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Original Article

The Gut Microbiota in Elderly Patients with Acute Hepatitis E Infection

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Abstract

Background and Aims: Gut dysbiosis has been reported in severe liver diseases. However, information on the impact of hepatitis E virus infection on the gut microbiota, and the association between enteric microbiota disturbances and acute hepatitis E (AHE), is limited, particularly in elderly patients with AHE (AHE-elderly). Our objective was to characterize the AHE-specific microbiome in elderly patients and evaluate its association with clinical outcomes. ***Methods:*** Fecal samples and clinical data were collected from 58 AHE-elderly patients (46 self-healing cases, 12 non-self-healing cases) and 30 elderly patients with healthy controls (hereinafter referred to as HCs-elderly). Gut microbiota composition was analyzed using 16S rRNA gene sequencing. Bioinformatic analyses, including alpha diversity and STAMP, were performed. The predictive potential of *Bacteroides fragilis* was assessed using statistical analysis and receiver operating characteristic curves. ***Results:*** Alpha diversity indices showed no significant differences in microbial diversity between the AHE-elderly and HCs-elderly groups, nor between self-healing and non-self-healing groups among AHE-elderly patients. Nevertheless, a trend toward altered species richness was observed. In the AHE-elderly group, the relative abundance of *Firmicutes*, *Lactobacillales*, and *Bacilli* increased significantly. Meanwhile, compared with the self-healing group, *Bacteroidetes* were more abundant in the non-self-healing group. At the species level, *Bacteroides fragilis* was the most abundant in the non-self-healing group, significantly contributing to the divergence in gut microbiota between the two groups. ***Conclusions:*** The relative abundance of *Bacteroidetes* significantly distinguished AHE-elderly patients from healthy controls and could more accurately predict recovery outcomes in elderly AHE patients. These findings suggest new strategies for preventing and managing AHE recurrence in the elderly patients.

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Introduction

Hepatitis E virus (HEV) is a spherical, single-stranded, positive-sense RNA virus belonging to the Hepeviridae family, and it is the causative agent of hepatitis E.[1] HEV has seven known genotypes, among which HEV-1, HEV-2, HEV-3, HEV-4, and HEV-7 can infect humans, while HEV-3, HEV-4, and HEV-7 can also infect animals. Currently, HEV-4 is the predominant genotype in China, with occasional cases of HEV-1.[2] According to the World Health Organization, there are approximately 20 million new HEV infections annually, with about 3.3 million cases exhibiting clinical symptoms of hepatitis.[3,4] China is considered a high-incidence region for HEV infection, reporting hundreds of thousands of new cases each year, particularly in rural areas and regions with poor sanitation.[5] The incubation period for acute hepatitis E (AHE) typically ranges from two to six weeks. Following infection, patients may experience symptoms such as fever, vomiting, jaundice, liver discomfort, and significantly elevated serum transaminase levels.[6,7] In immunocompetent individuals, AHE is generally self-limiting, with the immune system clearing the virus within weeks to months, leading to complete recovery—a process termed self-healing.[8] However, in high-risk populations (e.g., the elderly, pregnant women, patients with chronic liver disease, or immunocompromised individuals), AHE may progress to severe hepatitis, liver failure,

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decompensated cirrhosis, or death, referred to as non-self-healing.[9,10] It has been reported that the incidence of AHE among elderly patients is approximately 66%, with a mortality rate of up to 10%.[11] Currently, there is limited data on the mechanisms that distinguish self-healing from non-self-healing outcomes in HEV infection.

The gut microbiome plays a significant role in regulating the gut-liver axis.[12–16] Numerous studies have explored the role of gut microbiota in chronic hepatitis B and C. Patients with chronic hepatitis C, both with and without cirrhosis, have been found to exhibit reduced gut microbial diversity compared to healthy individuals.[17–19]

Our recent research revealed that gut microbiota dysbiosis in AHE and HEV-related acute liver failure (ALF) patients, as detected by 16S rRNA sequencing, is associated with more severe HEV infections. Compared with the healthy control (HC) group, *Proteobacteria*, *gamma-Proteobacteria,* and *Enterobacteriaceae* were the most abundant taxa in the AHE group.[20] Another study found that gut microbiota dysbiosis in AHE patients was associated with HEV infection severity, IFN-γ levels, and viral load. HEV-ALF patients exhibited higher levels of *Gammaproteobacteria*, *Proteobacteria*, *Xanthomonadaceae*, and *Stenotrophomonas* compared to AHE patients, suggesting that dysbiosis may critically drive the progression of severe HEV disease.[21] Notably, the composition of the gut microbiota in elderly individuals differs significantly from that of healthy younger adults. Aging is associated with a decline in gut microbial diversity and a disruption in the balance between beneficial and harmful bacteria, which has been identified as a contributing factor in the onset and progression of liver diseases.[22–24] However, most existing clinical studies examining the relationship between liver disease and gut microbiota have not accounted for the effects of aging. Therefore, this study specifically focuses on the variability of gut microbiota in elderly patients with AHE (AHE-elderly).

The aim was to investigate differences in gut microbiota diversity and composition between AHE-elderly patients and age-matched healthy controls (HCs-elderly) using 16S rRNA sequencing. Additionally, we compared gut microbiota profiles between self-healing and non-self-healing AHE-elderly patients. This study contributes to the development of novel therapeutic strategies for AHE. To the best of our knowledge, this is the first study to characterize the fecal microbiota in elderly patients with HEV infection.

Methods

Patient population

We recruited 58 AHE-elderly patients and 30 HCs-elderly patients from the First Affiliated Hospital of Zhejiang University School of Medicine between September 2020 and October 2021. At admission and discharge, we collected baseline clinical data, including gender and age; laboratory indicators such as blood biochemistry and viral load; length of hospital stay; and diagnostic information. All 58 AHE-elderly patients were additionally followed for 30 days after discharge.

