Article

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Identification and Analysis of Gut Microbiota in Different Severity of Sepsis Patients and CLP-induced Rats

Haiyong Zhu[1], Kailun Hu[2], Jiansong Yu[3], Dan Li[4,*]

[1]Department of Critical Care Medicine, The First People's Hospital of Linhai City of Zhejiang Province, 317000 Linhai, Zhejiang, China
[2]Department of Emergency, Taizhou Hospital of Zhejiang Province, 317000 Linhai, Zhejiang, China
[3]Department of Rehabilitation, Taizhou Hospital of Zhejiang Province, 317000 Linhai, Zhejiang, China
[4]Endoscopy Center, Taizhou Hospital of Zhejiang Province, 317000 Linhai, Zhejiang, China
*Correspondence: lid@enzemed.com (Dan Li)
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Background: Sepsis is a critical condition characterized by organ failure due to the body's inappropriate reaction to an infection. The disrupted gut microbiome is constantly changing and contributes to the development of sepsis. This research seeks to explore the makeup and role of gut microbiota in individuals experiencing sepsis or septic shock and the sepsis rat model induced by cecal ligation and puncture (CLP).

Methods: Fecal samples from persons in the healthy control group, sepsis, and septic shock patients were collected. The intestinal flora was examined using 16S rRNA-sequencing analysis. Besides, SD rats were subjected to CLP surgery to create a sepsis model. Post-surgery, mice were euthanized at 6, 12, 24, and 48 h to evaluate inflammatory mediators, intestinal microbiota, morphology, and intestinal barrier markers.

Results: In patients and rats with sepsis, the intestinal barrier was notably disrupted, intestinal permeability was significantly increased, and the inflammatory level was conspicuously elevated ($p < 0.05$). In sepsis patients and CLP-induced rats, the variety and balance of gut microbiota were reduced. Compared with the control group, sepsis patients had lower abundances of *Agathobacter*, *Coprococcus*, *Erysipelotrichaceae_UCG-003*, *Faecalibacterium*, *Fusicatenibacter*, *Haemophilus*, *Roseburia* and *Subdoligranulum*, but increased abundances of *Corynebacterium* and *Enterococcus* compared to the control group. CLP rats exhibited more severe cortical inflammation and enhanced intestinal permeability. The higher *Bacillus*, *Sutterella*, *Odoribacter*, *Pseudomonas*, *Brochothrix*, *Clostridium*, *Enterococcus*, and *Ruminococcus* abundances at the genus level were shown in CLP surgery after 6, 12, 24, and 48 h. KEGG analysis revealed a significant enrichment in carbohydrate metabolism, cofactors and vitamins metabolism, terpenoid and polyketide metabolism, as well as amino acid metabolism.

Conclusion: Taken together, beneficial bacteria like *Agathobacter*, *Coprococcus*, *Erysipelotrichaceae_UCG-003*, and *Faecalibacterium* decline, while harmful species such as *Corynebacterium* and *Enterococcus* increase, which might contribute to triggering intense inflammation and compromising intestinal barrier function in the progression of sepsis. These shifts highlight potential microbial targets for sepsis treatment.

Keywords: sepsis; gut microbiota; intestinal barrier; cecal ligation and puncture

Introduction

Sepsis is a systemic inflammatory response syndrome (SIRS) triggered by various infectious agents [1]. It can progress to Septic Shock and Multiple Organ Dysfunction Syndrome (MODS). Sepsis poses a significant threat to human health due to its three characteristics: high prevalence, high mortality rate, and high treatment cost. A previous study found sepsis incidence in the intensive care unit (ICU) varied from 13.6% to 39.3%, with ICU and hospital fatalities at 25.8% and 35.3%, respectively [2]. Infections, surgical procedures, traumatic injuries, burns, hemorrhages, or even bacterial translocation resulting from gut ischemia-reperfusion can all trigger sepsis, which may rapidly escalate to septic shock, multiple organ failure, and other severe complications [3]. That's precisely why it's considered one of the greatest challenges in intensive care. Therefore, early identification, diagnosis, and prevention of sepsis have emerged as critical clinical priorities that demand urgent resolution.

The gut is hypothesized to play a vital role in the development of sepsis [4]. It is widely recognized that the gastrointestinal (GI) microbiota significantly influences the host's metabolic, immune, and endocrine systems [5]. Research indicates that the intestines have a dual role in sepsis, serving both as a primary site of injury and as a key driver of systemic damage [6]. When gastrointestinal function is

impaired, pathogenic bacteria and toxins can breach the intestinal barrier and enter the bloodstream via the mesenteric lymph nodes [7]. This triggers a widespread inflammatory response, ultimately leading to multiple organ failure. Sepsis reduces microbial diversity, increases the abundance of potentially harmful pathogens, and decreases beneficial microorganisms associated with health [8]. This imbalance disrupts the microbiota's metabolic functions and contributes to intestinal barrier dysfunction. Thus, the microbiome may represent a critical link between infection and dysregulated systemic inflammation in sepsis [9]. Currently, the relationship between sepsis severity, intestinal barrier permeability, and gut microbiota alterations remains poorly understood. Identifying specific microbial signatures associated with different stages of sepsis may help develop innovative therapeutic strategies for this condition.

In the present study, we evaluated the function of the intestinal barrier in patients with sepsis and in a cecal ligation and puncture (CLP) rat model, characterized microbiome alterations through 16S rDNA sequencing, and investigated the patterns of intestinal flora changes and their correlations with disease severity and prognosis, thereby providing a theoretical basis for the clinical management of sepsis.

Materials and Methods

Patients and Study Design

This study included 20 patients with sepsis admitted to the First People's Hospital of Linhai City (Zhejiang, China) between March 2024 and December 2024. Among them, 10 patients with septic shock were admitted to the intensive care unit (ICU), and 10 patients with sepsis received standard antibiotic therapy. The diagnostic criteria for sepsis followed the Sepsis 3.0 guidelines outlined in the *International Guidelines for the Management of Sepsis and Septic Shock* (2016) [10]. The Sequential Organ Failure Assessment (SOFA) score was used to assess organ dysfunction after infection [11]. The subjects were divided into a sepsis group (n = 10) and a septic shock group (n = 10). Additionally, a control group of healthy individuals undergoing routine physical examinations (n = 10) was included, with no significant differences in age or gender among the groups. This study was approved by the Ethics Committee of the First People's Hospital of Linhai City, Zhejiang Province (No. 2020-0005), conducted in accordance with the Declaration of Helsinki, and written informed consent was obtained from all participants.

Inclusion and Exclusion Criteria

Sepsis was defined as infection-induced dysfunction of one or more organs. The SOFA score (0–3) was used to identify patients at risk. One point was assigned for each of the following: systolic blood pressure $\leq$100 mmHg, respiratory rate $\geq$22 breaths/min, or acute changes in mental status. Mental status was assessed using the Glasgow Coma Scale (GCS), with one point given for a GCS $<$13 [12]. A SOFA score $\geq$2 indicated that sepsis patients required urgent ICU admission for monitoring and intervention. Septic shock was defined by (1) sepsis, (2) persistent hypotension $<$90 mmHg, and (3) serum lactate $>$2 mmol/L despite adequate fluid resuscitation.

