https://doi.org/10.31925/farmacia.2025.1.7 **ORIGINAL ARTICLE**

VITAMIN C REGULATING HOST-MICROBE HOMEOSTASIS IN THE GUT OF PATIENTS WITH SEPSIS

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Abstract

This study examined the regulatory effects of vitamin C (VitC) on intestinal microbial homeostasis in patients with sepsis complicated by gastrointestinal perforation (GIP). Ninety-six septic GIP patients were divided into two groups: the VitC group (VCG) (GIP repair + conventional sepsis treatment + VitC) and the non-VitC group (nVCG) (GIP repair + conventional sepsis treatment). A control group (Ctrl) of 96 non-septic GIP patients was also included. Faecal samples were analysed using 16S rRNA sequencing to assess bacterial community structure, alpha and beta diversity, and relative abundance (RA). Clinical parameters, including inflammatory markers, organ function, and coagulation indices, were evaluated before and after treatment. The RA of *Firmicutes* and *Bacteroidetes* was higher, while *Actinobacteria* and *Proteobacteria* were lower in the VCG compared to nVCG ($p < 0.05$). Chao1 and Faith pd indices indicated improved microbial diversity in the VCG. After treatment, the VCG showed distinct enrichment of beneficial gut flora, while nVCG exhibited an overgrowth of *Pseudomonas* species. VitC also improved clinical outcomes, reducing TBIL, ALK-P and urea levels, while increasing PLT and lowering Cr. In conclusion, VitC enhances gut microbial diversity, restores intestinal homeostasis, and ameliorates clinical symptoms of sepsis in GIP patients.

Rezumat

Acest studiu a investigat efectele reglatoare ale vitaminei C (VitC) asupra homeostaziei microbiene intestinale la pacienții cu sepsis complicat cu perforație gastrointestinală (GIP). 96 de pacienți cu GIP septic au fost împărțiți în două grupuri: grupul VitC (VCG) (reparare GIP + tratament convențional pentru sepsis + VitC) și grupul fără VitC (nVCG) (reparare GIP + tratament convențional pentru sepsis). Un grup de control (Ctrl) format din 96 de pacienți non-septici cu GIP a fost de asemenea inclus. Probele fecale au fost analizate prin secvențierea genei 16S rRNA pentru a evalua structura comunităților bacteriene, diversitatea *alfa* și *beta*, precum și abundența relativă. Parametrii clinici, incluzând markerii inflamatori, funcția organelor și indicii de coagulare, au fost evaluați înainte și după tratament. Abundența relativă pentru *Firmicutes* și *Bacteroidetes* a fost mai mare, în timp ce *Actinobacteria* și *Proteobacteria* au fost mai scăzute în VCG comparativ cu nVCG ($p < 0,05$). Indicii Chao1 și Faith pd au indicat o diversitate microbiană îmbunătățită în VCG. După tratament, VCG a prezentat o îmbogățire a florei intestinale benefice, în timp ce nVCG a arătat o creștere excesivă a speciei *Pseudomonas*. VitC a îmbunătățit, de asemenea, rezultatele clinice, reducând nivelurile de TBIL, ALK-P și uree, crescând în același timp PLT și scăzând Cr. În concluzie, VitC crește diversitatea microbiană intestinală, restabilește homeostazia intestinală și ameliorează simptomele clinice ale sepsisului la pacienții cu GIP.

Keywords: Vitamin C, sepsis, microbiota

Introduction

Sepsis is a common clinical syndrome with high mortality and complex aetiology [1]. In 2017, the World Health Assembly and WHO listed sepsis as a major public health event of global concern [2]. It mainly affects immune homeostasis and organ function of the infected host. Due to its complex aetiology, sepsis affects systemic homeostasis and systemic organs, and existing treatments are difficult to effectively correct the systemic imbalance and organ damage caused by sepsis [3]. Therefore, early systemic intervention is particularly important.

