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Research article

Analysis of fecal microbiota and related clinical indicators in ICU patients with sepsis

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ABSTRACT

Background: To analyze the characteristics of fecal microbiota disturbance in the intensive care unit (ICU) patients with sepsis and the correlation with related clinical indicators.
Methods: This study included 31 patients with sepsis admitted to the emergency ICU ward between September 2019 and December 2021. They were divided into Group without septic shock (ND_NS group, 7 cases) and Group with septic shock (ND_S group, 24 cases) according to the presence or absence of septic shock. Furthermore, we divided these 31 sepsis patients into Clinical Improvement group (21 cases) and Death or DAMA group (10 cases) based on clinical outcome, 15 cases of Physical Examiner recruited in the same period were included as control group: ND_HC group (15 cases). The fecal samples of the patients with sepsis within 24 h of admission and random fecal samples of the control group were collected and analyzed by 16S rDNA gene sequencing used for the analysis of fecal microbiota. At the same time, the relevant clinical data of these patients with sepsis were also collected for analysis.
Results: There were 15 cases with drug-resistant bacteria in the ND_S group and only 2 cases in the ND_NS group ($P = 0.015$). There were significant differences in APACHE II score, length of ICU stay, lactate level, and oxygenation index of patients between the Death or DAMA group and Clinical Improvement group (all $P < 0.05$). For phylum level, the abundance of Firmicutes, Actinobacteria, and Bacteroidetes decreased in the ND group compared with the ND_HC group, while the abundance of Proteobacteria increased ($P < 0.05$). For genus level, the relative abundance of *Escherichia-Shigella* and *Klebsiella* were significantly increased in the ND group compared with the ND_HC group ($P < 0.05$). The top six genera in relative abundance in the ND_S group were *Escherichia-Shigella, Enterococcus, Bifidobacterium, Lactobacillus, Akkermansia,* and *Klebsiella.* Compared with the Clinical Improvement group, the relative abundance of *Escherichia-Shigella* and *Klebsiella* in the Death or DAMA group showed an increasing trend with no significant significance, while the relative abundance of *Enterococcus* and *Faecalibacterium* decreased in the Death or DAMA group ($P < 0.05$). Alpha diversity analysis showed that compared with the ND_HC group, the alpha diversity of the fecal microbiota in the ND group decreased. There were significant differences in the Observed_species index, Chao1 index, and ACE index of patients between the ND_HC group and ND group (all $P < 0.05$). Moreover, compared with the ND_NS group, the Alpha diversity of the ND_S group was more abundant. PCoA analysis showed significant differences in microbial community structure between the ND group and ND_HC group ($P = 0.001$). There also were significant differences in microbial community structure between

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the ND_S group and ND_NS group (P = 0.008). LEfSe analysis showed that compared with the ND_HC group, there were significant differences in the species of the ND group, including *Enterobacteriaceae, Escherichia-Shigella, Enterococcus, Elizabethkingia,* and *Family_XIII_AD3011_group*.

Conclusions: ICU patients with sepsis suffered intestinal microecological disturbances with significantly decreased abundance of fecal microbiota, diversity, and beneficial symbiotic bacteria. For these patients, the ratio of pathogenic bacteria, including *Escherichia-Shigella* and *Klebsiella* increased and became the main bacterial genus in some samples. Moreover, the increasing trend of these two pathogenic bacteria may be correlated with the development of septic shock and the risk of death in patients with sepsis.

1. Introduction

Sepsis is a potentially life-threatening condition that occurs due to organ dysfunction induced by a dysregulated host response to infection. Septic shock is sepsis complicated by severe circulatory and cellular metabolic disturbance. Patients with septic shock are more severely ill and at higher risk of death [1,2]. Sepsis and septic shock have become medical emergencies that affect millions of people worldwide each year. They are also among the leading causes of death in critically ill patients in the ICU [3–5]. The pathogenesis of sepsis is complex, and the various changes in cell function, microcirculation, and metabolism may involve pathophysiological processes such as inflammation, immunity, and coagulation [6–8].

The gut is the body's largest immune organ and the initiating organ of multiple organ dysfunction syndromes (MODS). Intestinal microecology, which is closely related to human health, is composed of intestinal mucosal tissue, intestinal immune system, and fecal microbiota, with the latter one having the greatest importance [9,10]. In recent years, relevant studies on the role of the gut microbiome in sepsis have made considerable progress, establishing a vicious cycle of fecal microbiota disturbance and sepsis worsening. Studies have also found that sepsis can lead to disturbance of fecal microbiota and activation of inflammatory factors, further leading to increased intestinal permeability that damages the intestinal mucosa and intestinal epithelial cells and contributes to the shift of fecal microbiota, finally resulting in the development of sepsis by inflammatory burst [11–15]. Previous studies on the role of the fecal microbiota in sepsis focused on disclosing novel therapies for sepsis and improving survival in patients with sepsis.

The aim of the current study was to analyze the characteristics of fecal microbiota disturbance in ICU patients with sepsis.

2. Methods

2.1. General data

This study included 31 sepsis patients (ND group) admitted to the emergency ICU ward of Zhongshan Hospital Xiamen University between September 2019 and December 2021. They were divided into Group without septic shock (ND_NS group, 7 cases) and Group with septic shock (ND_S group, 24 cases) according to the presence or absence of septic shock. In addition, we further divided these 31 sepsis patients into Clinical Improvement group (21 cases) and Death or DAMA group (10 cases) according to another classification based on clinical outcome. 15 cases of Physical Examiner recruited in the same period were included as control group: ND_HC group (15 cases). The diagnostic criteria for sepsis and septic shock were made based on the latest definition "Sepsis 3.0″ issued by the American Society of Critical Care Medicine (SCCM) in 2016 [2]. Inclusion criteria were the following: 1) age ≥18; 2) patients who met the diagnostic criteria of Sepsis 3.0. Exclusion criteria were: 1) patients with ICU stay for less than 24 h; 2) patients with end-stage irreversible diseases such as immunosuppressive therapy, immunodeficiency, and malignant tumors; 3) patients with perianal infection, gastroenterostomy, and chronic gastrointestinal diseases.

Requirements for the control group were the following: 1) patients matched for age with those in the ND group; 2) patients with no history of chronic gastrointestinal diseases and gastrointestinal surgery; 3) patients who did not use antibiotics or probiotics within 3 months before enrollment; 4) patients with a history of hypertension, diabetes mellitus, and coronary heart disease essentially matching the levels of the patients in sepsis group.

This study was approved by the hospital ethics committee (Scientific Research Subcommittee of Medical Ethics Committee, Zhongshan Hospital Affiliated to Xiamen University; Ethical approval number: xmzsyyky Lun Shen No.2019007; ethics approval date: February 15, 2019). All patients (or their proxies/legal guardians) provided informed consent to participate in the study.

2.2. Clinical outcome measures

The general clinical data were collected for the patients in ND group, including age, gender, main underlying diseases, main sources of infection, including respiratory system (ND_RS), urinary system (ND_US), and digestive system (ND_DS), length of ICU stay, length of overall hospital stay, clinical outcomes, acute physiology and chronic health evaluation II (APACHE II) score within 24 h of ICU admission, sequential organ failure assessment (SOFA) score within 24 h of ICU admission, and drug-resistant bacteria. In addition, the worst values of the following indicators within 24 h of ICU stay were collected: mean arterial pressure (MAP) level, white blood cell count (WBC), lymphocyte ratio (LY%), platelet count (PLT), C-reactive protein level (CRP), procalcitonin level (PCT), plasma lactate level (Lac), oxygenation index (OI), creatinine level (CREA), total bilirubin level (TBIL).

2.3. Samples collection and detection method

We collected fecal samples from sepsis patients within 24 h of admission, and collected random fecal samples from the control group,. The fecal samples of patients with sepsis were collected from the depths of the fresh feces after spontaneous defecation or enema. All samples were quickly collected and stored at −80 °C freezer.

The 16S rDNA gene sequencing of fecal microbiota was performed by LC-Bio Technologies (Hangzhou) Co., Ltd. Materials and methods of 16S rDNA sequencing were as follows: 1) the total microbiome DNA was extracted using the E. Z.N.A. ®Stool DNA Kit (D4015, Omega, Inc., USA) according to manufacturer's instructions; 2) PCR amplification area was V3V4 area of 16S rDNA. The primer sequences were 341F (5'_CCTACGGGNGGCWGCAG_3′) and 805R (5'_GACTACHVGGGTATCTAATCC_3′). The PCR products were confirmed by 2% agarose gel electrophoresis. The PCR products were then purified by AMPure XT beads (Beckman Coulter Genomics, Danvers, MA, USA) and quantified by Qubit (Invitrogen, USA). The amplicon pools were prepared for sequencing, and the size and quantity of the amplicon library were assessed on Agilent 2100 Bioanalyzer (Agilent, USA) and with the Library Quantification Kit for Illumina (Kapa Biosciences, Woburn, MA, USA), respectively. The libraries were sequenced on the NovaSeq PE250 platform.