Fifty-eight patients were diagnosed with HEV IgM/IgG and PCR positive, in conjunction with clinical manifestations consistent with acute hepatitis.[23] Exclusion criteria included: co-infection with hepatitis A virus, hepatitis B virus, or other hepatitis viruses; alcoholic liver disease, non-alcoholic fatty liver disease, or other non-viral liver disorders; recent (within one month) use of antibiotics, probiotics, prebiotics, or synthetic microbial agents; active bacterial, fungal, chlamydial, or viral infections; confirmed diagnosis of irritable bowel syndrome, inflammatory bowel disease, or other autoimmune diseases; and incomplete clinical data. Survival information was obtained from hospital records or through direct communication with patients and their families, with death or liver transplantation considered as primary endpoints. This study was registered in the National Human Resources Database (CJ1253) and approved by the Ethics Committee of the First Affiliated Hospital of Zhejiang University School of Medicine (2020454). All participants provided written informed consent.

Self-healing and non-self-healing types of AHE-elderly

Self-healing AHE-elderly: (1) Significant improvement in symptoms such as jaundice, nausea, vomiting, and abdominal pain, accompanied by recovery of liver function. (2) Marked improvement in liver function indicators (alanine aminotransferase (ALT)/aspartate aminotransferase (AST) ratio between 0.6 and 1.25), with total bilirubin < 5 mmol/L and international normalized ratio < 1.5. (3) Negative results for anti-HEV IgM, anti-HEV IgG, and hepatitis E ribonucleic acid.

Non-self-healing AHE-elderly: (1) Aggravation of clinical symptoms and signs, including jaundice, nausea, vomiting, and abdominal pain. (2) Deterioration in liver function indicators (ALT, AST). (3) Development of new complications and/or failure of extrahepatic organs. (4) Persistent positivity for anti-HEV IgM, anti-HEV IgG, and hepatitis E ribonucleic acid.

Specimen collection and processing

Fresh fecal samples from patients were collected immediately in sterile plastic containers and stored at −80°C. Bacterial genomic DNA was extracted within 15 m of thawing. The cetyltrimethylammonium bromide (CTAB) and sodium dodecyl sulfate method was employed for total genomic DNA extraction. DNA concentration and purity were assessed using 1% agarose gel electrophoresis. The total genomic DNA of fecal samples was extracted using the CTAB method. 1,000 μL of CTAB lysis buffer and 20 μL of lysozyme were added to a 2.0 mL microcentrifuge tube. An appropriate amount of fecal sample was added, and the mixture was incubated at 65°C for 2–3 h with periodic inversion. Following centrifugation, 0.95 mL of the supernatant was transferred to a new tube and mixed with an equal volume of phenol:chloroform:isoamyl alcohol (25:24:1). After centrifugation at 12,000 rpm for 10 m, the aqueous phase was transferred to a new tube and extracted again with an equal volume of chloroform:isoamyl alcohol (24:1). After another centrifugation at 12,000 rpm for 10 m, the supernatant was transferred to a 1.5 mL microcentrifuge tube, and isopropyl alcohol was added at 75% of the supernatant volume. The mixture was gently inverted and incubated at −20°C for precipitation. After a final centrifugation (12,000 rpm for 10 m), the supernatant was discarded, and the DNA pellet was washed twice with 1 mL of 75% ethanol. Any remaining liquid was removed by brief centrifugation and aspiration with a micropipette. The DNA pellet was air-dried and then dissolved in 51 μL of ultrapure water, followed by incubation at 55–60°C for 10 m. RNA was digested using 1 μL of RNase A at 37°C for 15 m.

PCR and pyrosequencing

Targeted amplification of hypervariable regions (V4, V3, V3–V4, and V4–V5) of the 16S rRNA gene was performed using region-specific primer pairs (e.g., 515F–806R for the V4 region). PCR reactions were conducted using Phusion® High-Fidelity PCR Master Mix (NEB), with 0.2 μM of each forward and reverse primer and approximately 10 ng of genomic DNA template. Thermal cycling conditions included an initial denaturation at 98°C for 60 s, followed by 30 cycles of denaturation, annealing, and extension. PCR products were pooled

Table 1. Analysis of baseline characteristics of AHE-elderly and HCs-elderly population

Variables	HCs-elderly (n = 30)	AHE-elderly (n = 58)	*p*
Age	54.83 ± 14.33	59.71 ± 11.14	0.111
Gender(M/F)	15/15	38/20	0.159
WBC	5.87 [4.94, 8.23]	5.99 [4.64, 7.71]	0.588
CRP	3.71 [2.91, 4.04]	10.48 [6.09, 16.40]	<0.001
PLT	241.33 ± 40.73	158.60 ± 77.70	<0.001
ALT	23.10 [15.60, 32.85]	512.20 [86.42, 1,033.00]	<0.001
AST	19.20 [15.98, 23.22]	186.05 [63.50, 565.60]	<0.001
TBIL	13.60 [11.01, 16.24]	156.55 [67.49, 240.88]	<0.001
ALB	46.56 ± 4.37	34.71 ± 5.70	<0.001
UREA	4.92 [4.00, 5.82]	4.65 [3.70, 6.18]	0.625
Cr	60.70 [54.20, 69.48]	64.00 [55.00, 78.25]	0.113
TCH	5.25 [4.62, 5.70]	3.04 [2.30, 4.10]	<0.001
AFP	3.34 [1.98, 4.81]	9.60 [2.93, 58.93]	<0.001
INR	1.06 [0.90, 1.23]	1.78 [1.62, 1.97]	<0.001

WBC, white blood cell; CRP, C-reactive protein; PLT, platelet; ALT, alanine aminotransferase; AST, glutamic oxaloacetic transaminase; TBIL, total bilirubin; ALB, albumin; UREA, urea nitrogen; Cr, creatinine; TCH, total cholesterol; AFP, alpha fetoprotein; INR, international normalized ratio.

in equimolar amounts and purified using the QIAquick Gel Extraction Kit. A sequencing library was then prepared using the TruSeq® DNA PCR-Free Sample Preparation Kit. Library quality was assessed with the Qubit® 2.0 Fluorometer and Agilent Bioanalyzer 2100. Sequencing was performed on the Ion S5™ XL platform.

