Coefficient) between clinical factors and relative abundance of bacteria. When Coefficient>0, it indicates a positive correlation; when Coefficient<0, it indicates a negative correlation; and when Coefficient is close to 0, it indicates no correlation. The *P*-value was used to evaluate the reliability of the test, and a P-value <0.05 was considered to indicate a relatively significant correlation between clinical factors and bacteria.

In terms of immune indicators, *Blautia_sp._N6HlT5, Veillonella unclassified,* and IgA showed negative correlations. The *Prevotella* and $CD3^{+}CD4^{+}$, CD4+/CD8+ showed negative correlations, while it showed a positive correlation with $CD3^{+}CD8^{+}$. *Escherichia coli.* showed a positive correlation with CD4+/CD8+, and a negative correlation with $CD3^{+}CD8^{+}$. In addition, CD4+/CD8+ showed a negative correlation with *Prevotella copri*. In terms of nutritional indicators, ALB showed negative correlations with unclassified species of *Prevotella*, *Streptococcus*, and *Lactobacillus*, and showed positive correlations with un-cultured species of *Roseburia* and *Dialister*. PA also showed a negative correlation with *Lactobacillus*, and a positive correlation with un-cultured species of *Treponema*. As for the inflammation indicators, SAA showed negative correlations with *Dialister* and *Actinomyces*. Specific values are shown in Table 5.

3.8. Correlation Analysis and Model Prediction Based on gut microbiome sequencing results using Random Forest

By analyzing gut microbiome sequencing results, we identified species that can serve as biomarkers and validated their accuracy in disease diagnosis. The Random Forest analysis was mainly used to select important microbes to build a disease diagnosis model, while ROC analysis was used to validate the accuracy of the constructed model. ROC analysis can also be used to verify the accuracy of biomarkers selected by other analysis methods.

The distribution and classification of Random Forest samples are shown in Fig. 6A, the statistical information of Random Forest species abundance is shown in Fig. 6B and 6C shows the validation information of Random Forest. Random Forest can effectively distinguish AECOPD, COPD, and normal individuals based on gut microbiome. The ROC curve based on gut microbiome is shown in Fig. 6D, with an AUC of 0.83 (95 % CI: 0.63–1).