Patients were excluded if they met any of the following: (1) pre-existing severe diseases affecting survival (e.g., unresectable tumors, hematologic diseases, cerebrovascular disease with long-term immobility, Alzheimer's disease); (2) acute myocardial infarction within one week; (3) acute poisoning; (4) diagnosed psychiatric disorders; (5) gastrointestinal failure, severe intra-abdominal infection, or complete intestinal obstruction; (6) refusal to participate or provide informed consent.

Data Collection

Patient characteristics, including age, gender, body temperature, respiratory rate, blood pressure, blood gas levels, and heart rate, as well as inflammatory, biochemical, and coagulation markers, were collected. Acute Physiology and Chronic Health Evaluation II (APACHE II) and SOFA scores were calculated using the worst recorded values [13]. Venous blood samples were collected within 24 h of admission to measure diamine oxidase (DAO), D-lactic acid, and intestinal fatty acid-binding protein (IFABP) using an enzyme-based intestinal barrier function analysis system (JY-DLT, ZhongShengJinYu, Beijing, China). Blood gas parameters ($PaCO_2$, PaO_2) were measured with a blood gas analyzer (PT1000, EasyDiagnosis Biomedicine Co., Ltd., Wuhan, China).

Animals and Establishment of Sepsis Rat Model

Forty specific pathogen-free (SPF) adult male SD rats (8 weeks old, 180–220 g) were purchased from SLAC Laboratory Animal Co., Ltd. (Shanghai, China) and housed under standard conditions with free access to food and water. All procedures were approved by the Animal Care and Welfare Committee of Zhejiang Chinese Medical University (SCXK (Zhe)2021-0012). Rats were randomly assigned to Group 1 (Sham, n = 8) or Group 2 (cecal ligation and puncture (CLP), n = 32). Both groups received normal saline by gavage for 5 days before surgery. After that, CLP was performed as previously described [14]. Briefly, rats were anesthetized with inhaled 3% isoflurane (Lot: 22-337, Baxter Healthcare Corporation, Deerfield, IL, USA) and maintained with a mixture of tiletamine/zolazepam (Zoletil 100, 10 mg/kg, i.p., Virbac, Milan, Italy). A midline laparotomy (1–2 cm) exposed the cecum. Feces were gently pressed toward the cecal tip. The cecum was ligated at the midpoint between the cecal valve and tip with a sterile silk suture (No. 4) and punctured with a 21-G sterile needle. A small amount of fecal content was extruded to ensure patency, and the cecum was replaced into the abdomen, which

was closed and sutured in layers. Sham-operated rats underwent identical anesthesia and laparotomy without ligation or puncture. All surgeries were performed aseptically within 10 min. After surgery, rats were monitored until recovery and returned to their cages. At 6, 12, 24, and 48 h after CLP, eight rats per time point were anesthetized with pentobarbital sodium (P3761, 50 mg/kg, i.p., SIGMA, St. Louis, MO, USA), and cardiac blood samples were collected. Euthanasia was performed by intraperitoneal injection of pentobarbital sodium (150 mg/kg). Death was confirmed by apnea, cardiac arrest, and absent corneal reflex. Ileum and colon tissues were collected, snap-frozen in liquid nitrogen, and stored at –80 °C.

Biochemical Markers Analysis

Serum α-amylase 1 (AMS1) (ML14832, Mlbio, Shanghai, China), C-reactive protein (CRP) (RX106373H, Ruixin Biotech, Quanzhou, China), interleukin (IL)-8 (KL-IL-8-Hu) and IL-10 (KL-IL-10-Hu, Kanglang Biotechnology, Shanghai, China) in patients were quantified by enzyme-linked immunosorbent assay (ELISA) per the manufacturer's instructions. Rat serum D-lactose (mlLTW48, Mlbio, Shanghai, China), DAO (CSB-E12634r, CUSABIO, Wuhan, China), IFABP (RX2D302576, Rui Xin Biotechnology, Quanzhou, China), and procalcitonin (PCT) (RX302580R, Rui Xin Biotechnology, Quanzhou, China) were also measured by ELISA. Rat serum and intestinal Tumour Necrosis Factor alpha (TNF-α, RX2D310636, Ruixin Biotech, Quanzhou, China), IL-1β (RX2D302066, Ruixin Biotech, Quanzhou, China), IL-6 (RXG60009, Ruixin Biotech, Quanzhou, China), and IL-8 (ml037351-1, Mlbio, Shanghai, China) were assessed by ELISA. Optical densities were read at 450 nm using a microplate reader (CMaxPlus, Molecular Devices, San Jose, CA, USA).

H&E Staining

Intestinal tissues were fixed with 4% paraformaldehyde (P0099, Beyotime, Shanghai, China), paraffin-embedded, and sectioned at 4 µm (RM2235, Leica, Shanghai, China). Sections were stained with Hematoxylin (H3136, SIGMA, St. Louis, MO, USA) and Eosin (E4009, SIGMA, St. Louis, MO, USA) and examined under a microscope (Eclipse Ci-L, Nikon, Tokyo, Japan) for histopathological assessment. Inflammation severity was scored as 0 (none), 1 (mild infiltration, no necrosis), 2 (marked infiltration with mucosal necrosis), or 3 (transmural necrosis) [15].

Fecal Collection and 16S rRNA Sequencing

Before euthanasia, fecal samples were collected from CLP rats at 0, 12, 24, and 48 h post-surgery, frozen at –80 °C, and sequenced at the Alkek Center for Metagenomics and Microbiome Research-CMMR. DNA was extracted using the MO BIO PowerMag Soil DNA kit (Lot: 12855-50; MO BIO Laboratories, Carlsbad, CA, USA). V4 region libraries were generated on an Illumina MiSeq platform with primers 515 F and 806 R. Sequencing data were processed using QIIME2 to generate OTUs and perform clustering and taxonomic analysis via the Majorbio Cloud platform (https://www.majorbio.com/), enabling α- and β-diversity analyses.

RT-qPCR Assay

Total RNA from rat ileum and colon was extracted using the FastPure Cell/Tissue Total RNA Isolation Kit V2 (RC112-01, Vazyme, Nanjing, China). cDNA synthesis was performed with the PrimeScript RT reagent Kit (RR037A, Takara, Tokyo, Japan). Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) was conducted with TB Green Premix Ex Taq II FAST qPCR (RR820A, Takara, Tokyo, Japan) on the LightCycler® 480 Instrument II (Roche, Switzerland). Primers (Tsingke, Beijing, China) were: Occludin Forward (F): 5′-TGAACAGCCCCCTAATGTGG-3′, R: 5′-CTTTCCCCTTCGTGGGAGTC-3′; zonula occludens-1 (ZO-1) F: 5′-TCGGAGCTCGGGCATTATTC-3′, Reverse (R): 5′-CAGGGCACCATACCAACCAT-3′; β-actin F: 5′-CCTGGACTTCGAGCAAGAGATGG-3′, R: 5′-CAGGAAGGAAGGCTGGAAGAGTG-3′. Relative mRNA levels were calculated by the $2^{-\Delta\Delta Ct}$ method with β-actin as the internal control.