Amplicon library construction

The variable regions (V3, V4, V3–V4, and V4–V5) of the bacterial 16S rRNA gene were PCR-amplified using unique barcoded primers designed for each segment. For instance, the primer pair 515F–806R was used for the V4 region. Amplification was performed using Phusion® High-Fidelity PCR Master Mix (New England Biolabs), containing 0.2 μM of each primer and approximately 10 ng of template DNA. The thermal cycling protocol consisted of an initial denaturation at 98°C for 1 m, followed by 30 cycles of denaturation at 98°C for 10 s, annealing at 50°C for 30 s, and extension at 72°C for 30 s with a final elongation step at 72°C for 5 m.

PCR amplicons were normalized to equimolar concentrations and purified using the QIAquick Gel Extraction Kit (Qiagen). The sequencing library was constructed following the TruSeq® DNA PCR-Free protocol (Illumina) with unique indexing. Library quality was assessed using the Qubit® 2.0 Fluorometer (Thermo Fisher Scientific) and the Agilent Bioanalyzer 2100 system.

HEV antibody detection

Anti-HEV IgM and IgG antibodies were detected using a commercial HEV enzyme-linked immunosorbent assay (ELISA) kit (Beijing Wantai Company). A sample with an optical density >1.1 was considered positive, while a sample with an optical density ≤1.1 was considered negative.

HEV RNA detection

Total RNA was extracted from serum samples using a viral nucleic acid purification kit (Hangzhou Aikang, China), according to the manufacturer's instructions. HEV RNA was detected by real-time fluorescent quantitative PCR. 348-nucleotide fragments of the HEV open reading frame 2 were amplified by nested PCR, and HEV genotypes were identified through sequencing. The viral load for each sample was quantified using a diagnostic HEV RNA detection kit (Hangzhou Aikang Company, China), following the manufacturer's protocol.

Bioinformatics analysis

QIIME software (version 1.9.0) was used for sequence length trimming, quality filtering, demultiplexing, and taxonomic classification. Operational taxonomic unit (OTU) estimations, rarefaction curves, Shannon indices, and Simpson indices were used to assess bacterial diversity and abundance. The OTU table was analyzed using LDA Effect Size (LEfSe) to identify taxa with significant differential abundance. Further analysis was performed using the STAMP metagenomic analysis software.

Statistical analysis

Statistical analyses were conducted using SPSS software (version 18.0; SPSS Inc., Chicago, IL, USA) and the STAMP metagenomic analysis software. The predictive value of *Bacteroides fragilis* was evaluated using receiver operating characteristic curve analysis, with the area under the curve (AUC) calculated; an AUC > 0.5 indicated predictive ability. Continuous variables were presented as mean ± standard deviation (SD) and analyzed using Student's t-test. A p-value < 0.05 was considered statistically significant.

Results

Baseline characteristics of study subjects and sequencing data quality

This study enrolled 88 participants, comprising 58 elderly patients with AHE and 30 age- and gender-matched HCs-elderly. No significant differences were observed between the AHE-elderly and HCs-elderly groups in terms of gender distribution, age, white blood cell count, or racial composition. Table 1 presents the baseline characteristics and clinical profiles of the study population. Compared to healthy controls, AHE-elderly