2.4. Bioinformatics analysis and statistical methods

After splicing, quality control, and chimeric filtration of raw data, operational taxonomic units (OTUs) were clustered based on 97% similarity. The Kruskal_Wallis rank-sum test combined with Benjamini_Hochbery correction was used to analyze the significance of differences in the α-diversity measures of different samples. Alpha-diversity mainly includes Observed species, Shannon, Simpson, ACE, Chao1, and Pielou's evenness (J′). β-diversity can estimate differences in community structure between samples. Nine hundred ninety-nine permutations of PERMANOVA and adonis in the R package were used to determine significant differences in β-diversity. Common OTUs were calculated and visualized using the VennDiagram in R. The taxa abundances were measured and plotted using ggplot2 software. LEfSe is an algorithm for discovering and interpreting high-dimensional biomarkers, which can identify and describe genomic features that differ between two or more biological conditions. LEfSe was used to identify taxa that differ in abundance among different taxa. Gene function was predicted via Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt), and ANOVA was performed using analysis of significant differences. Fuzhou TreatGut Biotechnology Co., Ltd. and LC-Bio Technologies (Hangzhou) Co., Ltd completed the above bioinformatics analysis and statistical methods. The data processing and analysis of clinical data were carried out with GraphPad Prism 9.0 software, and all data were first performed with normality or lognormality tests. The data with normal distribution were expressed as mean ± standard error of mean (Mean ± SEM), and a *t*-test was used for comparison. The data with non-normal distribution were expressed as M (P_{25}, P_{75}), and the rank-sum test was used for comparison. P-value <0.05 was considered to be statistically significant.

Table 1
Clinical features of patients with ND_HC group and ND group.

Characteristic	ND_HC group(n=15)	ND_NS group(n = 7)	ND_S group(n = 24)	p value (ND_NS_vs_ND_S)
Age(year, mean ± SD)	64.27 ± 4.644	74.86 ± 5.68	73.5 ± 3.285	
Sex(male/female)	9/6	3/4	14/10	
Major underlying diseases				
Hypertension	5	2	15	
Diabetes mellitus	3	4	5	
Coronary heart disease	1	0	3	
Rheumatic disease	1	0	1	
Major source of infection	/			
Respiratory system		1	13	
Urinary system		3	8	
Digestive system		3	3	
Clinical outcomes	/			0.379
Death or DAMA		1	9	
Clinical improvement		6	15	
Drug-resistant bacteria	/			0.015
Escherichia coli		0	3	
Klebsiella pneumoniae		0	3	
Acinetobacter baumannii		1	2	
Enterococcus faecium		1	1	
Aspergillus group		0	2	
Staphylococcus aureus		0	1	
Pseudomonas aeruginosa		0	1	
Others(Enterobacter kobe/Enterobacter cloacae)		0	2	

DAMA discharged against medical advice.

3. Results

3.1. Clinical general data

3.1.1. General data between ND group and ND_HC group

A total of 31 patients (17 males and 14 females with an average age of 73.81 ± 2.806 years) were enrolled in the ND group, and 15 cases (9 males and 6 females, with an average age of 64.27 ± 4.644 years) were enrolled in the ND_HC group. The main underlying diseases in the ND_HC group included 5 cases of hypertension, 3 cases of diabetes mellitus, 1 case of coronary heart disease, 1 case of rheumatic disease. The main underlying diseases in the ND group included 17 cases of hypertension, 9 cases of diabetes mellitus, 3 cases of coronary heart disease, and 1 case of rheumatic disease (Table 1).

3.1.2. Comparison between ND_S group and ND_NS group

There were no significant differences in age, gender, the main source of infection, length of ICU stay, length of overall hospital stay, clinical outcomes, APACHE II score, SOFA score, MAP level, WBC, LY%, PLT, CRP, PCT, Lac, OI, CREA, TBIL between ND_S group and ND_NS group (all $P > 0.05$). Results of drug-resistant bacteria in the clinical course of the ND group showed that the detected drug-resistant bacteria included *Escherichia coli* [3 cases, Extended spectrum beta-lactamases positive ($ESBL^{+}$)], *Klebsiella pneumoniae* [3 cases, Carbapenem-resistant enterobacteriaceae (CRE)], *Acinetobacter baumannii* (3 cases, CRE), *Enterococcus faecium* (2 cases), *Aspergillus group* (2 cases), *Staphylococcus aureus* [1 case, Methicillin resistente *Staphylococcus aureus* (MRSA)], *Pseudomonas aeruginosa* (1 case, CRE), *Enterobacter Kobe* (1 case), and *Enterobacter cloacae* (1 case). There were significant differences in the detection rate of

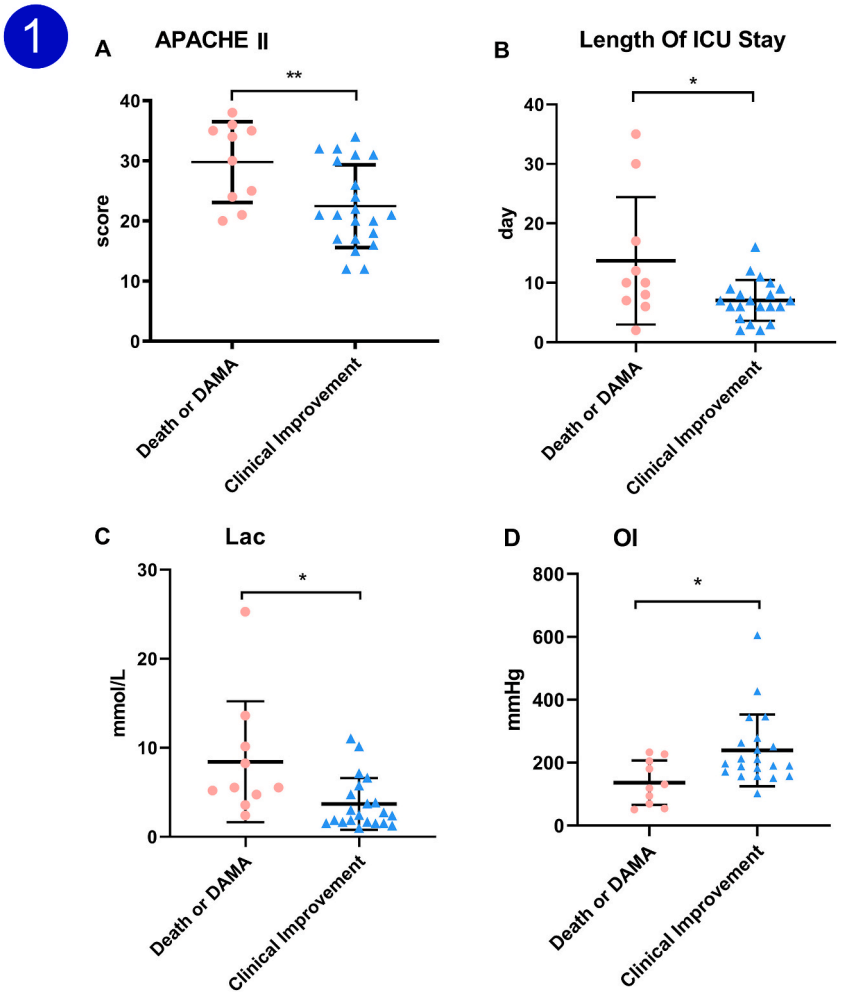

Fig. 1. ND group patients were grouped according to clinical outcomes with statistically different clinically relevant indicators. Patients in the ND group were divided into Clinical Improvement group and Death or DAMA group according to clinical outcome. Statistical analysis showed that the differences between the two groups were statistically significant in four indexes: APACHE II score, Length of ICU stay, lactate level, and oxygenation index. *$p < 0.05$, **$p < 0.01$.

drug-resistant bacteria between the ND_S group and the ND_NS group (P = 0.015). The distribution between the two groups is shown in Table 1.