In recent years, an increasing number of studies have shown that dysbiosis is closely associated with the onset and development of sepsis [4-5]. The balance of gut microbial composition is one of the key

factors in maintaining the health of the immune system and the overall host homeostasis [6]. In patients with sepsis, excessive proliferation of pathogenic microorganisms and a reduction in intestinal probiotics can lead to the destruction of the intestinal barrier function, allowing pathogens to easily cross the intestinal mucosa, enter the bloodstream and trigger a systemic immune response [7]. The imbalance of gut microbes may also trigger the release of inflammatory mediators and exacerbate the systemic inflammatory response, creating a vicious cycle [8]. This inflammatory state has a negative impact on the regulation of the immune system, making the host more susceptible to infection. In addition, gut microbial dysbiosis is associated with impaired function of multiple organs, including the cardiovascular, pulmonary and hepatic systems [9-11], exacerbating the severity of sepsis. Therefore, a better understanding of the role of the gut microbiome in sepsis is expected to provide new strategies for prevention and treatment, regulate the composition of the gut microbiome, maintain its homeostasis, and become a potential way to intervene in the development of sepsis.

As a water-soluble vitamin, vitamin C (VitC) is widely considered to play an important role in the regulation of the immune system [12]. Its function in antioxidant stress and inhibition of inflammatory response has attracted much attention [13]. It has been observed that serum VitC levels in sepsis patients are significantly lower than in normal patients, further suggesting the close association between VitC and sepsis [14]. This phenomenon is mainly due to the fact that non-human primates lack VitC synthetase and therefore rely mainly on intestinal absorption to meet the body's need for VitC [15]. In patients with sepsis, a visible decrease in VitC levels may be due to a variety of reasons, including a decrease in VitC intake, intestinal malabsorption, and an increase in VitC consumption due to the disease [16]. Recent evidence suggests that although VitC failed to improve the Sequential Organ Failure Assessment (SOFA) score in patients with sepsis, it was able to reduce 28-day in-hospital mortality in patients [17]. VitC supplementation has also been found to alter the abundance of Bacteroides in the human gut, further suggesting that VitC may play an immunomodulatory role by regulating the gut flora [18]. *Lactobacillus* may play an important regulatory role in this process. However, current clinical data are relatively limited, and results from larger clinical samples and correlation analysis are needed to verify the relevance of VitC in regulating immune dysregulation and promoting resolution of inflammation in sepsis by *Lactobacillus*. We anticipate these results to offer a more profound theoretical foundation for the application of VitC in sepsis.

To clarify the clinical value of VitC in sepsis, the microbial composition of the faeces of patients before and after VitC treatment and those of patients without VitC treatment was analysed and compared using a large number of clinical case samples. In addition, the influence of VitC in regulating flora and disease treatment was verified by comparing the faeces of sepsis and non-sepsis patients, and the regulatory effect of VitC on host-microbe interaction in sepsis patients was further explored, and its potential mechanism in the development of disease was revealed. Through systematic experimental design and analysis, the effects of VitC on host intestinal immune response and microbial composition were comprehensively analysed. The results are expected to provide new ideas and methods for the treatment of sepsis, as well as deeper theoretical support for the clinical application of VitC. A deeper understanding of the pathogenesis of sepsis may provide a solid scientific basis for future individualised treatment and prevention strategies.

Materials and Methods

Subjects

Ninety-six patients with sepsis complicated by GIP were hospitalised between March 2020 and March 2023 and 96 patients with non-septic GIP hospitalised during the same period were enrolled. A full and detailed explanation was given to the patients, and informed consent was signed by the subjects or their legal representatives (in the case of incapacitated subjects). The study was approved by the Ethics Committee of First Affiliated Hospital of Wenzhou Medical University, Wenzhou, Zhejiang, China.

Inclusion criteria: (1) age range: between 18 and 60 years old; (2) all patients were diagnosed with sepsis according to Sepsis-3 [19] published in 2016: SOFA: respiratory system, liver function, PLT, circulatory system, renal function and consciousness state, the total score was 0 - 24 points. When SOFA $\geq$ 2 points, they were considered to have sepsis; (3) they had not received any interventions affecting the gastrointestinal microbiome, such as antibiotics, probiotics; (4) patients could understand and follow the requirements of the protocol.