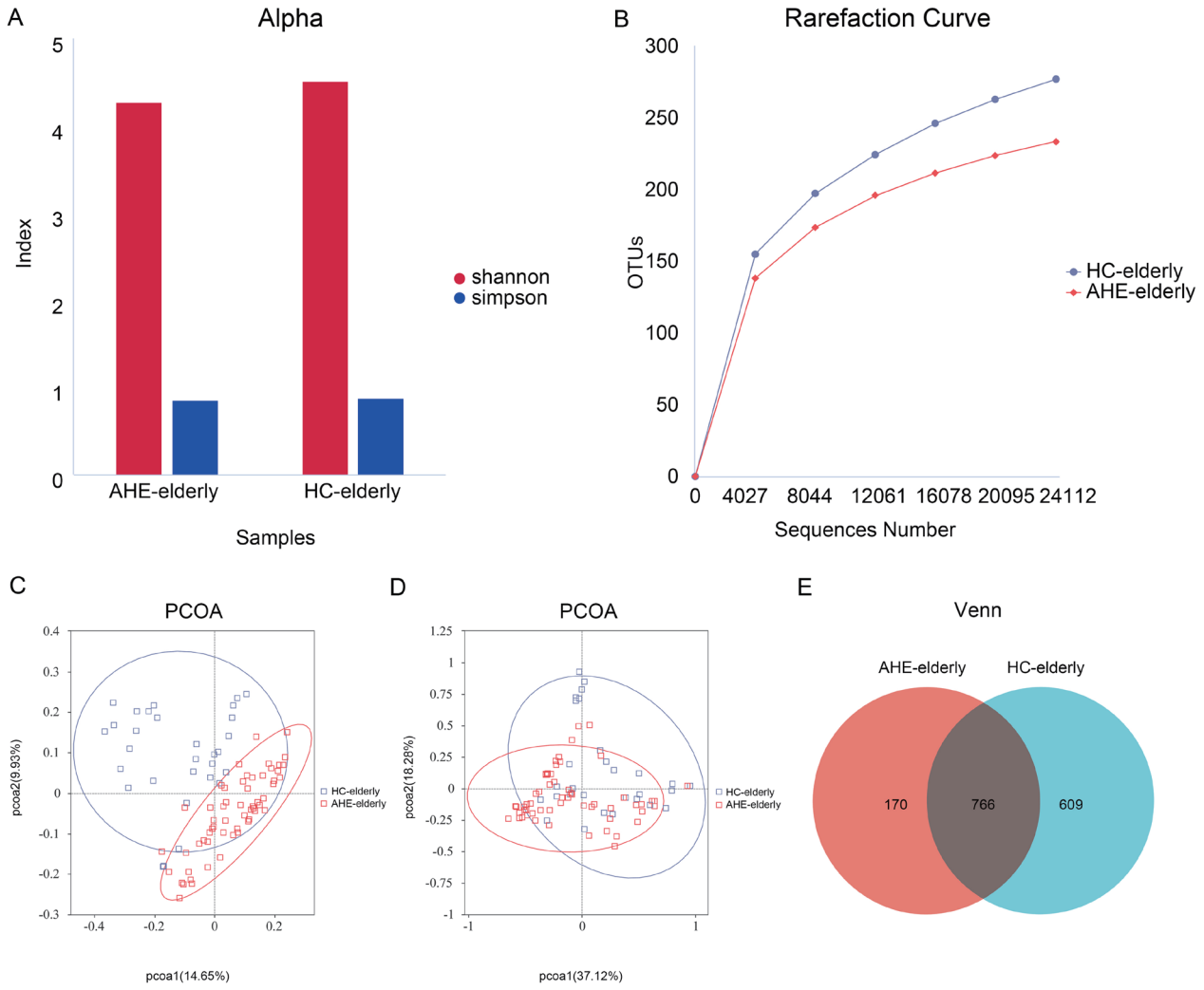

Fig. 1. Diversity of gut microbiota. (A) Shannon and Simpson indices between the AHE-elderly group and the HCs-elderly group. (B) Rarefaction curves between the AHE-elderly group and the HCs-elderly group. (C) PCoA plot based on unweighted UniFrac distance. (D) PCoA plot based on weighted UniFrac distance. (E) Venn diagram illustrating overlap in OTUs within the fecal microbiota between the AHE-elderly group and the HCs-elderly group. HC, healthy control; AHE, acute hepatitis E; PCoA, Principal Co-ordinates Analysis.

patients demonstrated significantly reduced platelet counts, total cholesterol, and albumin levels, as well as elevated levels of AST, ALT, total bilirubin, C-reactive protein, international normalized ratio, and alpha-fetoprotein. All samples were sequenced using the Ion S5TM XL sequencing platform with a single-end sequencing strategy to construct small fragment libraries. After trimming and filtering, an average of 83,374 reads per sample was obtained. Following quality control, an average of 78,641 valid reads was retained, resulting in a quality control efficiency of 94.32%. Across the 88 samples, a total of 7,336,872 raw reads and 6,920,442 clean reads were generated, with an average read length of 412.

Altered bacterial diversity in AHE-elderly group

Species richness refers to the number of bacterial species detected in the OTU distribution of a sample. Alpha diversity was assessed using the Simpson and Shannon indices to compare microbial diversity between the AHE-elderly and HCs-elderly groups, revealing no statistically significant differences (both $p > 0.05$; Fig. 1A). Rarefaction curves plateaued for both groups, indicating that sequencing depth was sufficient to capture most genera present in individual samples and to adequately reflect microbial diversity. Rarefaction analysis suggested a lower trend in species richness in the AHE-elderly group compared to the HCs-elderly group (Fig. 1B). Due to high inter-individual variability, clustering of the fecal microbiota based on community composition was not observed using either unweighted or weighted UniFrac distance metrics. Similarly, principal coordinates analysis did not reveal clear separation between the groups (Fig. 1C, D) Cluster analysis based on bacterial richness was conducted by comparing shared and unique OTUs between the two groups, visualized with a Venn diagram (Fig. 1E). A total of 766 OTUs were shared, while 170 OTUs were uniquely present in the AHE-elderly group.

Altered gut microbiota composition in the AHE-elderly group

We further characterized the gut microbiota composition in the AHE-elderly group compared to the HCs-elderly group using