3.1.3. Comparison between death or DAMA group and clinical improvement group

Considering the 31 sepsis patients, the clinical outcome in 5 patients was death, while the other 5 patients were discharged against medical advice (DAMA). Telephone follow-up showed that all 5 DAMA patients died on discharge. Therefore, they were assigned to the Death or DAMA group. Besides, the remaining 21 patients who achieved clinical improvement after treatment were assigned to the Clinical Improvement group. After statistical analysis, there were significant differences in APACHE II (29.80 ± 6.73 vs 22.48 ± 6.88, P = 0.009), length of ICU stay [10(6.75,20.25) vs 7(5,9), P = 0.039], Lac [5.52 (4.44, 11.02) vs 2.45 (1.59, 5.26), P = 0.048], OI [125 (65.25, 210.5) vs 197 (164, 270.5), P = 0.019] between the Death or DAMA group and the Clinical Improvement group (Fig. 1A–D). Results of drug-resistant bacteria in the clinical course of the Death or DAMA group showed that the detected drug-resistant bacteria mainly included *Klebsiella pneumoniae* (3 cases, CRE), *Acinetobacter baumannii* (2 cases, CRE), *Escherichia coli* (1 case, ESBL^{+}), *Pseudomonas aeruginosa* (1 case, CRE), *Enterobacter Kobe* (1 case), *Enterobacter cloacae* (1 case), and *Aspergillus group* (1 case).

3.2. Analysis of the composition of bacterial community

3.2.1. Comparison between ND group and ND_HC group

The top five phyla in the ND_HC group according to relative abundance were Firmicutes (relative abundance 66.73%),

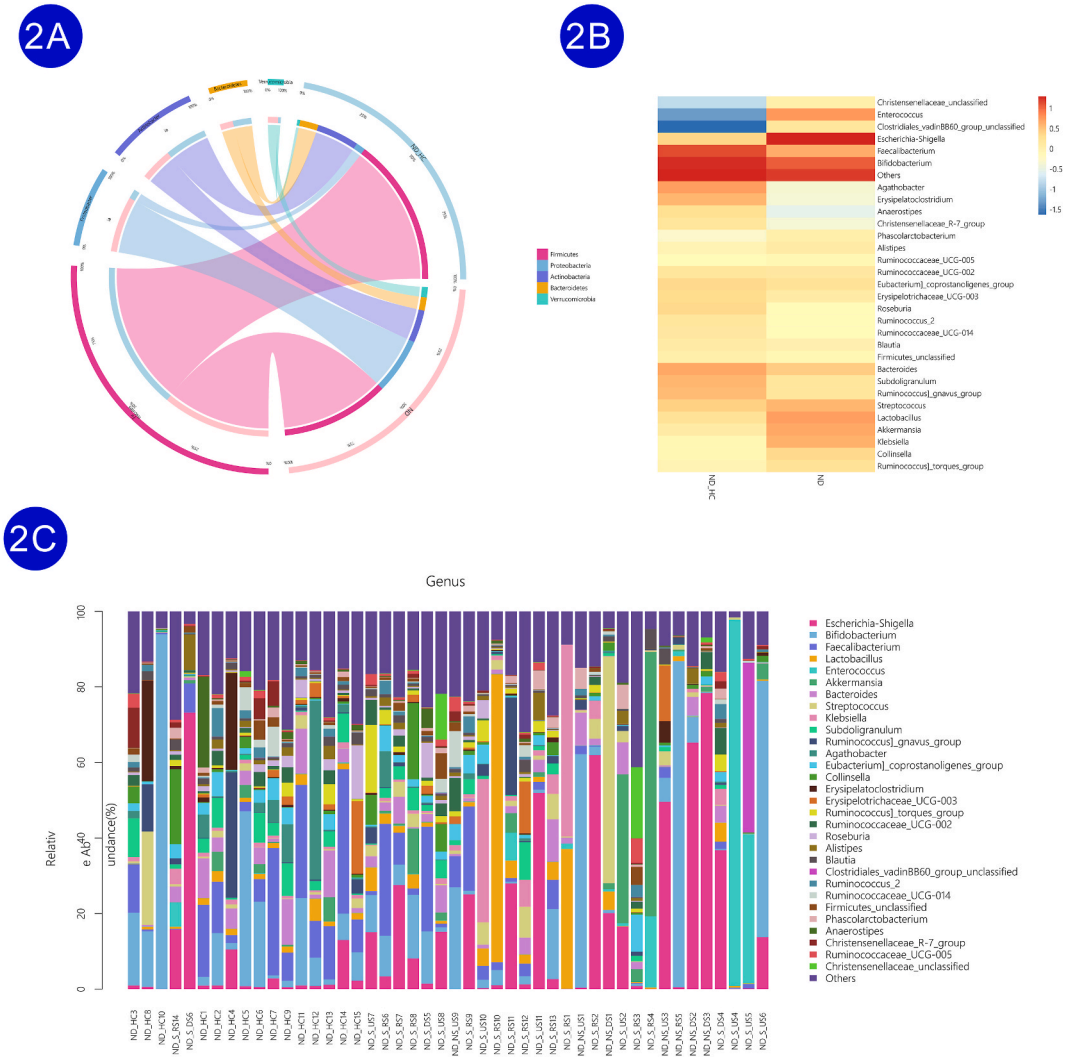

Fig. 2. Analysis of the composition of fecal microbiota in ND_HC group and ND group. (A) Circos map of the top five bacteria in relative abundances of the ND_HC group versus the ND group at the phylum level. (B)Heatmap of the relative abundances of bacterial groups in the ND_HC group versus the ND group at the genus level. (C)The top 30 bacteria in relative abundance of all samples in the ND_HC group and ND group at the genus level.

Actinobacteria (19.09%), Bacteroidetes (8.42%), Proteobacteria (4.24%), and Verrucomicrobia (1.24%). The abundance of Firmicutes (49.03%, as low as 6.04% in individual patients), Actinobacteria (14.72%, as low as 0.20% in individual patients), Bacteroidetes (5.98%, as low as 0.05% in individual patients) of ND group decreased, while the abundance of Proteobacteria (25.28%, as high as 78.54% in individual patients) significantly increased. There were significant differences in Proteobacteria between two groups [18.87 (5.13, 41.62) vs 2.73 (2.07, 4.41), P = 0.004] (Fig. 2A). Compared with the ND_HC group at the genus level, the relative abundance of *Escherichia-Shigella* [13.77(0.49, 27.95) vs 0.96(0.60, 2.22), P = 0.021] and *Klebsiella* [0.87(0.02, 1.49) vs 0.88 (0.64, 1.09), P = 0.025] of ND group significantly increased. The relative abundance of *Enterococcus, Streptococcus,* and *Akkermansia* was increased; however, the differences were not statistically significant (P > 0.05). The proportion of symbiotic bacteria such as *Faecalibacterium, Bifidobacterium, Bacteroides,* and *Ruminococcus* significantly decreased (Fig. 2B). The top 30 relative abundances of the genus in ND group and ND_HC group are shown in Fig. 2C.

3.2.2. Predominant flora and clinical etiological culture of some samples of ND group

3.2.2.1. Escherichia-Shigella. The relative abundance of *Escherichia-Shigella* in the fecal samples of the ND group was greater than 50% with ND_NS_DS3 (78.36%), ND_S_DS6 (73.19%), ND_NS_DS2 (65.25%), ND_S_RS2 (61.93%), and ND_S_US11 (51.96%). Besides, both the clinical blood culture and liver pus culture of ND_NS_DS3 patients showed *Escherichia coli* [Extended spectrum beta-lactamases negative ($ESBL^-$)]. Both sputum culture and bronchoalveolar lavage fluid culture of ND_S_RS2 were *Enterobacter Kobe* (non-CRE) and *Enterobacter Cloacae* ($ESBL^-$).

3.2.2.2. Enterococcus. The relative abundance of *Enterococcus* was >50% with ND_S_US4 (96.91%), and the clinical blood culture showed *Escherichia coli* ($ESBL^-$).

3.2.2.3. Klebsiella. The relative abundance of *Klebsiella* was >50% with ND_S_RS1 (50.71%). All the sputum culture (8 times), bronchoalveolar lavage fluid culture (2 times), and urine culture (3 times) during the course of sepsis showed *Klebsiella pneumoniae* (CRE) and 3 sputum cultures showed A*cinetobacter baumannii* (CRE).

3.2.3. Comparison between ND_S group and ND_NS group

At the genus level, the top 6 genera with the highest relative abundance in the ND_NS group included *Escherichia-Shigella, Bifidobacterium, Streptococcus, Erysipelotrichaceae_UCG-003, Bacteroides,* and *Ruminococcaceae_UCG-002*. In addition to *Escherichia-Shigella* with the highest relative abundance, the other five, including *Enterococcus, Bifidobacterium, Lactobacillus, Akkermansia,* and *Klebsiella* ranked the highest in the ND_S group, among which *Lactobacillus* (P = 0.031) was higher in the ND_S group with a significant increase in the relative abundance of ND_S_RS10 (76.23%) and ND_S_RS1 (37%). The relative abundance of some fecal microbiota in the ND_NS group and ND_S group at the genus level are shown in Table 2 and Fig. 3A.