Western Blot Assay

Total protein from rat ileum and colon was extracted with RIPA buffer (P0013B, Beyotime, Shanghai, China) plus protease inhibitor (CW2200S, CoWin Biotech, Taizhou, China). Protein concentration was determined by BCA assay (pc0020, Beyotime, Shanghai, China). Proteins were separated by SDS-PAGE and transferred to polyvinylidene fluoride (PVDF) membranes, blocked with 5% skimmed milk, and probed overnight at 4 °C with primary antibodies: anti-ZO-1 (AF5145, 1:1000, Affinity, Changzhou, China), anti-Occludin (DF7504, 1:1000, Affinity, Changzhou, China), anti-β-actin (81115-1-RR, 1:6000, Proteintech, Wuhan, China). Horseradish peroxidase (HRP)-conjugated secondary antibodies (#7074, Cell Signaling Technology, Beverly, MA, USA) and ECL (WP20005, Thermo Fisher Scientific, Waltham, MA, USA) were used for detection. Bands were quantified using ImageJ software (1.8.0, National Institutes of Health, Bethesda, MD, USA).

Statistical Analysis

Data are presented as mean $\pm$ standard deviation (SD). Statistical analyses were performed with SPSS 22.0 (IBM, Armonk, NY, USA). One-way analysis of variance (ANOVA) with LSD post hoc test was used for multiple comparisons. The Kruskal-Wallis test with Dunnett's post hoc test was applied when normality was not met. $p < 0.05$ was considered statistically significant.

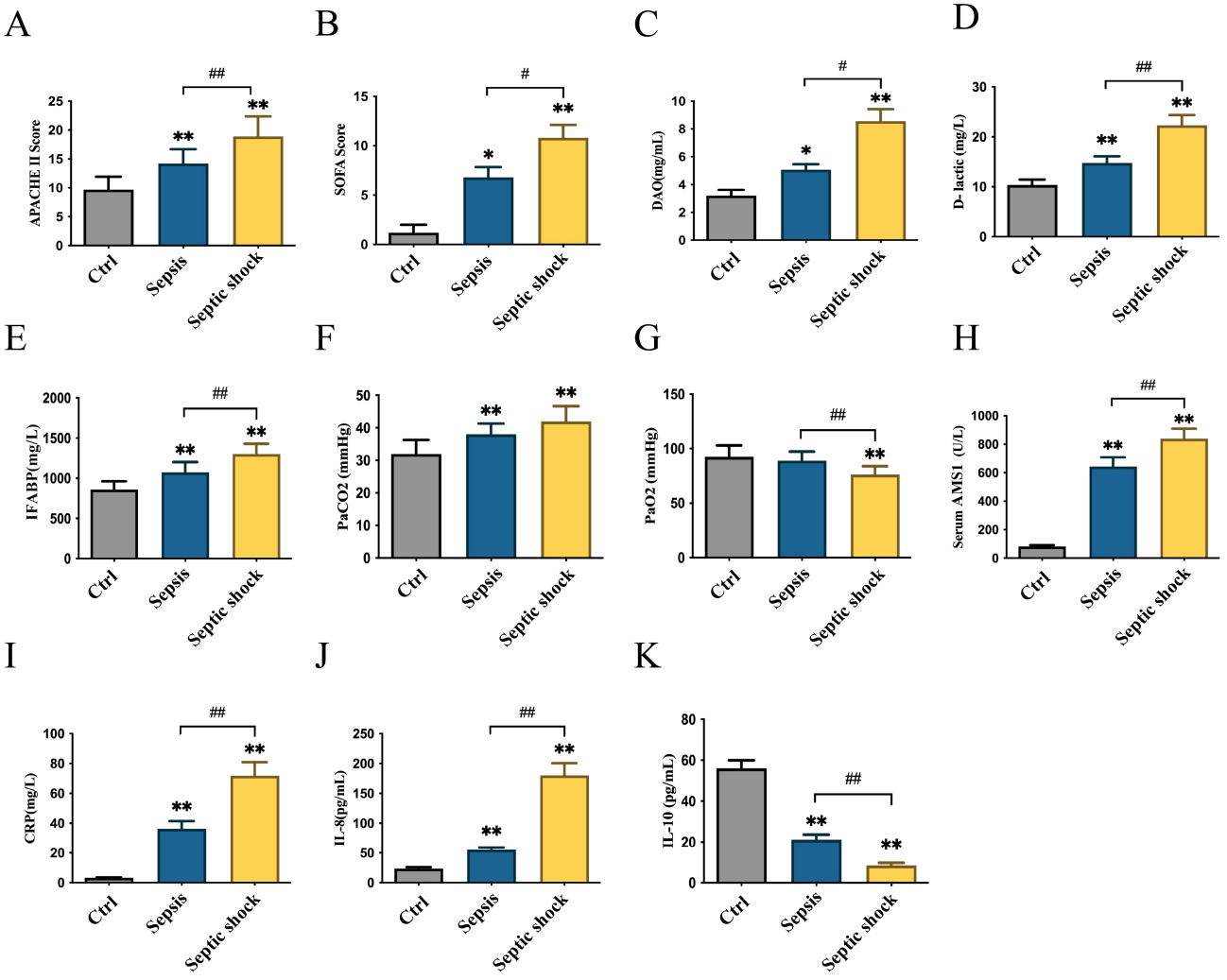

Fig. 1. The levels of DAO, D-lactic acid, IFABP, APACHE II score, SOFA score, and blood gas indicators in each group. (A) Acute Physiology and Chronic Health Evaluation II (APACHE II) score. (B) Sequential Organ Failure Assessment (SOFA) score. (C) Diamine oxidase (DAO) levels. (D) D-lactic acid levels. (E) Intestinal fatty acid-binding protein (IFABP) levels. (F) Partial pressure of carbon dioxide ($PaCO_2$). (G) Partial pressure of oxygen (PaO_2). (H) Serum α-amylase 1 (AMS1) level. (I) C-reactive protein (CRP) levels. (J) Serum interleukin (IL)-8 levels. (K) Serum interleukin-10 (IL-10) levels. Data are shown as the mean $\pm$ standard deviation (SD) (n=10). $^{*}p < 0.05$, $^{**}p < 0.01$ vs. the control group; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ vs. the sepsis group.

Results

Analysis of DAO, D-lactic Acid, IFABP, APACHE II, SOFA Scores, and Blood Gas Indexes in Patients With Sepsis

As shown in Fig. 1A–G, the APACHE II score, SOFA score, and the levels of DAO, D-lactic acid, IFABP, and $PaCO_2$ were significantly increased in both the sepsis and septic shock groups compared with the control group ($p < 0.05$). The PaO_2 level in the septic shock group was significantly decreased compared with the control group ($p < 0.05$). Furthermore, compared with the sepsis group, the septic shock group showed significantly higher APACHE II and SOFA scores, as well as elevated levels of DAO, D-lactic acid, and IFABP ($p < 0.05$), while the PaO_2 level was significantly lower ($p < 0.05$). In addition, the serum levels of AMS1, CRP, and IL-8 were significantly increased in both the sepsis and septic shock groups compared with controls ($p < 0.05$), whereas the serum IL-10 level was significantly decreased (Fig. 1H–K, $p < 0.05$). Notably, the CRP and IL-8 levels were significantly higher in the septic shock group than in the sepsis group ($p < 0.05$; Fig. 1I,J).