Exclusion criteria: (1) pregnant or lactating women; (2) severe liver or renal insufficiency or other vital organ diseases; (3) immune-related diseases; (4) patients allergic to VitC; (5) patients already involved in other clinical practice; (6) intestinal infection; (7) other chronic diseases that may affect the microbiome, such as inflammatory bowel disease; (8) severe cardiovascular disease, neurological disease, or other diseases unsuitable for participation; (9) individuals with alcohol or drug abuse problems.

Grouping and Treatment

Ninety-six patients with sepsis complicated by GIP were randomised into VCG and nVCG group (48 cases each) and 96 patients with non-septic GIP were included in the Ctrl group.

GIP repair: All subjects underwent GIP repair. Prophylactic anti-infection and rehydration treatments were administered before surgery. Approximately 30 min before surgery, 1 g of cefazolin (Zhongshan Branch, Zhuhai United Lab Pharmaceutical Co., LTD., China) and 300 mL of glucose solution combined with balanced salt solution (1.25% sodium bicarbonate solution, Huiyinbi Group (Jiangxi) East Asia Pharmaceutical Co., LTD., China): 0.9% sodium chloride solution (Zhejiang Kangle Pharmaceutical Co., LTD., China) = 1:2 were slowly infused intravenously at one time. A Foley catheter (Bard, USA) and a gastrointestinal decompression tube (Rüsch, Germany) were also inserted. General combined intravenous anaesthesia was performed with an endotracheal tube using inhaled sevoflurane (Fujian Haixi United Pharmaceutical Co., LTD., China) at an inhaled concentration of 4%. To perform the three-hole procedure, a mesoclavicular puncture was made in the midline of the clavicle at the inferior edge of the right rib and a 1.0 cm trocar (Medtronic, United States) was inserted to visualise the lesion. A second procedure hole was created by midline clavicle puncture at the inferior margin of the left rib and a 0.5 cm trocar was inserted. The gastric contents and effusion in the operative field were evacuated, and the appropriate absorbable suture was selected according to the diameter of the perforation and oedema status and sutured along the long axis of the stomach and duodenum. The pedicled omentum was then covered and secured. Postoperative gastrointestinal decompression was continued until anal evacuation was restored. 2 to 3 days later, the drainage tube was removed, and routine care was performed.

VCG: GIP repair + routine sepsis medication + VitC + normal saline. GIP repair was performed. Routine drug therapy for sepsis specifically includes: cefoperazone sulbactam (Pfizer, USA), intravenous infusion, 2 g each time, once every 8 h; sodium chloride injection (Hubei Tiansheng Pharmaceutical Co., LTD., China), intravenous infusion, 10 - 60 mL each time, 1 - 2 times a day; potassium chloride needle (10%) (Shandong Shenglu Pharmaceutical Co., LTD., China), intravenous drip/micropump maintenance, 10 ~ 40 mL each time, 1 - 2 times a day; magnesium sulfate solution (Jinling Pharmaceutical Co., LTD., China), (nutrition) intravenous drip, 5 ~ 100 mL each time, once a day; epinephrine solution (tartaric acid) (Shanghai Hefeng Pharmaceutical Co., LTD., China), micro pump maintenance, 2 - 18 mg each time, maintenance medication; Low molecular weight heparin sodium injection (Qilu Pharmaceutical Co, LTD., China), subcutaneous injection, 0.2 - 0.4 mL each time, 1 - 2 times a day; insulin (RI) (Novo Nordisk Pharmaceuticals Co., LTD., China), subcutaneous injection/intravenous drip, 4 ~ 24 u each time, once a day. A total dose of 50 mg/(kg/day) of VitC (Shanxi Pude Pharmaceutical, China) and 0.9% normal saline (Zhejiang Kangle Pharmaceutical Co., LTD., China) were mixed into 30 mL solution by intravenous injection, once a day, at a constant speed within 30 minutes. Each treatment course lasts four weeks, with a minimum of two courses, totalling eight weeks.

nVCG: GIP repair + routine drugs for sepsis + normal saline. The treatment methods of GIP repair and routine drug therapy were the same as VCG. Normal saline treatment: 30 mL of 0.9% normal saline was injected into the body by intravenous injection, once a day. Each treatment course lasts four weeks, with a minimum of two courses, totalling eight weeks.