3.2.4. Comparison between death or DAMA group and clinical improvement group

The top 6 genera with the highest relative abundance in the Clinical Improvement group included *Escherichia-Shigella, Bifidobacterium, Enterococcus, Akkermansia, Faecalibacterium,* and *Lactobacillus.* The Death or DAMA group showed an increased relative abundance of *Escherichia-Shigella* and *Klebsiella* compared with the Clinical Improvement group, but the difference was not statistically significant. However, the relative abundance of *Bifidobacterium, Enterococcus,* and *Faecalibacterium* was reduced in the Death or DAMA group, with *Enterococcus* (P = 0.019), *Faecalibacterium* (P = 0.048) being statistically significant between the two groups (Table 3, Fig. 3B).

3.3. Analysis of bacterial diversity

3.3.1. Analysis of alpha diversity

Alpha diversity is a measure of how many microbial species exist in a single sample (i.e., species richness, richness) and the proportion of each microbial sample (i.e., evenness, evenness), which can be used for intuitive comparison of the diversity changes in the microbiota of different samples (or groups). Alpha analysis showed that compared with the ND_HC group, the alpha diversity of the fecal microbiota in the ND group decreased with significant differences in Observed_species index (p = 0.015), Chao1 index (p = 0.011), and ACE index (p = 0.0046) (Fig. 4A). Compared with the ND_NS group, the ND_S group had higher bacterial flora abundance

Table 2
Relative abundance of some fecal microbiota in the ND_NS group and ND_S group at the genus level (*p < 0.05).

Group	Relative abundance at the genus level					
	Escherichia-Shigella	Enterococcus	Bifidobacterium	Lactobacillus	Akkermansia	Klebsiella
ND_NS group (*n* = 7)	20.13(0.38,65.25)	0.05(0.03,0.61)	6.77(0.82,61.79)	0.07(0.04,1.30)	0.00(0.00,1.21)	0.09(0.00,0.91)
ND_S group (*n* = 24)	10.94(0.87,26.92)	0.09(0.03,1.25)	1.08(0.11,8.72)	1.25(0.38,4.63)	0.08(0.03,3.06)	0.90(0.02,4.16)
Statistical value	−0.857	−0.234	−0.642	0.785	−0.453	0.414
p value	0.687	0.99	0.578	0.031*	0.687	0.093

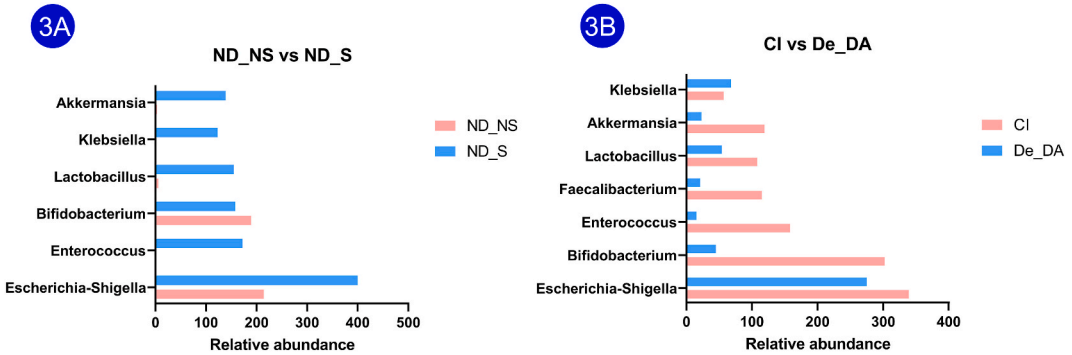

Fig. 3. Differences in the composition of the fecal microbiota in the ND group of patients in different grouping conditions at the genus level. (A)The total percentage of relative abundance of some fecal microbiota in the ND_NS group versus the ND_S group at the genus level (ND_NS group n = 7; ND_S group n = 24). (B)The total percentage of relative abundance of some fecal microbiota in the Clinical Improvement (CI) group versus the Death or DAMA (De_DA) group at the genus level (Clinical Improvement group n = 21; Death or DAMA group n = 10).

Table 3
Relative abundance of some fecal microbiota in the Clinical Improvement group and Death or DAMA group at the genus level (*p < 0.05).

Group	Relative abundance at the genus level						
	Escherichia - Shigella	Bifidobacterium	Enterococcus	Faecalibacterium	Lactobacillus	Akkermansia	Klebsiella
Clinical Improvement (*n* = 21)	3.37(0.32,22.6)	2.23 (0.29,12.32)	0.08 (0.03,0.68)	1.89(0.02,5.68)	0.79 (0.11,3.67)	0.08 (0.00,1.22)	0.20 (0.01,1.30)
Death or DAMA (*n* = 10)	21.68 (2.20,54.45)	0.70(0.24,8.27)	0.10 (0.03,2.66)	0.59(0.07,3.46)	1.56 (0.21,4.66)	0.83 (0.04,3.84)	1.27 (0.46,4.92)
Statistical value	−0.139	0.733	0.412	−0.151	−0.369	0.311	0.060
p value	0.193	0.105	0.019*	0.048*	0.99	0.193	0.769

with significant differences in Observerd_species index (p = 0.045) and ACE index (p = 0.04) (Fig. 4B). Yet, there was no significant difference in alpha diversity between the Death or DAMA group and the Clinical Improvement group (Fig. 4C).

3.3.2. Analysis of beta diversity

Beta diversity is a measure of the similarity in the composition of bacterial groups between different samples that focuses on the differences in the composition of bacterial groups between samples. In this study, principal coordinates analysis (PCoA) was used to distinguish differences in the composition of microbiota between samples. PCoA analysis showed that the ND group and ND_HC group could cluster together, and the bacterial community structure between the two groups was significantly different (P = 0.001) (Fig. 5A). In addition, PCoA analysis showed that the ND_S group and ND_NS group could cluster together, and there was a significant difference in the bacterial community structure between the two groups (P = 0.008) (Fig. 5B). However, there was no significant difference in beta diversity between the Death or DAMA group and the Clinical Improvement group (Fig. 5C).

3.4. Analysis of flora difference

LDA Effect Size (LEfSe) is an analytical tool for discovering and interpreting biomarkers in high-dimensional data, enabling comparisons between two or more groups to find biomarkers with significant differences between groups. The phylogenetic diagram showed that the relative abundance of dominant bacteria in the ND_HC group included *Clostridiales, Ruminococcaceae, Lachnospiraceae, Bifidobacteriaceae*, etc. The relative abundance of dominant bacteria in the ND group included mainly *Enterobacteriaceae, Enterococcaceae, Clostridia Family_XIII*, etc. The distribution of LDA value suggested that, compared with the ND_HC group, the species with significant differences in the ND group mainly included *Enterobacteriaceae, Escherichia_Shigella, Enterococcus, Elizabethkingia,* and *Family_XIII_AD3011_group* (Fig. 6A and B).

4. Discussion

Sepsis is a clinical syndrome accompanied by multiple organ dysfunction and high mortality, which occurs due to the imbalance in the body's response to inflammation caused by microbial infection. Septic shock is also involved in abnormal circulatory, cellular and metabolic disorders, in addition to the imbalance in the body's response to inflammation. According to the latest diagnostic criteria for sepsis ("Sepsis-3.0") [2] and "Surviving Sepsis Campaign: international guidelines for the management of sepsis and septic shock 2021" [1], multiple organ dysfunction is an important cause of poor prognosis in sepsis, so focusing on early identification of multiple