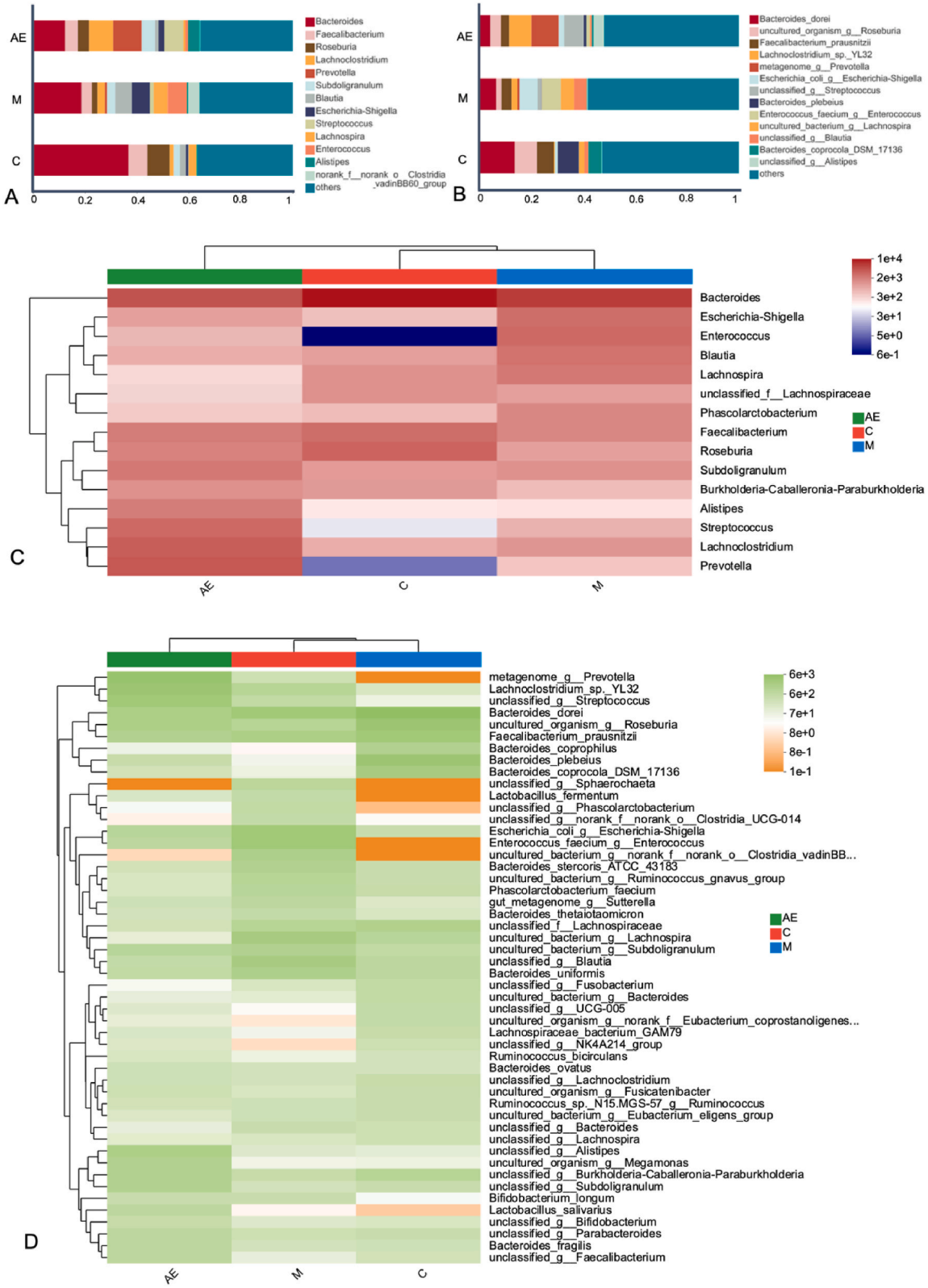

Fig. 2. Gut microbiota composition from the healthy, stable COPD patients, and AECOPD patients in Genus (A) and Species (B) level. Community heatmap analysis from the healthy, stable COPD patients, and AECOPD patients in Genus (C) and Species (D) level.