Analysis of Intestinal Flora in Patients With Sepsis

To explore the gut microbiota in patients with sepsis and septic shock, 16S rRNA sequencing was performed. The alpha diversity indices showed that the septic shock group had lower Ace, Chao1, and Shannon indexes compared with the other groups, although the differences were not statistically significant (Fig. 2A–C). Principal coordi-

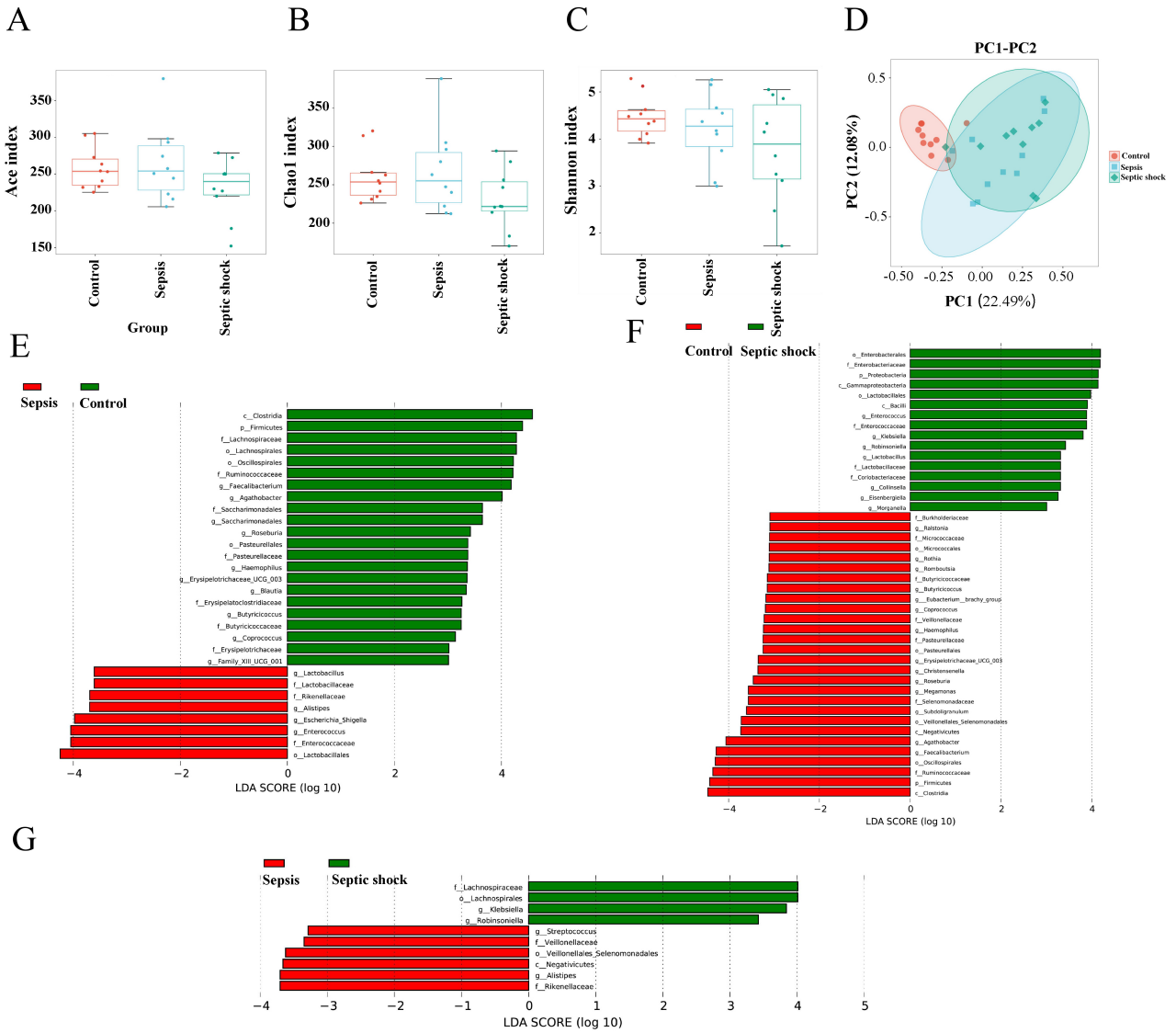

Fig. 2. The gut microbiota in sepsis patients. (A–C) Ace, Chao1, and Shannon indexes. (D) Principal coordinate analysis (PCoA) of three groups. (E–G) Linear Discriminant Analysis Effect Size (LEfSe) analysis. PC1, Principal component 1; PC2, Principal component 2.

nate analysis (PCoA) demonstrated clear separation between the control group and the sepsis groups (Fig. 2D). Linear Discriminant Analysis Effect Size (LEfSe) analysis revealed that patients with sepsis had increased abundances of *Lactobacillus*, *Enterococcus*, *Escherichia-Shigella*, and *Alistipes* at the genus level (Fig. 2E). Moreover, the septic shock group showed higher abundances of *Enterococcus*, *Klebsiella*, *Robinsoniella*, and *Lactobacillus* than the control group (Fig. 2F). Notably, *Klebsiella* and *Robinsoniella* were markedly more abundant in the septic shock group than in the sepsis group (Fig. 2G). Analysis of differential bacterial genera showed a decreased abundance of *[Eubacterium]_hallii_group*, *Agathobacter*, *Blautia*, *Butyricicoccus*, *Dorea*, *Erysipelotrichaceae_UCG-003*, *Fusicatenibacter*, *Monoglobus*, and *Roseburia*, but an increased abundance of *Hungatella* in the sepsis group compared with controls (Fig. 3A, $p < 0.05$). In the septic shock group, the abundance of *Agathobacter*, *Coprococcus*, *Erysipelotrichaceae_UCG-003*, *Faecalibacterium*, *Fusicatenibacter*, *Haemophilus*, *Roseburia*, and *Subdoligranulum* was significantly decreased, while *Corynebacterium* and *Enterococcus* were increased compared with controls (Fig. 3B, $p < 0.05$). Compared with the sepsis group, patients in the septic shock group had higher levels of *Klebsiella* and *Robinsoniella*, but lower levels of *[Eubacterium]_nodatum_group*, *Alistipes*, *Christensenella*, *Desulfovibrio*, *Intestinimonas*, *Odoribacter*, *Pyramidobacter*, and *Streptococcus* (Fig. 3C, $p < 0.05$). These findings indicate that the diversity of the intestinal microbiome was markedly reduced in patients with sepsis and septic shock.