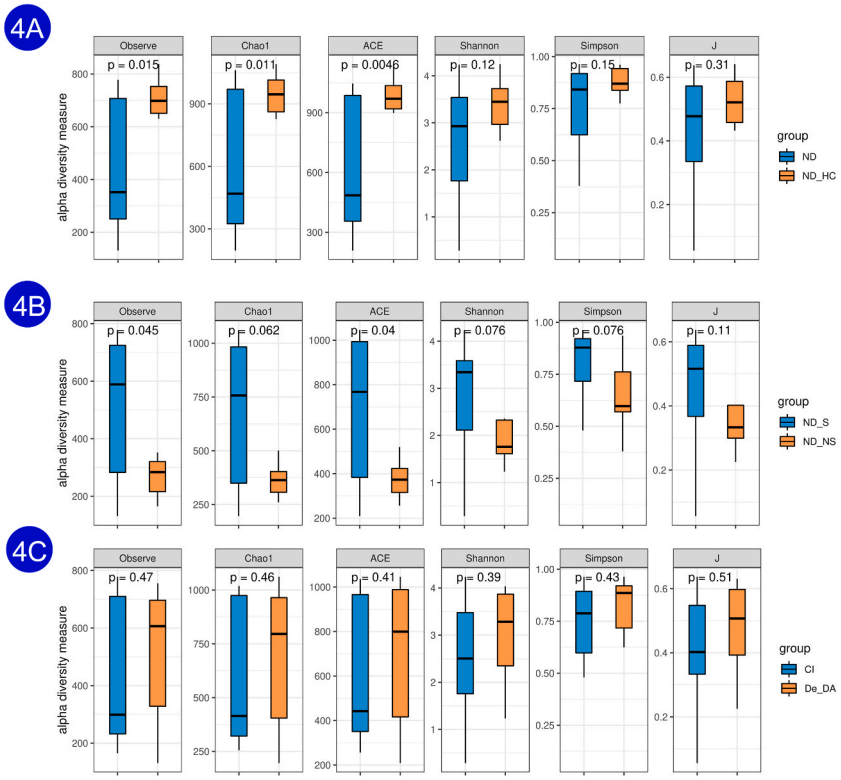

Fig. 4. Alpha diversity analysis of fecal microbiota in patients of ND group under different grouping conditions. (A)Alpha diversity analysis(as accessed by Observed_species index, Chao1 index, ACE index, Shannon index, Simpson index, and J index) of fecal microbiota in the ND_HC group (n = 15) and the ND group (n = 31). (B)Alpha diversity analysis(as accessed by Observed_species index, Chao1 index, ACE index, Shannon index, Simpson index, and J index) of fecal microbiota in the ND_NS group (n = 7) and the ND_S group (n = 24). (C)Alpha diversity analysis(as accessed by Observed_species index, Chao1 index, ACE index, Shannon index, Simpson index, and J index) of fecal microbiota in the Clinical Improvement (CI) group (n = 21) and the Death or DAMA (De_DA) group (n = 10).

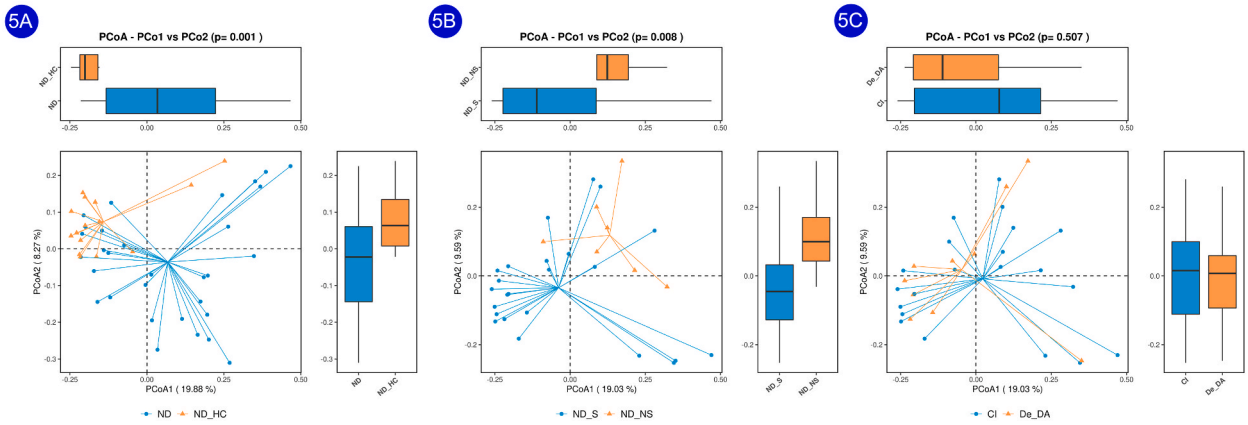

Fig. 5. Beta diversity analysis of fecal microbiota in patients of ND group under different grouping conditions. (A)Scatter plot of unweighted UniFrac PCoA showing Beta diversity of fecal microbiota in the ND_HC group (n = 15) and the ND group (n = 31). (B)Scatter plot of unweighted UniFrac PCoA showing Beta diversity of fecal microbiota in the ND_NS group (n = 7) and the ND_S group (n = 24). (C)Scatter plot of unweighted UniFrac PCoA showing Beta diversity of fecal microbiota in the Clinical Improvement (CI) group (n = 21) and the Death or DAMA (De_DA) group (n = 10).

organ dysfunction and appropriate treatment could improve the prognosis of these patients. As early as the 1980s, the gastrointestinal tract was considered the "motive force" organ for sepsis and multiple organ dysfunction syndromes in critically ill patients with shock, multiple trauma, burns, etc. The gastrointestinal tract was also considered to be largest "bacteria bank". About 10^{13} to 10^{14} bacteria

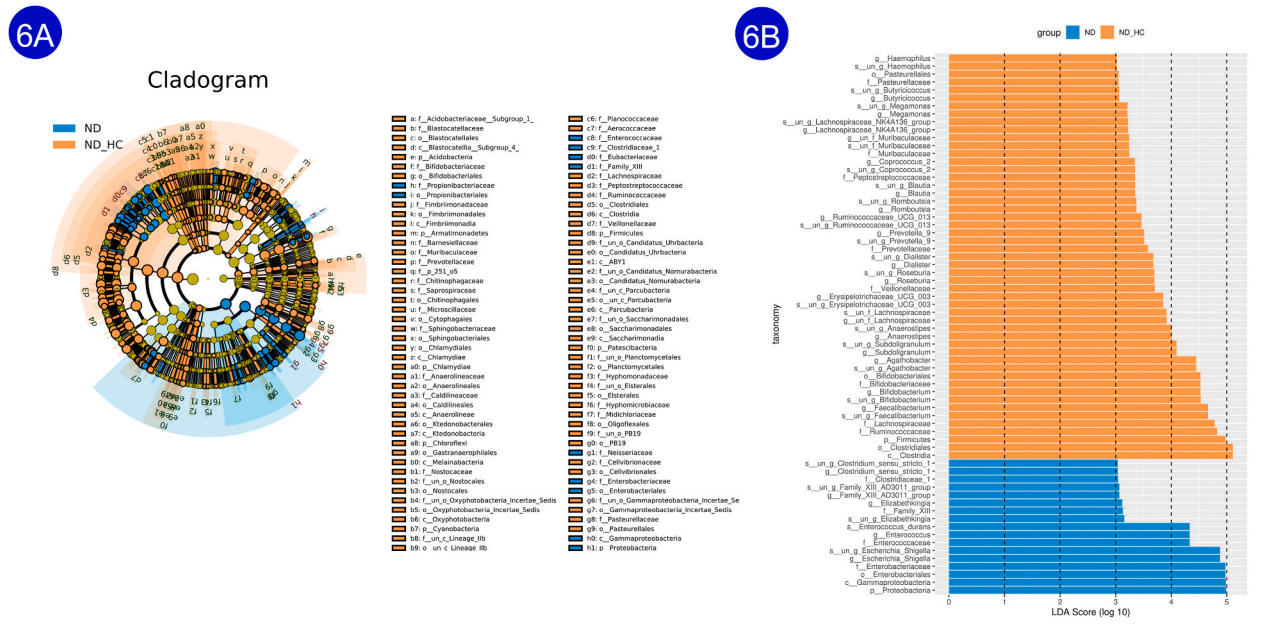

Fig. 6. Differential analysis of fecal microbiota between ND group and ND_HC group. (A)The circles radiating from inside to outside in the evolutionary branching diagram represent the taxonomic rank from phylum to genus (or species). The diameter of the small circles is proportional to the relative abundance. (B)Linear discriminant analysis Effect Size (LEfSe) analysis demonstrated species with significantly different relative abundance in the ND_HC group versus the ND group. LDA Score >3, $p < 0.05$.

live in the normal human intestine, which approximately equals the total number of human cells [16–18]. Under physiological conditions, these fecal microbiotas are relatively stable as an important part of the intestinal microecology, having an important role in intestinal immunity, nutrient metabolism, digestion and absorption, and maintenance of intestinal mucosal barrier function. Previous studies have confirmed that the intestinal flora participates in the pathophysiological process of sepsis by relying on the unique physiological environment of the intestine [19–22]. Prescott et al. [23] found a clear dose-response relationship between the imbalance of fecal microbiota and subsequent severe sepsis. A multicenter trial also showed that critical illness in the ICU could lead to a severe, rapid imbalance of fecal microbiota, manifested by reduced bacterial diversity and overgrowth of pathogenic bacteria [24]. Several related studies have also shown that the intestine is the first organ to be involved when sepsis is complicated by multiple organ dysfunction. Sepsis is often accompanied by intestinal barrier dysfunction, increased intestinal mucosal permeability, and bacterial shift, activation of the intestinal immune system, and similar, which promote the occurrence and development of sepsis [25–27].