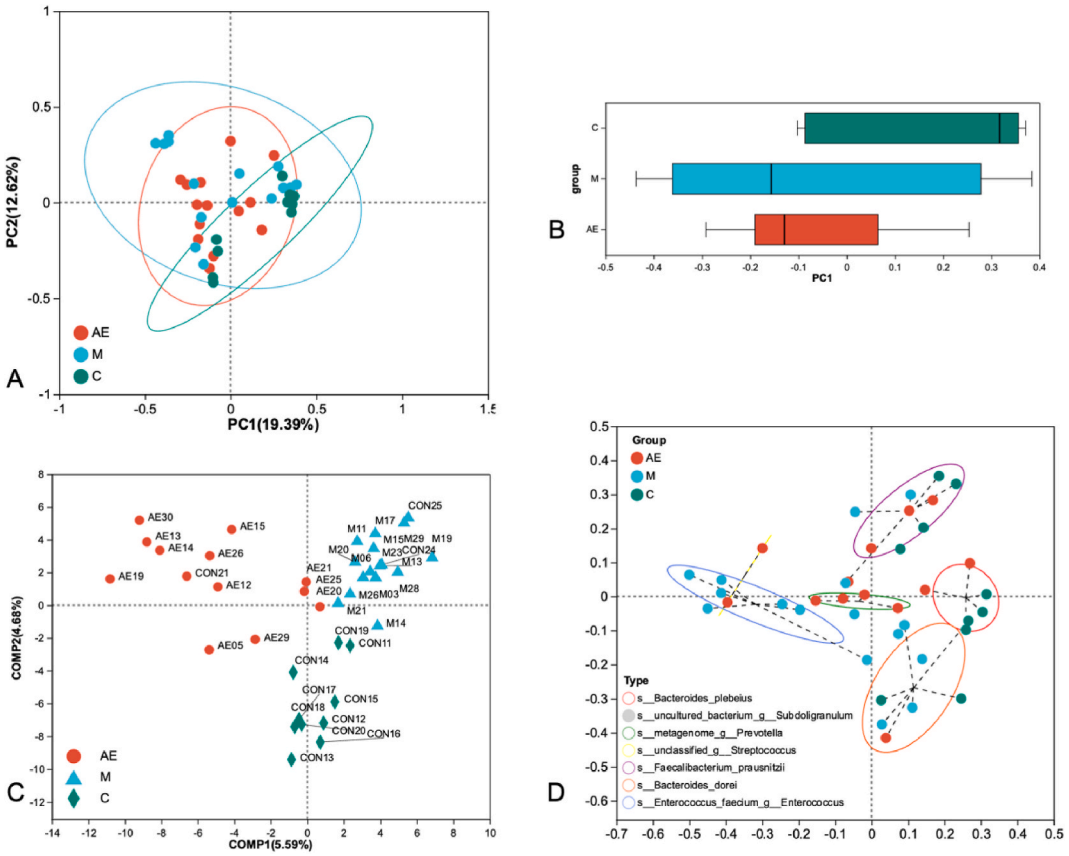

Fig. 3. Gut microbiota alterations in the healthy control, stable COPD patients and AECOPD patients. (A) PCoA on Genus level; (B) Box-plot of PCoA; (C) PLS-DA analysis of the three groups; (D) Typing analysis on Species level.

4. Discussion

COPD has always been a major public health issue because of its high prevalence, morbidity, and mortality rates, presenting great challenges to the healthcare system [8]. AECOPD is characterized by symptoms like cough, wheezing, purulent sputum, fever, and general malaise, leading to airflow limitation and development of dynamic hyperinflation [9]. Bacterial infections are the leading cause of infectious AECOPD, with a prevalence rate ranging from 26 % to 81 % [10]. Cumulative evidence from both human and animal studies suggests that the development of respiratory diseases (including COPD) may depend on the bacterial community in the gut [11]. The gut microbiota interacts with the host immune system through bacterial structural components and secreted metabolites, and these interactions have the ability to regulate local and systemic immune responses in the gastrointestinal tract, affecting various distal sites, including the lung.

In the present study, we observed significant differences in the gut microbiota of patients between the AECOPD and COPD groups. Specifically, an increased relative abundance of *Firmicutes* and *Bacteroidetes*, were observed in the AECOPD group. At the genus level, this change appears to be driven by a corresponding increase in *Lachnoclostridium, Alistipes, Streptococcus,* and *Prevotella*, although other potentially pathogenic genera, may also be involved. According to research reports, *Bacteroidetes* and *Firmicutes* are the most abundant phyla in healthy adults [12]. Most *Firmicutes* are Gram-positive bacteria that can be transformed into pathogenic pathogens by symbiosis. *Bacteroidetes* mainly act on steroids, polysaccharides, and bile acids that contribute to polysaccharide absorption and protein synthesis, it has been reported to promote the differentiation of regulatory T cells (Treg) and protect against inflammatory reactions. Studies have demonstrated a link between various diseases and increased *Firmicutes/Bacteroidetes* ratio [13]. In particular, a rise in the *Firmicutes/Bacteroidetes* ratio is related to inflammation [14]. At the phylum level, the COPD, AECOPD group showed significantly higher abundances of *Firmicutes*, but a lower *Bacteroidetes* than the healthy control, which could imply impaired disorder of intestinal flora and inflammation in the patients with AECOPD.