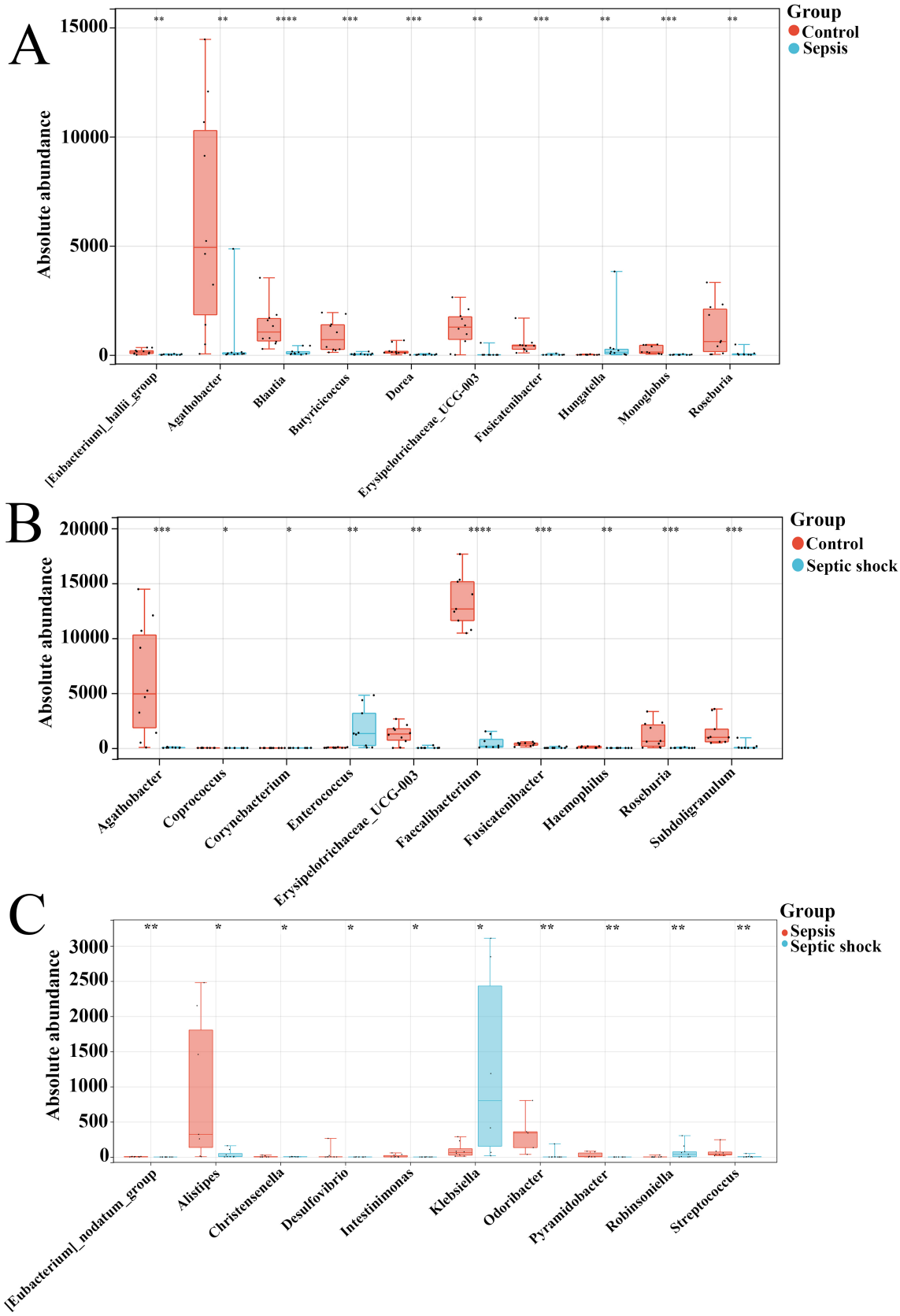

Fig. 3. The top 10 bacterial abundances among the control, sepsis, and septic shock groups. (A) The top 10 bacterial genera between the control and sepsis groups. (B) The top 10 bacterial genera between the control and septic shock groups. (C) The top 10 bacterial genera between the sepsis and septic shock groups. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$ *vs.* the control or sepsis group. TNF-α, Tumour Necrosis Factor alpha.

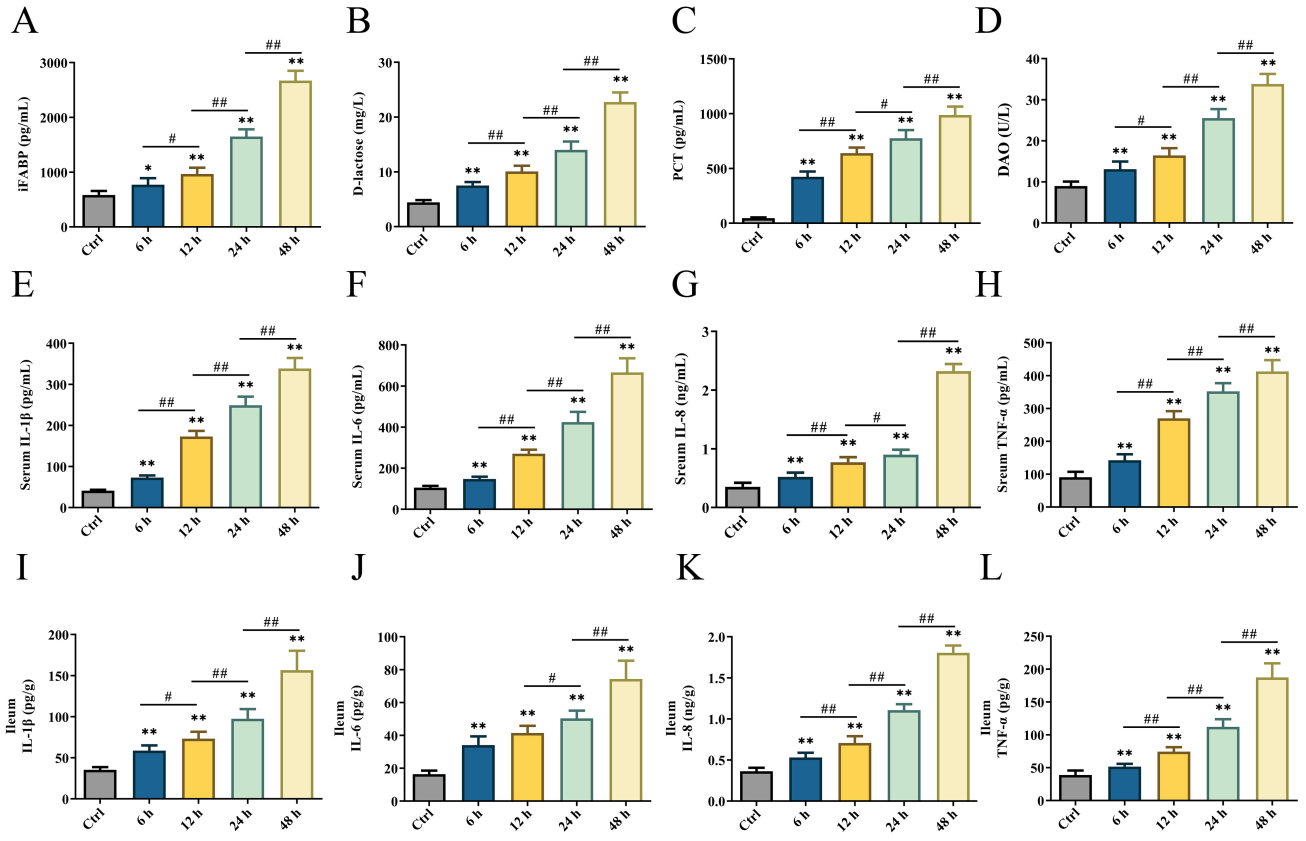

Fig. 4. Effect of cecal ligation and puncture (CLP) on intestinal barrier function, inflammation level, and systemic infection markers in rats. (A–D) The serum level of IFABP, D-lactose, procalcitonin (PCT), and diamine oxidase (DAO). (E–L) The serum and ileum tissue levels of IL-1β, IL-6, IL-8, and TNF-α in sepsis rats. Data are shown as the mean $\pm$ SD (n=8). *$p < 0.05$, **$p < 0.01$ *vs.* the control group. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$.

Effects of CLP on Function of the Intestinal Barrier, Inflammation, and Systemic Infection Markers in Rats

As shown in Fig. 4A–D, the levels of IFABP, D-lactose, PCT, and DAO were increased at 6, 12, 24, and 48 hours after CLP compared with the control group. Similarly, the serum concentrations of IL-1β, IL-6, IL-8, and TNF-α were significantly elevated at each time point following CLP (Fig. 4E–H, $p < 0.05$). In addition, the concentrations of these inflammatory factors were measured in ileum tissue, and the levels of IL-1β, IL-6, IL-8, and TNF-α were also markedly increased in the CLP groups compared with the control group (Fig. 4I–L, $p < 0.05$).