There are 100–1000 species of bacteria in the intestine of healthy people. Under physiological conditions, the fecal microflora in the intestinal cavity is mainly composed of Firmicutes, Bacteroidetes, Actinomycetes, and Proteobacteria. Especially *Bifidobacterium* and other beneficial bacteria are involved in normal intestinal barrier function, nutrient metabolism, and immune regulation [28,29]. Under pathological conditions of a variety of acute and chronic diseases such as recurrent Clostridium difficile infection (RCDI), inflammatory bowel disease (IBD), obesity, and similar, the fecal microbiota can be changed in terms of flora composition, quantity, diversity, and virulence, having an important role in these diseases [30–32]. Sepsis-related studies have also found multiple changes in the fecal microbiota in patients with sepsis, including reduced bacterial diversity, overgrowth of pathogenic bacteria, and increased abundance of potentially pathogenic genera [24,33–36]. This is in line with our results revealing that the alpha diversity of the fecal microbiota of the patients with sepsis significantly decreased. Beta diversity analysis also showed that the bacterial community structure of the patients with sepsis significantly changed. The patients in the control group were dominated by Firmicutes, Actinobacteria, and Bacteroidetes at the phylum level, accounting for 94.24% of the total. However, the relative abundance of Firmicutes and Bacteroidetes in patients with sepsis decreased, while the relative abundance of Proteobacteria increased significantly. The results at the genus level showed that compared with the control group, the pathogenic bacteria, including *Escherichia-Shigella* and *Klebsiella,* in patients with sepsis significantly increased, while the abundance of *Enterococcus* also increased in some patients. In addition, the proportion of intestinal obligate anaerobic bacteria such as *Faecalibacterium* and *Bifidobacterium* decreased. LEfSe analysis also showed that the major differences between the ND group and the ND_HC group were in *Enterobacteriaceae, Escherichia-Shigella, Enterococcus*, etc. The study confirmed that ICU patients with sepsis could develop intestinal microecological disturbance, which is manifested by decreased abundance and diversity of fecal microbiota, decreased proportion of obligate anaerobic bacteria, and increased proportion of pathogenic bacteria such as facultative anaerobic or aerobic bacteria.

Animal studies have confirmed that burnt mice suffer from impaired intestinal mucosal barrier function and abnormal fecal microbiota within 5 min of being burned [37]. De Souza et al. [38] found that the intestinal function of patients with sepsis was abnormal upon diagnosis. With the widespread application of broad-spectrum antibiotics in critically ill patients treated in the ICU, a large number of sensitive flora have been removed, while non-dominant flora such as opportunistic pathogens has proliferated in large

numbers, and many bacterial colonization targets on the intestinal mucosa have been exposed, thus leaving colonization space for pathogenic bacteria. In addition, during sepsis, due to endotoxin strikes, intestinal ischemia and hypoxia, weakened peristalsis, and decreased ability of the intestine to remove pathogenic bacteria, opportunistic pathogenic bacteria and pathogenic bacteria become dominant flora, and enter the circulatory system and various tissues and organs through the damaged intestinal mucosal barrier, eventually causing intestinal infection [39,40]. This study collected fecal samples from sepsis patients within 24 h of ICU admission. Our results showed that in patients with a relative abundance of *Escherichia-Shigella* in the ND group of fecal microbiota >50%, pathogenic cultures at multiple clinical sites (including blood, sputum, alveolar lavage fluid, and pus) in some patients were suggestive of *Enterobacteriaceae* growth. Clinical sputum, alveolar lavage fluid, and urine cultures repeatedly showed growth of *Klebsiella pneumoniae* in patients with a relative abundance of *Klebsiella* > 50% in the fecal microbiota, which suggested that some of the *Enterobacter* and *Klebsiella* clinically cultured in these patients with sepsis may be of gut origin, thus further supporting the premise that the gut is a "platform" for bacterial dissemination for infections in multiple sites such as blood, urethra, respiratory tract, and surgical wounds. Interestingly, in the present study, the clinical blood culture of patients with a relative abundance of *Enterococcus* as high as 96.91% suggested *Escherichia coli*. The clinical data of this patient were reviewed. The patient was admitted to the hospital because of "dysuria, diarrhea with fever", and his clinical diagnosis was septic shock, urinary tract infection, gastrointestinal tract infection, and diabetes mellitus. Considering that the patient had septic shock caused by multi-site infection, it was speculated that the blood culture of *Escherichia coli* might be the source of the urinary system. However, at this time, the patient's fecal microbiota underwent significant changes, which were manifested by the overgrowth of a single *Enterococcus* and clinical symptoms of the gastrointestinal tract such as diarrhea. Thus, if the antibiotic was selected only based on the patient's blood culture of *Escherichia coli* for treatment, the disease could be delayed, which suggests that fecal microbiota may provide some reference for clinicians to guide the empirical use of antibiotics. Our study also confirmed that the fecal microbiota of ICU patients with sepsis could exhibit overgrowth of a single pathogenic genus, which was similar to results reported by Lankelma et al. [41] and Zaborin et al. [34].

Septic shock tends to be accompanied by persistent hypotension induced by sepsis, and vasoactive drugs are still required to maintain mean arterial pressure (MAP) $\geq$ 65 mmHg and blood lactate level >2 mmol/L after adequate volume resuscitation, which is indicative of the sepsis combined with severe circulatory, cellular and metabolic disturbances. Thus, the death risk of septic shock is higher than that of sepsis [2]. Septic shock lacks characteristic clinical manifestations in the early stage and can easily progress to refractory hypotension in the late stage, which can be life-threatening. In fact, every second must be counted for treatment. Early and effective antibiotic treatment is vital to reduce mortality and improve prognosis. The latest guideline [1] still recommends immediate infusion of antibacterial drugs for suspected septic shock or high possibility of sepsis, preferably within 1 h after diagnosis; for those at risk of methicillin-resistant staphylococcus aureus (MRSA) infection, it is recommended to empirically apply antibacterial drugs covering MRSA, while for those at risk of multiple drug resistance (MDR), it is recommended to use two kinds of anti-gram-negative bacilli drugs empirically. For those at high risk of fungal infection, empiric antifungal therapy is recommended. Nevertheless, there is an obvious lag in obtaining etiological evidence in clinical practice. The choice of initial antibiotics should not only be based on patients' clinical characteristics but also attention should be paid to the prevalence of bacteria in hospitals and the region and the trend of drug resistance. Previous studies have reported that the correct choice of empiric antibiotics is a key factor affecting the prognosis of patients, and inappropriate empiric antibiotic therapy is associated with significantly higher mortality [42,43]. Currently, among clinical studies on the changes in fecal microbiota in sepsis at home and abroad, there are few studies that distinguish the changes in sepsis and septic shock microbiota. In our study, the enrolled patients were divided into the ND_NS group and the ND_S group. No significant differences were found in the level of relevant indicators between the two groups. However, the 16S rDNA assay showed that the two groups' relative abundance of bacteria at the genus level was different. In addition to the higher relative abundance ratio of *Escherichia-Shigella* in both groups, the ND_S group still showed an increasing trend in the relative abundance ratio of *Klebsiella* and *Enterococcus*, which were lower in the ND_NS group, thus suggesting that the increasing trend of the ratio of *Klebsiella* and *Enterococcus* may be associated with septic shock. Although there was no significant difference in the above bacterial groups between the two groups, there was a significant difference in drug-resistant bacteria between the two groups. Fifteen cases in the ND_S group were clinically detected with multidrug-resistant bacteria, including *Escherichia coli, Klebsiella pneumoniae*, and *Enterococcus* (Table 1), which was consistent with the relatively higher abundance ratios of *Escherichia-Shigella, Klebsiella*, and *Enterococcus* in the flora of ND_S group. According to the statistics of the China antimicrobial surveillance network (CHINET), the top five drug-resistant strains from 2019 to 2022 were *Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus, Pseudomonas aeruginosa,* and *Acinetobacter baumannii*. Studies have shown that *Escherichia coli* and *Klebsiella pneumoniae* are common Gram-negative bacteria that lead to the development of sepsis [44,45]. With the wide application of antimicrobial drugs and the existence of variability between different regions and individuals, sepsis-resistant strains have gradually increased, exacerbating the difficulty of sepsis treatment. Our study suggested that, in addition to the recommendations of the guideline [1], the choice of empirical antibiotics for patients with septic shock in this region should be considered to cover pathogens such as drug-resistant *Escherichia coli* and *Klebsiella pneumoniae*.