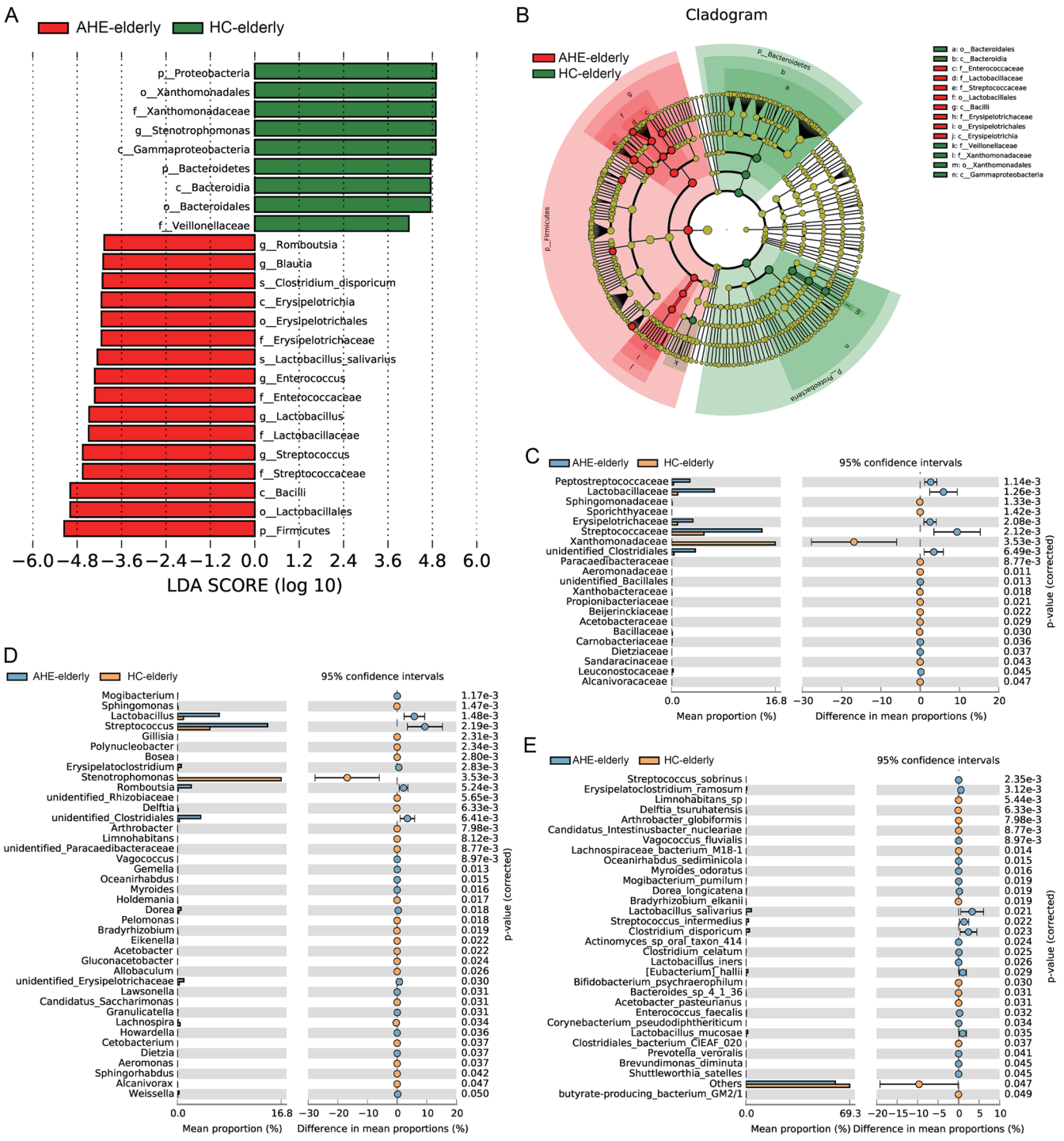

Fig. 2. Taxonomic differences in gut microbiota composition between AHE-elderly and HCs-elderly groups. Linear discriminant analysis and effect size measurements identified the greatest differences between the two groups (A). A linear discriminant analysis-based model was used to determine the evolution of bacterial rank characteristics (B). The relative abundance of bacteria at the family (C), genus (D), and species (E) levels in the two groups of samples was compared. HC, healthy control; AHE, acute hepatitis E.

LEfSe metagenomic analysis. An increased abundance of *Firmicutes*, *Lactobacillales*, and *Bacilli* was observed in AHE-elderly patients, suggesting a potential role of these taxa in disease progression. Conversely, *Proteobacteria*, *Xanthomonas*, *Gammaproteobacteria*, and *Bacteroidetes* were more abundant in the HCs-elderly group (Fig. 2A, B). At the family level, the relative abundances of *Peptostreptococcaceae*, *Lactobacillaceae*, *Erysipelotrichaceae*, and *Streptococcaceae* were higher in the AHE-elderly group, while *Xanthomonadaceae* was more abundant in the HCs-elderly group (Fig. 2C). At

Table 2. Analysis of baseline characteristics of self-healing and non-self-healing in AHE-elderly

Variables	Self-healing (n = 46)	Non-self-healing (n = 12)	*p*
Age	57.98 ± 11.18	66.33 ± 8.42	0.019
Gender	29/17	9/3	0.515
WBC	6.04 [4.66, 7.82]	5.80 [4.54, 7.20]	0.652
CRP	10.34 [5.37, 18.47]	10.50 [8.02, 14.34]	0.810
PLT	162.65 ± 74.33	143.08 ± 91.38	0.442
ALT	468.50 [81.78, 1,033.00]	869.60 [359.92, 1,375.00]	0.219
AST	161.50 [61.25, 499.58]	444.95 [68.00, 1,104.25]	0.304
TBIL	97.97 [52.32, 222.75]	283.40 [187.05, 470.40]	<0.001
ALB	34.65 ± 5.81	34.95 ± 5.52	0.872
UREA	4.50 [3.67, 5.93]	5.40 [4.28, 16.75]	0.132
Cr	63.60 [54.72, 73.72]	71.80 [62.42, 103.08]	0.052
TCH	3.04 [2.37, 4.10]	2.98 [1.95, 4.12]	0.514
AFP	9.60 [3.54, 79.72]	10.11 [2.27, 34.15]	0.571
INR	1.72 [1.58, 1.94]	2.10 [1.77, 2.71]	0.003
Hospital stay	13.93 ± 6.85	19.33 ± 10.55	0.035

WBC, white blood cell; CRP, C-reactive protein; PLT, platelet; ALT, alanine aminotransferase; AST, glutamic oxaloacetic transaminase; TBIL, total bilirubin; ALB, albumin; UREA, urea nitrogen; Cr, creatinine; TCH, total cholesterol; AFP, alpha fetoprotein; INR, international normalized ratio.

the genus level, *Lactobacillus*, *Streptococcus*, and *Roseburia* were more prevalent in the AHE-elderly group, whereas *Stenotrophomonas* was more abundant in the HCs-elderly group (Fig. 2D). At the species level, *Lactobacillus salivarius* and *Clostridium disporicum* were significantly more abundant in the AHE-elderly group compared to the HC group, while the relative abundance of other species was lower (Fig. 2E).