CLP Enhanced the Intestinal Permeability in Septic Rats

As illustrated in Fig. 5A, the overall morphology of the small intestine in the control group was intact, with closely arranged cells and no visible damage. In contrast, the small intestinal tissue of rats at 6, 12, 24, and 48 hours after CLP showed varying degrees of injury, including villus degeneration, edema, irregularity, fusion, and even disappearance of villi, along with inflammatory cell infiltration. The severity of these changes increased over time. Moreover, the hematoxylin and eosin (H&E) scores of small intestinal tissues in the 12, 24, and 48-h groups were significantly higher than those in the control group (Fig. 5B, $p < 0.05$). Furthermore, the mRNA levels of Occludin and ZO-1 in the ileum and colon were markedly decreased after CLP. Consistently, the protein expressions of Occludin and ZO-1 in the ileum and colon tissues were also significantly reduced in the 12, 24, and 48-h groups (Fig. 6A–D, $p < 0.05$). These findings indicate that CLP can increase intestinal barrier permeability in rats, and the increase deepens over time.

Effect of CLP on the Intestinal Flora of Sepsis Rats

To explore the changes of intestinal microorganisms in septic rats at each time point and discover their potential biomarker flora. The alpha diversity metrics indicated a progressive reduction in the diversity of gut microbiota in rats with sepsis induced by CLP (Fig. 7A). PCoA analysis showed a good degree of separation between different groups (Fig. 7B). Heatmap and LEfSe analyses showed the control group had higher *Bifidobacterium*, *Allobaculum*, *Turicibacter*, and *Candidatus_Arthromitus* abundances at the genus level compared with the different CLP groups

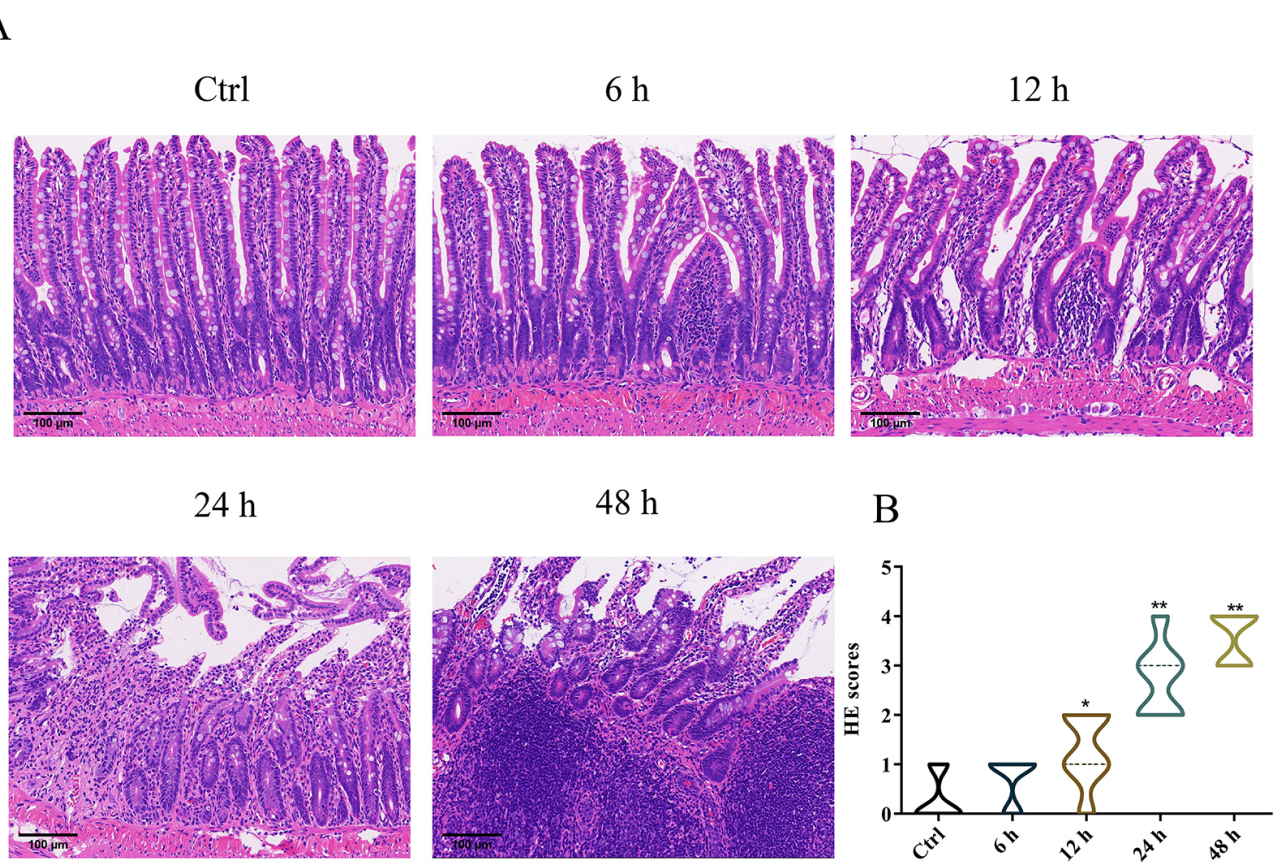

Fig. 5. Effect of CLP on histopathological changes in intestinal tissues of rats. (A) Representative photographs of hematoxylin and eosin (H&E) staining (Scale bar, 100 μm). (B) H&E staining score. Data are shown as the mean ± SD (n=3). *$p < 0.05$, **$p < 0.01$ *vs.* the control group.

(Fig. 7C). LEfSe analysis showed the 6-h group had higher *Oscillospira* and *Desulfovibrio* abundances at the genus level. *Clostridium*, *Pseudomonas*, and *Blautia* at the genus level were greater in the 12, 24, and 48-h groups, respectively (Fig. 7D,E). KEGG analysis revealed that CLP-induced sepsis in rats may act through cellular processes, metabolism, and genetic information processing pathways. Interestingly, the metabolism of carbohydrates, terpenoids and polyketides, as well as amino acids, showed significant enrichment in rats with sepsis induced by CLP (Fig. 7F).

Discussion

Substantial evidence indicates that sepsis-induced dysbiosis of the intestinal microbiota can directly and indirectly impair the function of mucosal immune barriers and immune cells. This immune dysregulation further exacerbates microbial imbalance, thereby aggravating disease progression [16]. The intestinal microbiome is now recognized as crucial for maintaining metabolic and immune homeostasis in the human body [17]. Consequently, disruptions to the gut microbial community during critical illness can significantly affect the recovery of ICU patients and may lead to adverse outcomes [18]. Consistent with this, our study found that alpha diversity indices in the septic shock group were lower than those in the control group, although the differences were not statistically significant. Furthermore, beta diversity analysis demonstrated a distinct clustering between the septic shock and control groups. These findings align with previous reports showing reduced gut microbial diversity in sepsis patients [19].