We also utilized LEfSe analysis to identify species associated with AECOPD. Consistently, we observed higher relative abundances of *Prevotella* and *Lactobacillus* in the AECOPD group than in the other two groups, while the relative abundances of *Enterococcus* and *Lachnospira* were higher in the COPD. *Prevotella* and *Enterococcus faecalis* differed relatively significantly in AECOPD. *Prevotella* is a conditionally pathogenic bacterium, which is a gram-negative anaerobic bacterium and has an antagonistic relationship with *Mycobacterium* spp. in the intestinal tract [15]. Increased relative abundance of *Prevotella* activates Toll-like receptors, leading to the

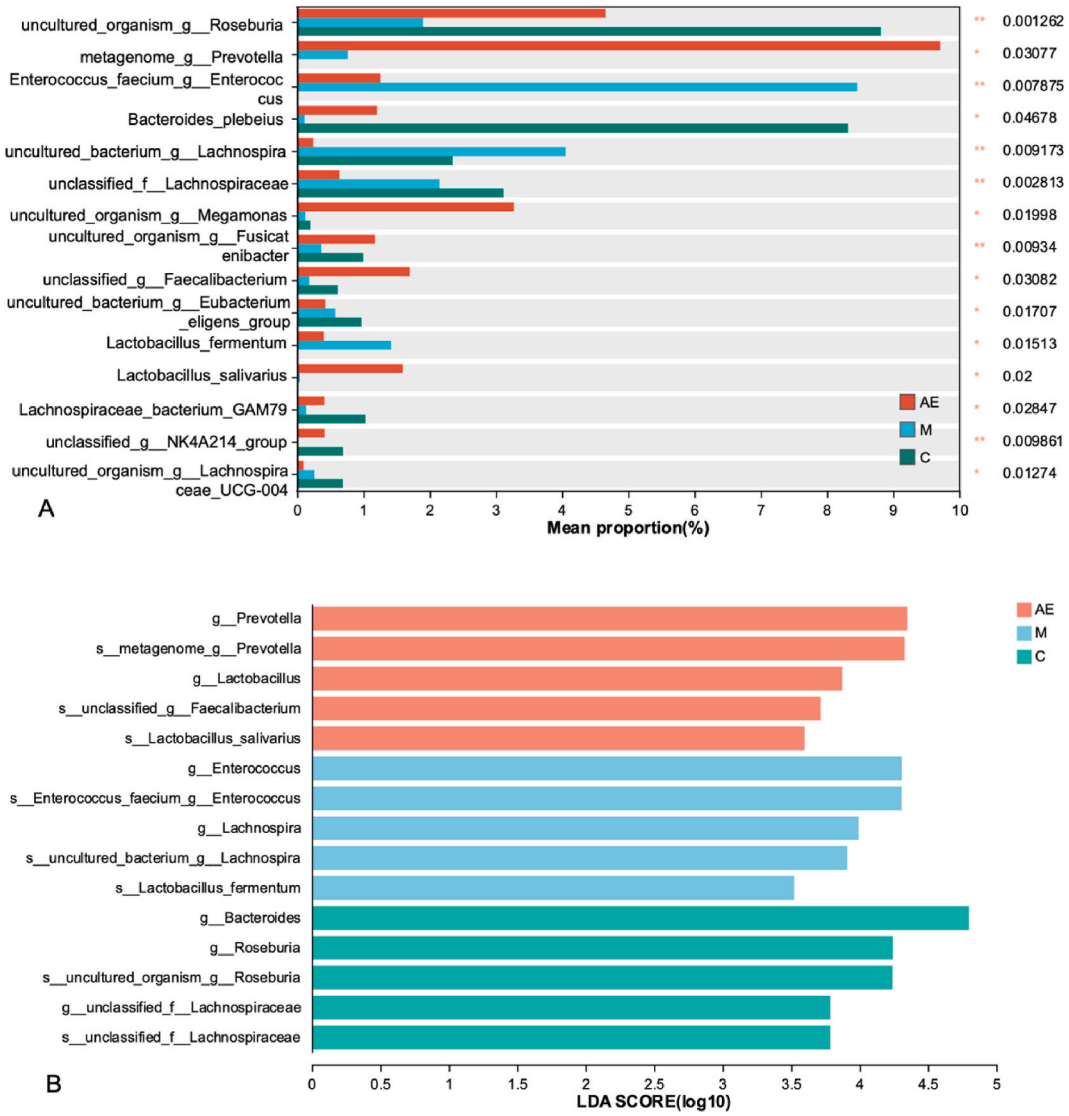

Fig. 4. Specific microbial signatures of stable COPD patients and AECOPD patients. (A) Inter-group difference significance test.(B) Lefse multilevel species difference analysis.

production of Th17-polarizing cytokines by antigen-presenting cells on one hand [16,17]; on the other hand, it stimulates epithelial cells to produce cytokines, such as IL-6 and IL-8, to promote mucosal immune responses and to recruit neutrophils, resulting in a sustained inflammatory response [18]. Since *Prevotella* differed relatively significantly in AECOPD, it was hypothesized that patients with frequent acute exacerbation phenotypes of COPD would be more susceptible to airway fibrillation and have a poorer prognosis. *Enterococcus* spp. is one of the normal flora colonizing the human intestinal tract [19], and according to the isolation rate, it is mainly classified into *Enterococcus faecalis*, of which *Enterococcus faecalis* is closely associated with catheter-associated urinary tract infections [17]. When host immunity is dysfunctional, *enterococci* can leave their normal colonization sites and enter other tissues and organs, causing infections, and are resistant to a wide range of antibiotics [20]. In the M group, the intestinal flora was dominated by *Bacteroides* Genus which has been shown to be involved in the up-regulation of genes involved in intestinal barrier function, which may reduce chronic inflammation associated with the accumulation of lipopolysaccharides in the body [21].

Intestinal flora plays an important role in the formation and regulation of the host's immune system [22], and the differences in the intestinal flora of each group can reflect the immunity of the host, while the changes in immune factors can also reflect the trend of intestinal flora to a certain extent. CCA (Canonical Correlation Analysis) and db-RDA (distance-based redundancy analysis) analyses were employed to identify crucial clinical drivers influencing sample distribution, aiming to discover key species associated with the disease. In terms of humoral immunity, the difference in IgG levels between the chronic obstructive pulmonary group and the control group was statistically significant ($P < 0.05$). IgA, as one of the important components of mucosal intrinsic immunity [23], was negatively correlated with *Lachnospira*, and the poor lung function and low IgA secretion level of frequently acutely exacerbated