An article published in the Lancet [46] showed that there were approximately 48.9 million sepsis cases worldwide in 2017, of which 11 million patients died from sepsis, amounting to 19.7% of all deaths worldwide. Sepsis has become one of the major diseases threatening global health, and a variety of biomarkers and disease severity scales have been widely used in the diagnosis and prognostic evaluation of sepsis. Previous studies have shown [47–49] that the abnormal increase of lactate levels and the duration of high lactate levels in sepsis are positively correlated with the severity of the disease and mortality of patients. Critical illness scales such as the APACHE II and SOFA scales can be used to judge sepsis [50,51]. Currently, biomarkers such as PCT and CRP are commonly used in clinical practice to predict the mortality of critically ill patients such as sepsis [52,53]. Hyperoxemia or hypoxemia has also been associated with mortality in patients with sepsis [54–56]. Yet, the mortality rate of sepsis remains high, which is in part due to the lack of clinically effective means for early diagnosis and disease prediction. In this study, the clinical outcomes of patients with sepsis

included death, DAMA, and improvement of clinical condition. Telephone follow-up showed that all 5 DAMA patients died on discharge. Therefore, according to clinical outcomes, patients were subdivided into the Death or DAMA group and the Clinical Improvement group. The statistical analysis showed significant differences in APACHE II score, length of ICU stay, lactate level, and oxygenation index between the two groups. Meanwhile, we tested the changes in the flora composition of the Death or DAMA group and the Clinical Improvement group, finding the relative abundance of beneficial bacteria such as *Faecalibacterium* and *Bifidobacterium* in the Death or DAMA group had a decreasing trend. Interestingly, the relative abundance of *Enterococcus* in the Death or DAMA group was significantly lower, which was in contrast to the results of a recent study [57] that the abundance of *Enterococcus* increased to varying degrees in deceased patients with sepsis. *Enterococci* are members of the human immune system that are associated with diseases such as urinary tract infections, abdominal and pelvic infections, sepsis, and infective endocarditis [58], and are also common drug-resistant bacteria in the ICU. In this study, one case of blood cultured *Enterococcus faecium* and one case of urine cultured *Enterococcus faecium* were clinically positive in the Clinical Improvement group, which was not found in the Death or DAMA group. As the above contradictory results may be related to small sample size, subsequent multicenter studies with larger sample sizes are needed to verify the reported findings further. In addition, it was also found that the relative abundance of *Enterobacteriaceae* such as *Escherichia-Shigella and Klebsiella* in the Death or DAMA group showed an increasing trend, and the clinically detected drug-resistant bacteria in the Death or DAMA group were mainly *Enterobacteriaceae*, which indicated that the changes in this flora might be correlated with the risk of death in patients with sepsis. Subsequent studies should further investigate this with larger sample sizes. Our study also showed that, both the Alpha diversity and Beta diversity of fecal microbiota in patients of the Clinical Improvement (CI) group ($n = 21$) and the Death or DAMA group ($n = 10$) were no significant differences between these two groups, this may be related to the fact that our fecal specimens were collected only once within 24 h of the patient's admission to the hospital and were not collected dynamically. Studies have shown that the diversity of the fecal microbiota and the structural composition of the flora change significantly over time in patients with sepsis after admission to the ICU [24,37,38,59], especially after various interventions such as medications in the ICU, and that the clinical outcomes of patients may be different. However, this was not further observed because of the timing of our sampling. Subsequently, we will continue to expand our sample sizes to further validate our main findings.

5. Conclusion

Through 16s rDNA sequencing of fecal samples from patients with sepsis, the study achieved the following results: (1) it was confirmed that ICU patients with sepsis have intestinal microecological disturbances, which were manifested as a decreased abundance of fecal microbiota, decreased diversity, and even overgrowth of a single pathogen; (2) the fecal samples of patients in the early stage of sepsis were assessed, revealing that the main composition of the bacterial flora of some patients was consistent with the clinical etiological results of the patients, which suggested that the positive pathogenic bacteria in the clinical culture of patients with sepsis may be of gut origin and can provide certain ideas and guidance for the selection of clinical empirical antimicrobials; (3) in ICU patients with sepsis, the trend of elevated relative abundance of some pathogenic gut microbes such as *Escherichia-Shigella* and *Klebsiella* may be correlated with septic shock and the risk of death. However, there were still many limitations to our study. First of all, the 16s rDNA sequencing method is limited by factors such as differences in sample collection/processing processes, limited versatility of amplification primers, and limitations of analysis methods. Moreover, our study was limited by the small sample size, the regionality of the research subjects, and only one detection of fecal flora in patients with sepsis. The application of molecular biology techniques in the field of microecology and the in-depth understanding of the intestinal microecology of patients with sepsis in the future research on the mechanism of fecal microbiota in the occurrence and development of sepsis are expected to become important approaches for therapy for sepsis.

Ethics statement

This study was reviewed and approved by the hospital ethics approving committee (Scientific Research Subcommittee of Medical Ethics Committee, Zhongshan Hospital Affiliated to Xiamen University; Ethical approval number: xmzsyyky Lun Shen No.2019007; ethics approval date: February 15, 2019). All patients (or their proxies/legal guardians) provided informed consent to participate in the study.

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Data availability statement

Data will be made available on request.

Additional information

No additional information is available for this paper.

CRediT authorship contribution statement

Huaying Chen: Writing – review & editing, Writing – original draft, Validation, Software, Resources, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Huiheng Liu:** Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Yujing Sun:** Software, Resources, Methodology, Investigation, Data curation, Conceptualization. **Meiqin Su:** Writing – review & editing, Validation, Supervision, Methodology, Investigation. **Jinzhou Lin:** Visualization, Software, Resources, Project administration, Methodology, Investigation. **Junsheng Wang:** Visualization, Supervision, Software, Project administration, Methodology, Investigation, Conceptualization. **Jueying Lin:** Resources, Methodology, Investigation. **Xiaoyan Zhao:** Resources, Methodology, Investigation.

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.

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References

[1] L. Evans, A. Rhodes, W. Alhazzani, et al., Surviving sepsis campaign: international guidelines for management of sepsis and septic shock 2021, Intensive Care Med. 47 (2021) 1181–1247.
[2] M. Singer, C.S. Deutschman, C.W. Seymour, et al., The third international consensus definitions for sepsis and septic shock (Sepsis-3), JAMA 315 (2016) 801–810.
[3] M. Shankar-Hari, G.S. Phillips, M.L. Levy, et al., Developing a new definition and assessing new clinical criteria for septic shock: for the third international consensus definitions for sepsis and septic shock (Sepsis-3), JAMA 227 (2016) 775–787.
[4] A. Perner, M. Cecconi, M. Cronhjort, et al., Expert statement for the management of hypovolemia in sepsis, Intensive Care Med. 44 (2018) 791–798.
[5] D.F. Gaieski, J.M. Edwards, M.J. Kallan, et al., Bench marking the incidence and mortality of severe sepsis in the United States, Crit. Care Med. 41 (2013) 1167–1174.
[6] S. Inoue, T. Sato, K. Suzuki_Utsunomiya, et al., Sepsis-induced hypercytokinemia and lymphocyte apoptosis in aging-accelerated klotho knockout mice, Shock 39 (2013) 311–316.
[7] A.N. Rizzo, S.M. Dudek, Endothelial glycocalyx repair: building a wall to protect the lung during sepsis, Am. J. Respir. Cell Mol. Biol. 56 (2017) 687–688.
[8] Y.C. Liu, X.B. Zou, Y.F. Chai, et al., Macrophage polarization in inflammatory diseases, Int. J. Biol. Sci. 10 (2014) 520–529.
[9] M.H. Floch, Intestinal microecology in health and wellness, J. Clin. Gastroenterol. 45 (2011) 108–110.
[10] M. Vancamelbeke, S. Vermeire, The intestinal barrier : a fundamental role in health and disease, Expet Rev. Gastroenterol. Hepatol. 11 (2017) 821–834.
[11] M.A. Haseeb, M.J. Salwen, Collateral damage : sepsis-induced gut injury . Crit, Care Med 33 (2005) 2439–2440.
[12] K. Shimizu, H. Ogura, T. Hamasaki, et al., Altered gut flora are associated with septic complications and death in critically ill patients with systemic inflammatory response syndrome, Dig. Dis. Sci. 56 (2011) 1171–1177.
[13] J. Cabrera-Perez, V.P. Badovinac, T.S. Griffith, Enteric immunity, the gut microbiome, and sepsis : Rethinking the germ theory of disease, Exp. Biol. Med. 242 (2017) 127–139.
[14] C. Vaishnavi, Translocation of gut flora and its role in sepsis, Indian J. Med. Microbiol. 31 (2013) 334–342.
[15] N.J. Klingensmith, C.M. Coopersmith, The gut as the motor of multiple organ dysfunction in critical illness, Crit. Care Clin. 32 (2016) 203–212.
[16] R. Sender, S. Fuchs, R. Milo, Revised estimates for the number of human and bacteria cells in the body, PLoS Biol. 14 (2016) e1002533.
[17] E. Bianconi, A. Piovesan, F. Facchin, et al., An estimation of the number of cells in the human body, Ann. Hum. Biol. 40 (2013) 463–471.
[18] R. Sender, S. Fuchs, R. Milo, Are we really vastly outnumbered? Revisiting the ratio of bacterial to host cells in humans, Cell 164 (2016) 337–340.
[19] S.E. Blutt, M.K. Estes, Gut bacterial bouncers:keeping viral pathogens out of the epithelium, Cell Host Microbe 26 (2019) 569–570.
[20] M.W. Adelman, M.H. Woodworth, C. Langelier, et al., The gut microbiome's role in the development, maintenance, and outcomes of sepsis, Crit. Care 24 (2020) 278.
[21] H. Kayama, R. Okumura, K. Takeda, Interaction between the Microbiota,Epithelia,and immune cells in the intestine, Annu. Rev. Immunol. 38 (2020) 23–48.
[22] V. Tremaroli, F. Bäckhed, Functional interactions between the gut microbiota and host metabolism, Nature 489 (2012) 242–249.
[23] H.C. Prescott, R.P. Dickson, M.A. Rogers, et al., Hospitalization type and subsequent severe sepsis, Am. J. Respir. Crit. Care Med. 192 (2015) 581–588.
[24] D. McDonald, G. Ackermann, L. Khailova, et al., Extreme dysbiosis of the microbiome in critical illness, mSphere 1 (2016) e00199–16.
[25] Y. Chen, H.S. Zhang, G.H. Fong, et al., PHD3 stabilizes the tight junction protein occludin and protects intestinal epithelial barrier function, J. Biol. Chem. 290 (2015) 20580–20589.
[26] M. Meng, N.J. Klingensmith, C.M. Coopersmith, New insights into the gut as driver of critical illness and organ failure, Curr. Opin. Crit. Care 23 (2017) 143–148.
[27] R. Mittal, C.M. Coopersmith, Redefining the gut as the motor of critical illness, Trends Mol. Med. 20 (2014) 214–223.
[28] L.V. Hooper, T. Midtvedt, J.I. Gordon, How host-microbial interactions shape the nutrient environment of the mammalian intestine, Annu. Rev. Nutr. 22 (2002) 283–307.
[29] D. Mariat, O. Firmesse, F. Levenez, et al., The Firmicutes/Bacteroidetes ratio of the human microbiota changes with age, BMC Microbiol. 9 (2009) 123.
[30] P.J. Turnbaugh, R.E. Ley, M.A. Mahowald, et al., An obesity-associated gut microbiome with increased capacity for energy harvest, Nature 444 (2006) 1027–1031.
[31] E.G. Pamer, Fecal microbiota transplantation : effectiveness , complexities , and lingering concerns, Mucosal Immunol. 7 (2014) 210–214.
[32] T.J. Borody, A. Khoruts, Fecal microbiota transplantation and emerging applications, Nat. Rev. Gastroenterol. Hepatol. 9 (2012) 88–96.
[33] Z.G. Liu, N. Li, H. Fang, et al., Enteric dysbiosis is associated with sepsis in patients, Faseb. J. 33 (2019) 12299–12310.
[34] A. Zaborin, D. Smith, K. Garfield, et al., Membership and behavior of ultra-low-diversity pathogen Communities present in the gut of humans during prolonged critical illness, mBio 5 (2014) 1361–1414.
[35] Y.D. Wan, R.X. Zhu, Z.Q. Wu, et al., Gut microbiota disruption in septic shock patients: a pilot study, Med. Sci. Mon. Int. Med. J. Exp. Clin. Res. 24 (2018) 8639–8646.