During sepsis, the local gut immune system becomes dysregulated, leading to excessive proliferation of gut microbiota and increased production of metabolites and toxins, which contribute to intestinal barrier dysfunction [20]. The integrity of the intestinal barrier primarily depends on tight junctions (TJs) between epithelial cells [21]. In sepsis, bacterial translocation and endotoxins activate the intestinal mucosal immune response, resulting in increased intestinal permeability through alterations in tight junction proteins [22]. Our results showed that circulating levels of intestinal barrier function markers—D-lactose, diamine oxidase (DAO), and intestinal fatty acid-binding protein (IFABP)—were significantly elevated in sepsis patients, indicating intestinal barrier damage and increased permeability.

Inflammation plays a pivotal role in the development of intestinal injury during sepsis [23]. Sepsis modulates the expression of tight junction proteins such as claudins, junc-

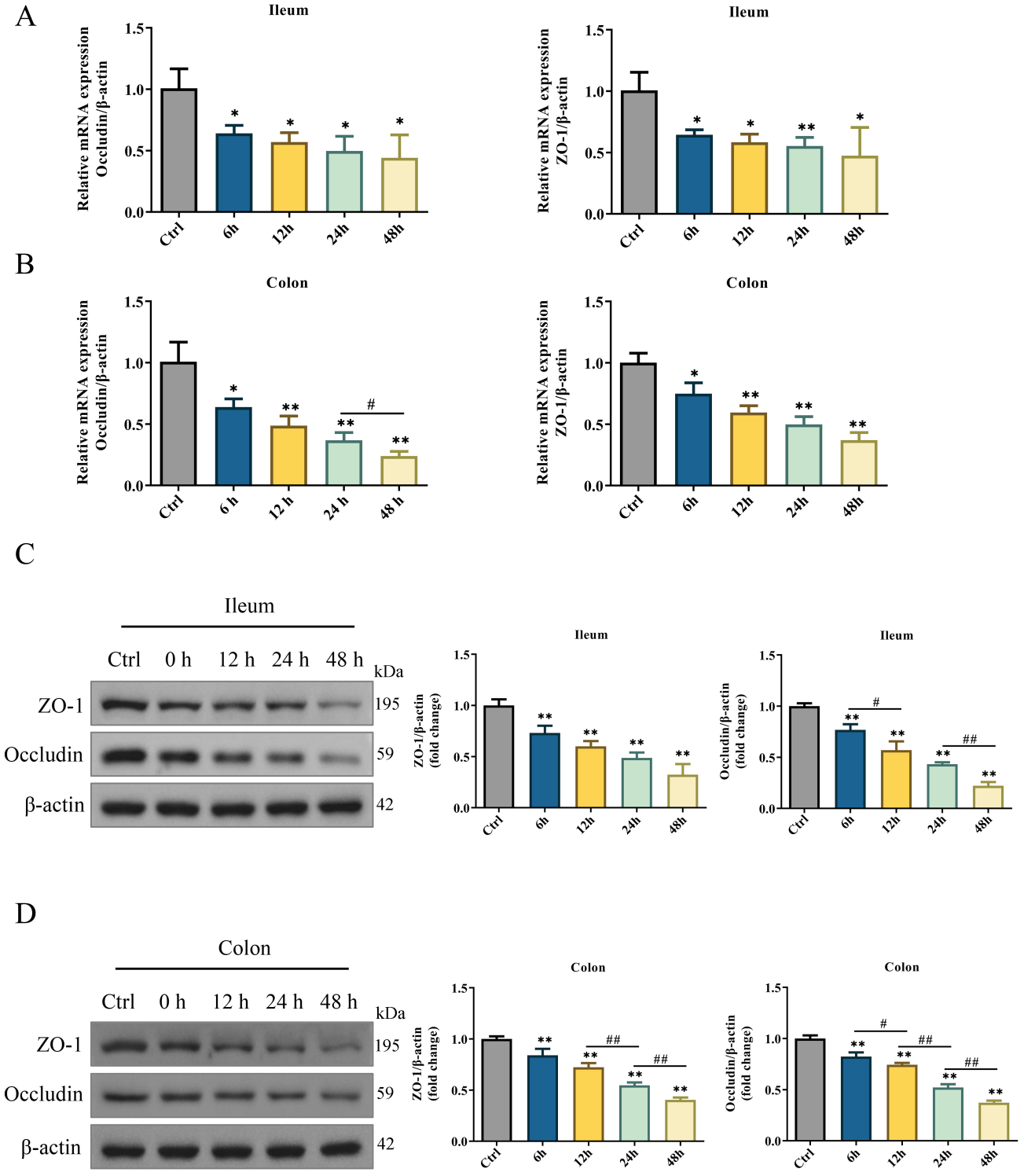

Fig. 6. Effect of CLP on the expression of barrier markers in the ileum and colon tissues of rats. (A,B) The Occludin and zonula occludens-1 (ZO-1) mRNA levels in the ileum and colon tissues of rats. (C,D) The protein levels of ZO-1 and Occludin in the ileum and colon tissues of rats. Data are shown as the mean ± SD (n=3). $*p < 0.05$, $**p < 0.01$ *vs.* the control group. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$.

tional adhesion molecule A (JAM-A), occludin, and zonula occludens-1 (ZO-1), and activates myosin light-chain kinase (MLCK), which regulates intestinal permeability [24]. In our study, serum levels of CRP and IL-8 were significantly increased in sepsis patients. Furthermore, CLP in rats significantly elevated intestinal permeability and inflammatory cytokine concentrations in both serum and intestinal tissues. The severity of ileal pathological injury also worsened with prolonged CLP duration. Additionally, CLP markedly reduced ZO-1 and Occludin protein expression in