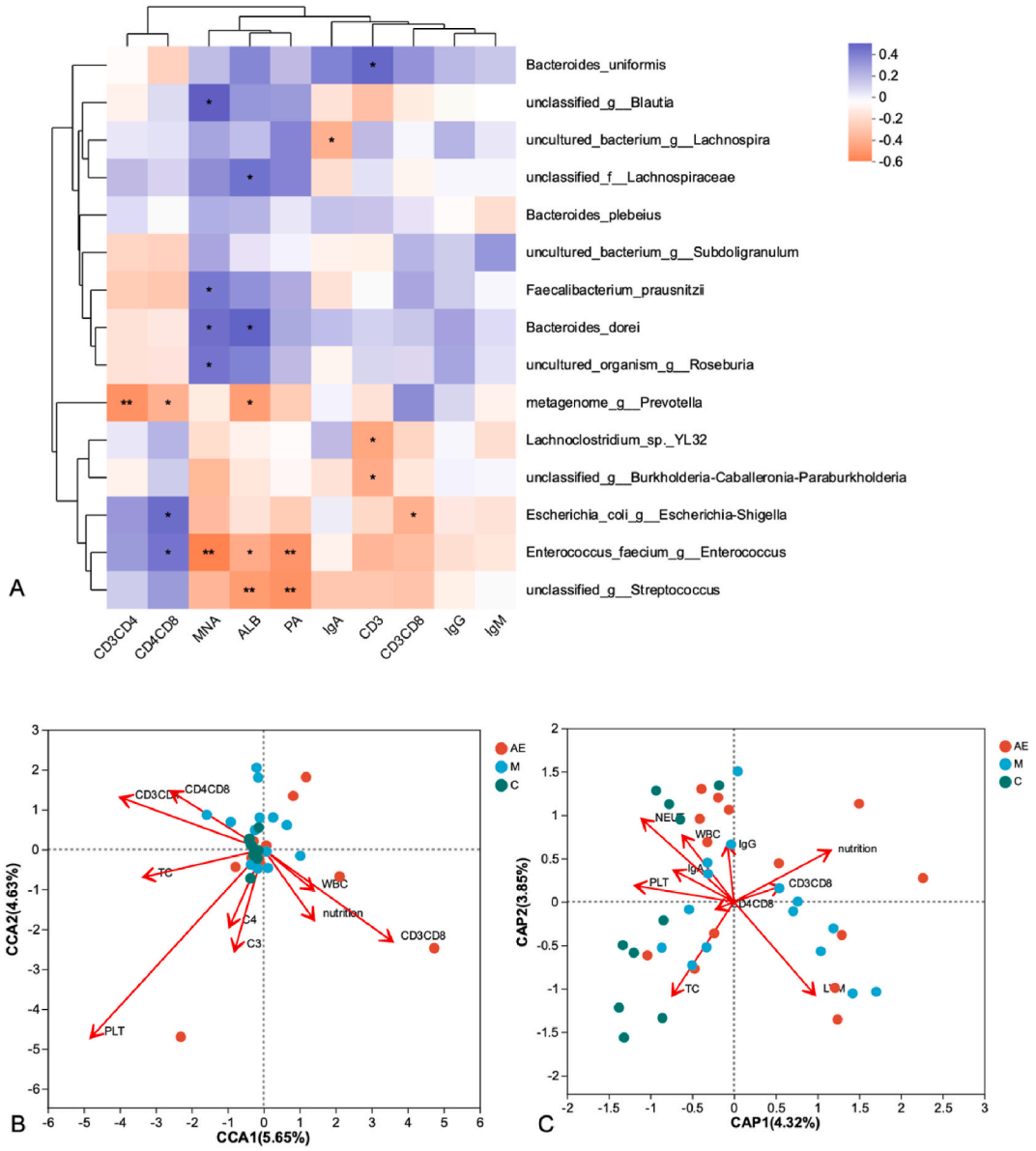

Fig. 5. Relationship between differences in Gut Microbiota and Clinical Factors by group. (A) Canonical Correlation Analysis and distance-based redundancy analysis. CCA analysis(B) and db-RDA (C) analysis was used to determine the important clinical drivers affecting the distribution of the samples with a view to finding key species associated with the disease.

chronic obstructive pulmonary disease (COPD) caused an increase in the relative abundance of *Lachnospira*, which affected the relative abundance of *Lachnospira* to a certain extent through the common mucosal immune system. In terms of cellular immunity, $CD3^+$ $CD4^+$, $CD3^+$ $CD8^+$ and CD4+/CD8+ play an important role in the immunoregulation of COPD. CCA analysis also indicated a negative correlation between $CD3^+$ $CD8^+$ and IgA, $CD3^+$ $CD4^+$ and $CD4^+$/CD8+. When the degree of airway obstruction is high, it can be hypothesized that IgA and $CD4^+$/CD8+ are not secreted sufficiently, which affects the immunity. As a conditionally pathogenic bacterium, *Prevotella,* showed a positive correlation with the $CD3^+$ $CD8^+$ level and a negative correlation with the CD4+/CD8+ ratio, and it was hypothesized that the $CD8^+$ level was higher in frequent acute exacerbations than in infrequent acute exacerbations, and that there might be an increase in *Prevotella*.