[36] M. Ojima, D. Motooka, K. Shimizu, et al., Metagenomic analysis reveals dynamic changes of whole gut microbiota in the acute phase of intensive care unit patients, Dig. Dis. Sci. 61 (2016) 1628–1634.
[37] T. Eaves-Pyles, J.W. Alexander, Rapid and prolonged impairment of gut barrier function after thermal injury in mice, Shock 9 (1998) 95–100.
[38] D. De-Souza, L.J. Greene, Intestinal permeability and systemic infections in critically ill patients: effect of glutamine, Crit. Care Med. 33 (2005) 1125–1135.
[39] S.A. Condotta, J. Cabrera-Perez, V.P. Badovinac, et al., T-cell mediated immunity and the role of TRAIL in sepsis-induced immunosuppression, Crit. Rev. Immunol. 33 (2013) 23–40.
[40] J. MacFie, C. O'Boyle, C.J. Mitchell, et al., Gut origin of sepsis: a prospective study investigating associations between bacterial translocation,gastric microflora, and septic morbidity, Gut 45 (1999) 223–228.
[41] J.M. Lankelma, L.A. van Vught, C. Belzer, et al., Critically ill patients demonstrate large interpersonal variation in intestinal microbiota dysregulation: a pilot study, Intensive Care Med. 43 (2017) 59–68.
[42] M. Paul, V. Shani, E. Muchtar, et al., Systematic review and meta-analysis of the efficacy of appropriate empiric antibiotic therapy for sepsis, Antimicrob. Agents Chemother. 54 (2010) 851–4863.
[43] G.S. Martin, Sepsis, severe sepsis and septic shock: changes in incidence, pathogens and outcomes, Expert Rev. Anti Infect. Ther. 10 (2012) 701–706.
[44] M. Koupetori, T. Retsas, N. Antonakos, et al., Bloodstream infections and sepsis in Greece: over-time change of epidemiology and impact of de-escalation on final outcome, BMC Infect. Dis. 14 (2014) 272.
[45] J. Rodríguez-Baño, M.D. López-Prieto, M.M. Portillo, et al., Epidemiology and clinical features of community-acquired, healthcare-associated and nosocomial bloodstream infections in tertiary-care and community hospitals, Clin. Microbiol. Infect. 16 (2010) 1408–1413.
[46] K.E. Rudd, S.C. Johnson, K.M. Agesa, et al., Global, regional, and national sepsis incidence and mortality, 1990-2017:analysis for the global burden of disease study, Lancet 395 (2020) 200–211.
[47] Z.H. Zhang, X. Xu, Lactate clearance is a useful biomarker for the prediction of all-cause mortality in critically ill patients:a systematic review and meta-analysis, Crit. Care Med. 42 (2014) 2118–2125.
[48] M.E. Mikkelsen, A.N. Miltiades, D.F. Gaieski, et al., Serum lactate is associated with mortality in severe sepsis independent of organ failure and shock, Crit. Care Med. 37 (2009) 1670–1677.
[49] J.P. Green, T. Berger, N. Garg, et al., Serum lactate is a better predictor of short-term mortality when stratified by C-reactive protein in adult emergency department patients hospitalized for a suspected infection, Ann. Emerg. Med. 57 (2011) 291–295.
[50] H.B. Nguyen, C. Van Ginkel, M. Batech, et al., Comparison of predisposition, insult/infection, response, and organ dysfunction, acute physiology and chronic health evaluation II , and mortality in emergency department sepsis in patients meeting criteria for early goal-directed therapy and the severe sepsis resuscitation bundle, J. Crit. Care 27 (2012) 362–369.
[51] J.M. Williams, J.H. Greenslade, K. Chu, et al., Severity scores in emergency department patients with presumed infection: a prospective validation study, Crit. Care Med. 44 (2016) 539–547.
[52] C. Lichtenstern, T. Brenner, H.J. Bardenheuer, et al., Predictors of survival in sepsis: what is the best inflammatory marker to measure? Curr. Opin. Infect. Dis. 25 (2012) 328–336.
[53] C.P. Schneider, Y. Yilmaz, A. Kleespies, et al., Accuracy of procalcitonin for outcome prediction in unselected postoperative critically ill patients, Shock 31 (2009) 568–573.
[54] D.K. Chu, L.H.-Y. Kim, P.J. Young, et al., Mortality and morbidity in acutely ill adults treated with liberal versus conservative oxygen therapy (IOTA): a systematic review and meta-analysis, Lancet 391 (2018) 1693–1705.
[55] B. Popoff, E. Besnier, B. Dureuil, et al., Effect of early hyperoxemia on mortality in mechanically ventilated septic shock patients according to Sepsis-3 criteria: analysis of the MIMIC-III database, Eur. J. Emerg. Med. 28 (2021) 469–475.
[56] M. Martín-Fernández, M. Heredia-Rodríguez, I. González-Jiménez, et al., Hyperoxemia in postsurgical sepsis/septic shock patients is associated with reduced mortality, Crit. Care 26 (2022) 4.
[57] G.M. Agudelo-Ochoa, B.E. Valdés-Duque, N.A. Giraldo-Giraldo, et al., Gut microbiota profiles in critically ill patients, potential biomarkers and risk variables for sepsis, Gut Microb. 12 (2020) 1707610.
[58] K. Fisher, C. Phillips, The ecology, epidemiology and virulence of enterococcus, Microbiology 155 (2009) 1749–1757.
[59] M. Hayakawa, T. Asahara, N. Henzan, et al., Dramatic changes of the gut flora immediately after severe and sudden insults, Dig. Dis. Sci. 56 (2011) 2361–2365.