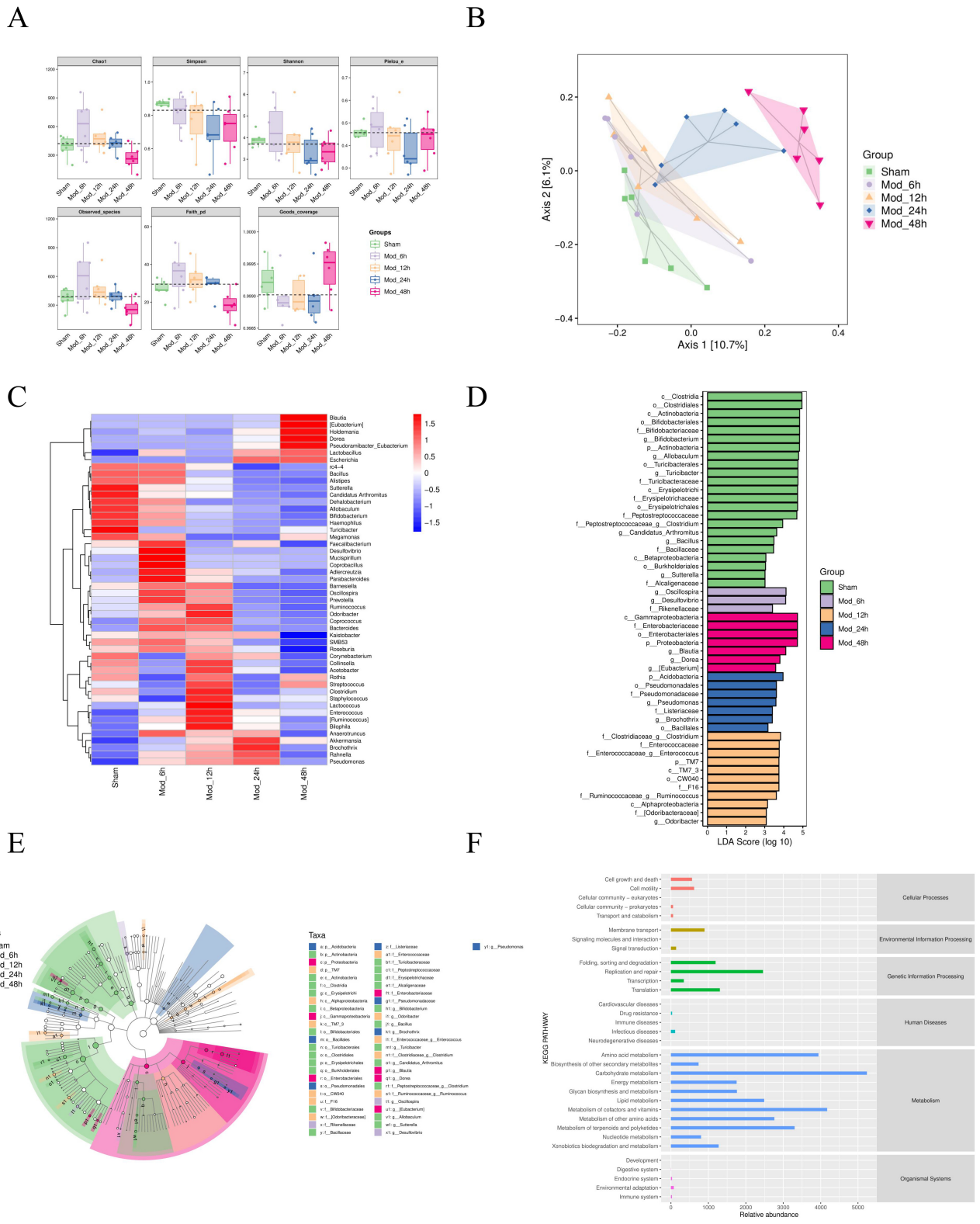

Fig. 7. The gut microbiota in CLP rats. (A) Chao1, Simpson, Shannon, Pielou_e, Observed_species, Faithe_pd, and Goods_coverage indexes in the CLP rats. (B) Principal coordinate analysis (PCoA) of all groups. (C) Heatmap depicting the variations in bacterial genera across different groups. (D,E) LEfSe analysis in three groups. (F) KEGG analysis.

the ileal and colonic tissues of rats. These findings suggest that CLP induces increased intestinal mucosal permeability in septic rats. The CLP-induced inflammatory response maintains a state of severe infection, leading to a significant increase in pathogenic and invasive bacteria, immune dysregulation, and subsequent intestinal mucosal injury [25].

Firmicutes and *Bacteroidetes* constitute the primary phyla of the normal gut microbiome [26]. Previous research has reported a reduction in *Firmicutes* abundance in sepsis patients [27]. Consistent with this, our findings showed that the control group had a significantly higher abundance of *Firmicutes* compared to the sepsis group. Moreover, the control group demonstrated a marked increase in *Proteobacteria* abundance relative to the septic shock group. The *Bacteroidetes* phylum, composed of anaerobic bacteria closely associated with intestinal inflammation and involved in immunity and infection, shows a significant decrease in abundance during sepsis [28]. Notably, in rats subjected to cecal ligation and puncture (CLP), *Bacteroidetes* abundance significantly increased at 6 h and 12 h post-surgery but decreased significantly at 24 h and 48 h. This suggests that CLP induces acute inflammation and stress responses that may initially promote proliferation or relative dominance of *Bacteroides*. Over time, as systemic inflammation intensifies and the intestinal environment deteriorates, the abundance of *Bacteroides* is inhibited.

At the genus level, there was a marked decrease in the abundance of *Agathobacter*, *Coprococcus*, *Erysipelotrichaceae_UCG-003*, *Faecalibacterium*, *Fusicatenibacter*, *Haemophilus*, *Roseburia*, and *Subdoligranulum*, whereas *Corynebacterium* and *Enterococcus* increased significantly in the septic shock group. *In vivo*, rats exhibited a higher abundance of *Bacillus* and *Sutterella* at 6 h post-CLP. The 12 h, 24 h, and 48-h groups showed increased abundances of *Odoribacter*, *Pseudomonas*, and *Brochothrix*, and *Blautia* and *Dorea*, respectively, at the genus level. In pediatric sepsis patients, *Agathobacter* abundance was lower compared to the non-septic group and negatively correlated with inflammatory markers such as white blood cell count (WBC), C-reactive protein (CRP), and interleukin-6 (IL-6) [29]. In CLP + saline mice, populations of *Sutterella*, *Oscillospira*, *Lactobacillus*, and *Desulfovibrio* were reduced, while *Bacteroides*, *Pseudomonas*, *Parabacteroides*, *Ruminococcus*, *Mucispirillum*, and *Vagococcus* increased relative to sham mice [30]. Similarly, *Corynebacterium* is significantly associated with sepsis onset by promoting pro-inflammatory cytokine release via activation of the TLR2/4 signaling pathway, exacerbating systemic inflammation [31,32]. Therefore, *Corynebacterium*, *Blautia*, and *Enterococcus* may serve as important microbial markers for sepsis.

PICRUSt analysis identified several functional pathways linking gut microbiota to sepsis, including metabolism, cell cycle regulation, and genetic information processing. Among these, carbohydrate metabolism was most significantly enriched. In sepsis, hepatic gluconeogenesis may be influenced by the release of glucogenic substrates from peripheral tissues [33]. Oxygen consumption, as well as glucose and pyruvate production and oxidation rates, were markedly elevated in septic patients compared to healthy individuals [34]. Meanwhile, the metabolism of terpenoids, polyketides and amino acids was also significantly enriched in CLP rats. Sun and colleagues [35] demonstrated abnormalities in the metabolism of gut amino acids in sepsis patients, suggesting that the metabolic phenotype plays a critical role in the context of sepsis.

Nonetheless, this study has limitations. First, functional experiments such as fecal microbiota transplantation are necessary to establish causal relationships between identified changes in gut microbiota and the progression of sepsis. Second, a larger clinical sample size is necessary for further validation.

Conclusion

In conclusion, the abundance of beneficial bacteria, including *Agathobacter*, *Coprococcus*, *Erysipelotrichaceae_UCG-003*, and *Faecalibacterium*, are reduced in septic shock, while the abundance of potentially pathogenic bacteria such as *Corynebacterium* and *Enterococcus* is increased. These microbial taxa may serve as important targets or biomarkers for therapeutic intervention in sepsis.

Availability of Data and Materials

The data supporting this study's findings are available on request from the corresponding author.

Author Contributions

DL: Conceptualization, Funding acquisition, Project administration, Writing-review & editing. HYZ: Investigation, Writing-original draft. KLH: Data curation, Investigation. JSY: Data curation, Investigation, Methodology. All authors contributed to the critical revision of the manuscript for important intellectual content. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

The clinical protocol was reviewed and approved by the Ethics Committee of the First People's Hospital of Linhai City, Zhejiang Province (No.2020-0005), conducted in accordance with the Declaration of Helsinki and written informed consent was obtained from all participants.. The animal experiment protocol in rats was performed according to the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978), all procedures were approved by the Animal Care and Welfare Committee of Zhejiang Chinese Medical University (SCXK (Zhe)2021-0012).

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Conflict of Interest

The authors declare no conflict of interest.

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