MaAsLin analysis of differential species showed relatively significant differences in nutritional indicators between the chronic obstructive pulmonary group and the control group, and between the AECOPD group and the COPD group, and this difference was likewise reflected in the correlation with intestinal flora. Patients with COPD commonly suffer from different degrees of malnutrition [24,25], which is often manifested as insufficient synthesis and secretion of ALB and PA, which has a serious impact on the quality of life of patients. Therefore, under the premise of nutritional support, the combination of immunomodulators can reduce the possibility

Table 5
MaAsLin analysis of differential species at the species level and clinical factors.

clinical factors	bacterial species	Coeffencient	*P*
IgA	*Blautia_sp._N6H1~15*	−0.001	0.012
IgA	*Veillonella unclassified*	−0.021	0.014
$CD3^+CD4^+$	*Prevotella*	−0.013	0.006
$CD3^+CD8^+$	*Prevotella*	0.014	0.000
$CD3^+CD8^+$	*Escherichia coli*	−0.004	0.007
CD4+/CD8+	*Prevotella*	−0.097	0.027
CD4+/CD8+	*Escherichia coli*	0.037	0.009
CD4+/CD8+	*Faecalibacterium prausnitzii*	−0.024	0.044
ALB	*Prevotella unclassified*	−0.005	0.005
ALB	*Streptococcus unclassified*	−0.003	0.011
ALB	*Roseburia uncultured organism*	0.015	0.030
ALB	*Bacteroides dorei*	0.016	0.050
ALB	*Lactobacillus fermentum*	−0.000	0.049
PA	*Lactobacillus fermentum*	−0.000	0.001
PA	*Lachnospira uncultured bacterium*	0.001	0.027
SAA	*Bacteroides dorei*	−0.000	0.038
SAA	*Bacteroides uniformis*	−0.000	0.049

of secondary infections in elderly patients with chronic obstructive pulmonary disease. We also employed the Random Forest method to predict acute exacerbations using the gut microbiota. This suggests that the prediction model based on gut microbiome has a certain degree of accuracy. Gut microbiome sequencing is a non-invasive test that can indicate the risk of AECOPD to a certain extent.

In conclusion, our results suggested that a distinct separation in bacterial community composition between the AECOPD, COPD and the healthy control. The increased abundance of bacteria such as *Lachnoclostridium* and *Prevotella* might indicate an early indication of acute exacerbations of COPD. Our study characterized the systemic composition of gut microbiota in AECOPD patients, uncovered the microbial signature associated with AECOPD, and identified the specific microbial biomarkers which showed a good discrimination capability for AECOPD. The study provides a reference to the early diagnosis and early treatment of AECOPD.

However, our research still has some limitations. The sample size is relatively small, with samples collected only from a single center, lacking a multicenter study. 16S rRNA gene sequencing typically provides relatively low resolution, making it unable to distinguish certain bacterial taxa within microbial communities. This limitation may lead to an incomplete or blurry understanding of the microbial community structure. 16S sequencing primarily focuses on the 16S rRNA gene, but not all bacteria possess this gene. Some microorganisms may be overlooked in the study due to the absence of the 16S rRNA gene. While 16S sequencing provides information on the relative abundance of microbes, it does not offer precise microbial cell counts. This makes accurate quantitative comparisons between different samples challenging.

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Ethical approval

This study has been approved by the ethics committee of Guangdong Provincial Hospital of Traditional Chinese Medicine (Approval number ZF2019-219-03).

CRediT authorship contribution statement

Jiali Yan: Writing – original draft, Investigation, Data curation. **Zhenhu Wu:** Writing – original draft, Validation, Data curation. **Li Deng:** Writing – review & editing, Writing – original draft, Data curation. **Chunzhen Huang:** Writing – review & editing. **Yuting Jing:** Validation, Data curation. **Xiao-yin Chen:** Resources, Funding acquisition, Conceptualization. **Yinji Xu:** Resources, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