Collection of 16S data in AHE-elderly patients: Self-healing and non-self-healing groups

No statistically significant differences were observed in gender, age, white blood cell count, or race between the self-healing and non-self-healing groups among elderly patients with acute AHE ($p > 0.05$; Table 2). A total of 4,886,844 raw reads and 4,600,398 clean reads were obtained from the 58 AHE samples, with an average read length of 412 base pairs.

Gut microbiota diversity in self-healing and non-self-healing AHE-elderly groups

The ecological characteristics of the fecal bacterial communities were assessed using multiple metrics based on OTU levels in both the self-healing and non-self-healing AHE-elderly groups. The results indicated no significant differences in microbial diversity between the two groups. Both rarefaction analysis and alpha diversity indices revealed no statistically significant variation ($p > 0.05$; Fig. 3A, B).

Furthermore, principal coordinates analysis showed no clear separation between the two groups when analyzed using unweighted UniFrac and weighted UniFrac (Fig. 3C, D). The Venn diagram illustrated the overlap in OTUs between the self-healing and non-self-healing groups, confirming a high degree of similarity in microbial composition (Fig. 3E).

Alterations in gut microbiota composition between self-healing and non-self-healing AHE-elderly groups

Taxonomic differences in gut microbiota composition between the two groups were analyzed using LEfSe, applying a logarithmic LDA score cutoff of 3.0. Significant variations were identified in the relative abundance of several bacterial taxa. *Firmicutes, Bacillus, Lactobacillus, Streptococcus,* and *Peptostreptococcus* were enriched in the self-healing group. In contrast, *Bifidobacteriaceae* and *Bacteroidia* were significantly more abundant in the non-self-healing group (Fig. 4A, B). At the family level, *Bacteroidaceae* was significantly elevated in the non-self-healing group compared to the self-healing group (Fig. 4C). Moreover, *Bacteroides* and *Bacteroides fragilis* were notably more abundant in the non-self-healing group (Fig. 4D, E). These findings demonstrate that *Bacteroides* represents the key microbial taxon driving compositional differences in intestinal microbiota between the two cohorts

The diagnostic potential of *Bacteroides fragilis* was evaluated using receiver operating characteristic curve analysis of microbiota data, yielding an AUC of 0.873 (Fig. 4F). This result indicates that *Bacteroides fragilis* abundance could effectively distinguish self-healing from non-self-healing AHE-elderly patients.

Discussion

This study represents the first systematic investigation of the gut microbiota in elderly HEV-infected patients compared to healthy controls, while also analyzing differences between self-healing and non-self-healing individuals. Specific microbial taxa and functional changes associated with the disease were identified, which may serve as key factors in HEV-induced gut microbiota dysbiosis and potential biomarkers for distinguishing self-healing from non-self-healing in elderly patients with AHE, particularly through the abundance of *Bacteroides*.

In this study, we assessed the ecological characteristics of gut microbial communities in elderly patients with AHE and HCs at the OTU level using the Shannon and Simpson indices. The analysis revealed no significant differences in microbial diversity between the two groups. Additionally, we compared gut microbiota diversity between the self-healing and non-