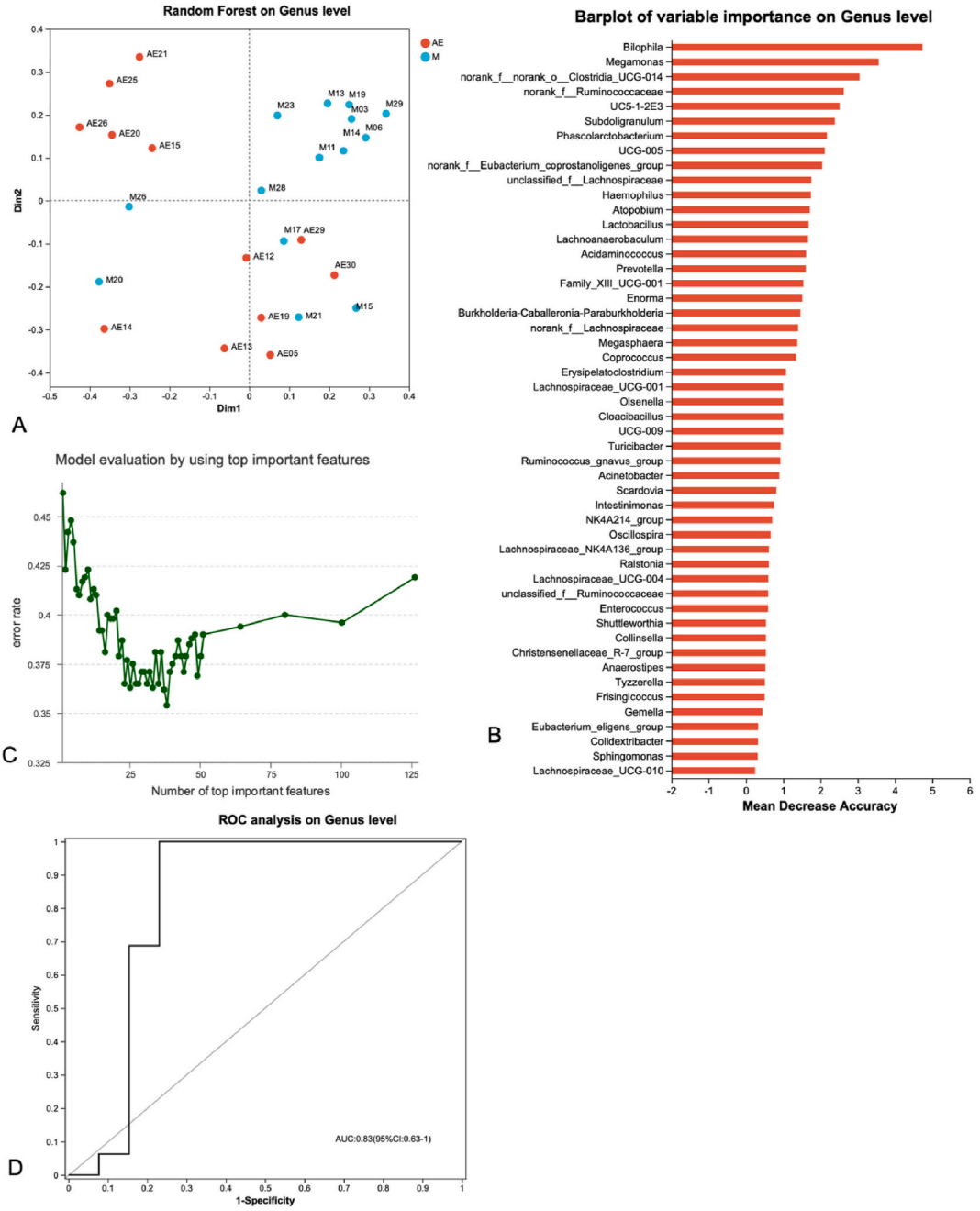

Fig. 6. Correlation Analysis and Model Prediction Based on Gut Microbiome Sequencing Results Using Random Forest. (A) The distribution and classification of Random Forest on Genus level; (B)Barplot of variable importance on Genus level. (C) Model evaluation by using top important features. (D) The ROC curve on Genus level.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e31512.

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