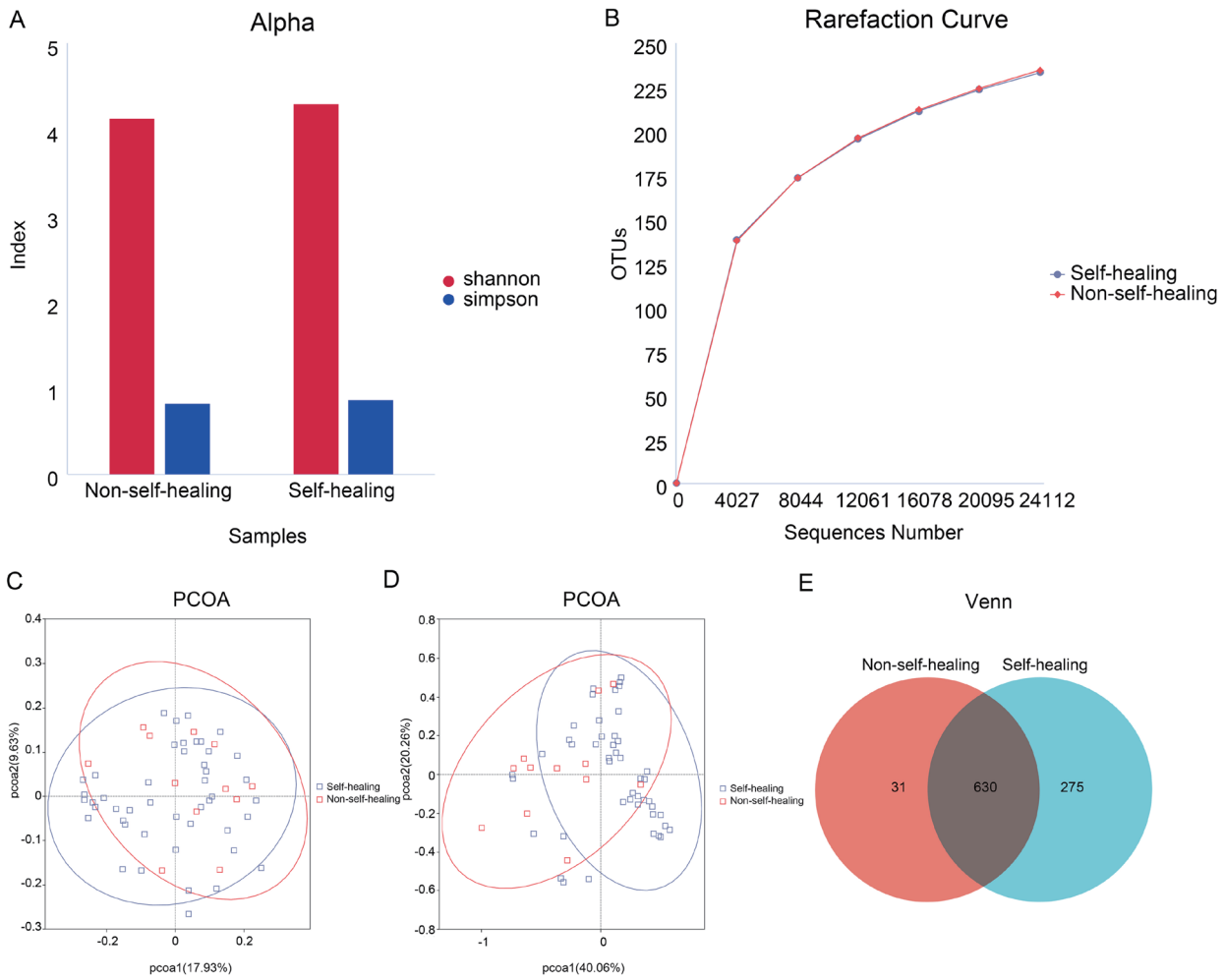

Fig. 3. Diversity of gut microbiota differs between self-healing and non-self-healing in AHE-elderly. (A) Shannon and Simpson indices between self-healing and non-self-healing AHE-elderly. (B) Rarefaction and genera accumulation curves between self-healing and non-self-healing groups. (C, D) PCoA plot based on unweighted and weighted UniFrac distances. (E) Venn diagram illustrating overlap in OTUs within the fecal microbiota between self-healing and non-self-healing groups. AHE, acute hepatitis E; PCoA, Principal Co-ordinates Analysis; OUT, Operational Taxonomic Unit.

self-healing groups in AHE-elderly patients but again found no significant differences. These findings align with our previous studies, which also reported no significant variations in microbial diversity between AHE patients, healthy controls, and HEV-ALF groups.[20,21] These results collectively suggest that alterations in gut microbiota diversity may not play a critical role in the clinical progression or outcomes of HEV infection. Instead, the functional and compositional changes in specific microbial taxa, rather than overall diversity, may hold greater clinical significance in understanding disease mechanisms and identifying potential therapeutic targets.

The compositional analysis of the gut microbiota demonstrated that, in contrast to the HCs-elderly group, the gut microbial composition of the AHE-elderly group was characterized by an enrichment of *Firmicutes*, *Lactobacillales*, *Bacilli*, and *Streptococcaceae* (Fig. 3). Simultaneously, we identified disparate and specific microbiota in various biological categories, such as *Peptostreptococcaceae, Erysipelotrichaceae, Lactobacillus, Streptococcus, Roseburia, Lactobacillus salivarius*, and *Clostridium disporicum*. These microbial taxa were more abundant in AHE-elderly individuals than in HCs-elderly individuals. This finding aligns with a previous study by Heidrich *et al*., which demonstrated elevated *Streptococcus* and *Lactobacillus* levels in cirrhotic patients, suggesting a potential link between these bacteria and liver-related conditions.[17] Yan *et al*. demonstrated that patients with hepatitis B virus-related hepatocellular carcinoma showed a significant increase in the abundance of *Bacillales*, *Lactobacillales*, and *Lactobacillus.*[25] The abundance of *Firmicutes* was found to be higher, while that of *Bacteroidetes* and *Proteobacteria* was lower, in AHE-elderly patients compared to HCs-elderly. These findings are inconsistent with prior observations regarding gut microbial diversity in chronic hepatitis B patients.[26,27] We hypothesize that this outcome might be associated with the infection pathway of the hepatitis E virus. Furthermore, gut microbial diversity is influenced by multiple variables, including age, dietary habits, and antibiotic exposure. As individuals age, there is a notable decline in both the diversity and richness of the gut microbiota.[28,29] The study by Wu and colleagues revealed a significant reduction in the diversity of gut microbiota among the oldest participants, specifically centenarians. Several beneficial bacterial strains exhibited either increased or decreased abundance, while harmful bacterial species followed a comparable pattern.[30]

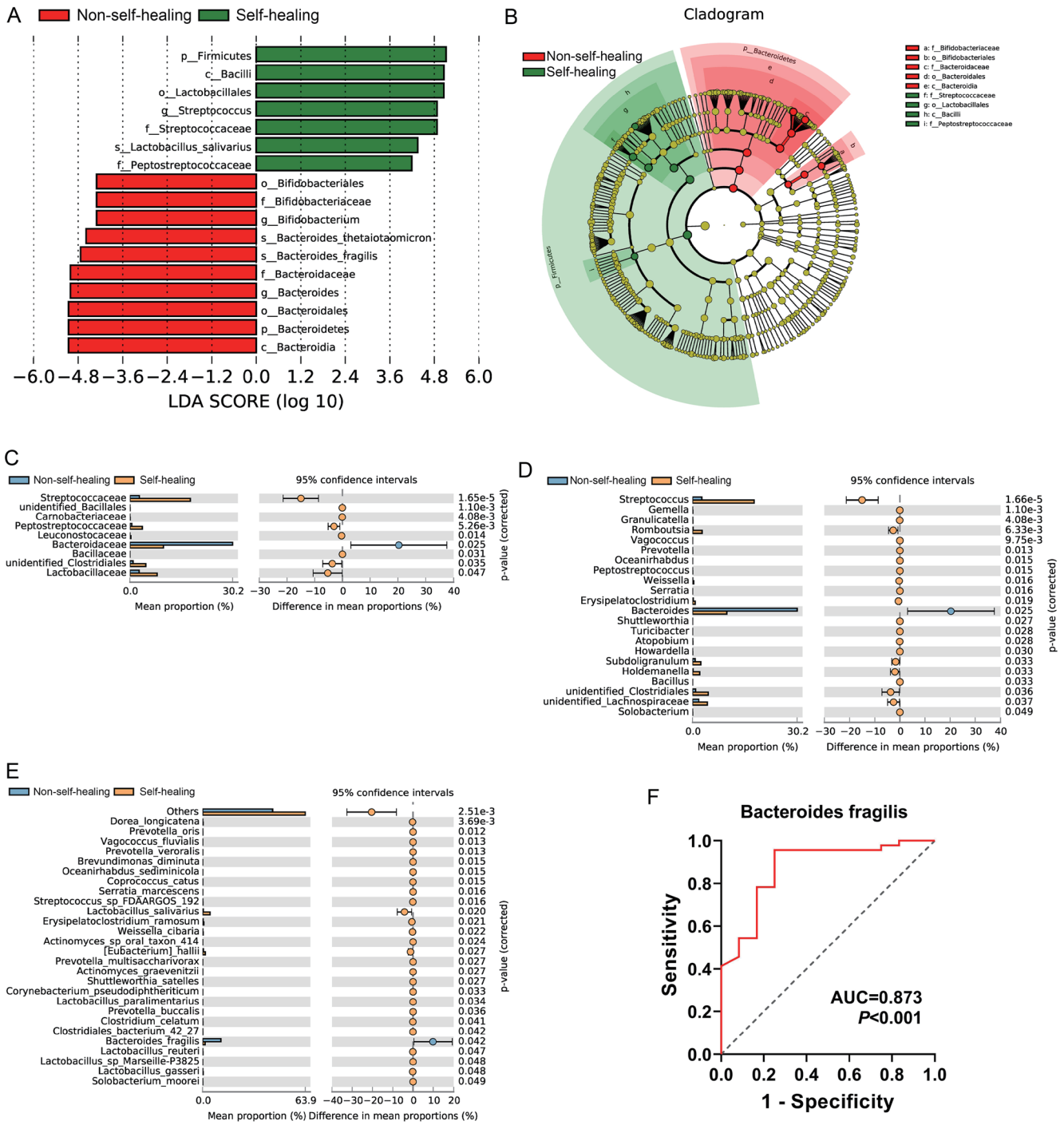

Fig. 4. Taxonomic differences in fecal microbiota composition between the self-healing and non-self-healing groups of AHE-elderly. Linear discriminant analysis and effect size measurements identified the groups with the greatest differences between the two groups (A). An evolutionary map (B) of bacterial rank characteristics determined by a linear discriminant analysis-based model was used. The relative abundance of bacterial family (C), genus (D), and species (E) in the two groups of samples was compared. *Bacteroides fragilis* distinguished the self-healing group from the non-self-healing group in AHE-elderly (F). AHE, acute hepatitis E; LDA, Linear Discriminant Analysis.

In an effort to explore the association between the development outcome of AHE in the elderly population and the intestinal microbiota, we examined the alterations of the intestinal microbial community in elderly patients belonging to the self-healing AHE group and the non-self-healing AHE group. The results indicated that, in contrast to the self-healing group, the abundances of *Bifidobacteriaceae* and *Bacteroidia* in the non-self-healing group were significantly higher. At the species level, *Bacteroides fragilis* was most abundant in the non-self-healing group of AHE-elderly patients. This element

substantially contributed to the microbial divergence characterizing the two cohorts.

The association between the gut microbiota composition and clinical outcomes in elderly patients with hepatitis E suggests that *Bacteroides* plays a pivotal role. The abundance of *Bacteroides fragilis* in fecal microbiota samples can serve as a predictor for the severity of AHE in elderly patients, achieving an AUC of 0.873. Species within the *genus Bacteroides* are major colon inhabitants and form a significant part of the gut microbiome.[31–34] While they can be beneficial in maintaining gut homeostasis, they may also act as opportunistic pathogens in other parts of the body.[32,33] Therefore, we hypothesize that in the context of HEV infection, *Bacteroides* may play a dual role: modulating the host's immune response through the production of metabolites such as polysaccharide degradation products, while simultaneously exacerbating liver injury by promoting inflammatory responses.

While our findings reveal microbiome-disease associations, the absence of longitudinal comparison data on microbiota changes between baseline and follow-up periods limits our understanding of microbial community dynamics, warranting future longitudinal investigations. The effect of these results in specific populations, such as those with cerebrovascular diseases and underlying liver conditions, requires stratified research. In addition, the causal relationship between microbial imbalance and HEV infection requires clarification through longitudinal studies and germ-free animal models. Future research should also investigate whether HEV genotype variations induce distinct microbial alterations and explore potential microbiome-based antiviral or immunomodulatory therapeutic strategies.

Conclusions

Our study reveals significant differences in the gut microbiota composition between elderly patients with AHE and healthy controls. The relative abundances of *Bacteroidetes*, *Lactobacillales*, and *Bacilli* can effectively distinguish AHE patients from HC individuals. Furthermore, the abundance of *Bacteroides* can differentiate self-healing cases from non-self-healing cases among elderly AHE patients. This study identifies Bacteroides fragilis as a potential biomarker for disease outcomes. Future studies should explore the causal relationships between gut microbiota and HEV infection in larger, longitudinal cohorts.

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Conflict of interest

The authors have no conflict of interests related to this publication.

Author contributions

Guaranteeing of the work (JW, RJ, MG), experiment design data acquisition, data analysis, data interpretation, drafting of the manuscript, statistical analysis (ML, MS, GJ, MG), performing the main experiments (LW, YW, ZX), critical revision of the manuscript (JW, HW, RJ). All authors have approved the final version and publication of the manuscript.

Ethical statement

This study was registered in the National Human Resources Database (CJ1253) and approved by the Ethics Committee of the First Affiliated Hospital of Zhejiang University School of Medicine (2020454) in accordance with the Helsinki Declaration as revised in 2024. All subjects provided signed informed consent forms.

Data sharing statement

All data relevant to the study are included in the article.

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