Ctrl: GIP repair + normal saline. The treatment of GIP repair was the same as VCG. The treatment of normal saline was the same as that of nVCG, intravenous injection.

Stool Collection and Preservation

Faecal samples were collected. After cleaning the anal area, the anus was exposed. A disposable sterile cotton swab (Shenzhen Huachenyang Technology Co., LTD., China) was inserted into the anorectal area, gently rotated and slowly removed after ensuring that sufficient stool sample had been collected. It was placed in a Thermo Scientific Nalgene sterile frozen storage tube (Thermo Electron Corporation, USA) and the tube cap was quickly and tightly closed. The sterile frozen tubes were immediately stored in liquid nitrogen containers. It was transferred to a deep freezer at -80℃ for storage.

16S rRNA Gene High-throughput Sequencing Technology Detection

Before performing the experiment, the stool sample was removed to avoid the freeze-thaw process and ensure that the sample remained stable. During the experimental operation, the necessary aseptic operation was carried out. The genomic DNA of the samples was extracted using the bacterial genome total DNA extraction kit. After extraction, agarose gel electrophoresis and fluorometer were used to determine the DNA concentration of the samples.

DNA extraction: total DNA was extracted from stool samples using bacterial genomic total DNA extraction kit (M3291-02; Omega Bio-tek, USA) according to the instructions provided by the manufacturer. To check the quality of the DNA extraction, the DNA bands can be visualised by agarose gel electrophoresis to ensure that the extracted DNA is intact and free of degradation. The DNA concentration was determined using a fluorometer to

ensure the amount of DNA required for subsequent steps.

DNA library construction and high-throughput sequencing: the 16S V4 region was selected for PCR amplification, and the primer 343F-798R was used for PCR amplification to obtain the V4 region fragment of 16S rRNA gene. The PCR products were then purified. Libraries were constructed and adapters and sample tags were added. Library average molecular lengths and concentrations were determined using an Agilent 2100 biochip analysis system. Libraries were quantified by real-time PCR and Paired-End sequencing (PE250) of the libraries was performed using an Illumina HiSeq 2500 platform.

Bioinformatics analysis: The original double-end sequences were dehybridised using Trimomatic software, and the sequences were clustered using Vsearch software to obtain representative sequences of operational taxonomic units (OTUs). QIIME software was used to align and annotates the representative sequences with the 16S database, and the RDP classification algorithm was used to annotates the representative sequences. The relative abundance (RA), Alpha diversity index, and Beta diversity were calculated to analyse the structure and diversity of bacterial communities. PCoA was drawn using R software, and the LEfSe online analysis program was adopted to find species with visible differences in abundance between groups.

Assessment of Disease Indicators

The condition changes of the subjects in VCG and nVCG were evaluated by detecting sepsis-related indicators, mainly by detecting the subjects' general vital signs and related laboratory indicators. General vital signs included body temperature, HR, RR, blood pressure (DBP, SBP). Related laboratory indicators mainly included blood routine, including CRP, WBC and PLT. Coagulation function indexes mainly included PT, APTT and INR. Liver function indices evaluated were AST, ALT, TBIL and ALK-P and the renal function indices evaluated were Cr, urea nitrogen, urine volume and lactate level.

CRP was determined by ELISA analyser (Model: XYZ-123, ABC Company, Germany), WBC and PLT by automated blood cell analyser (Model: Sysmex XN-1000, Sysmex Corporation, Japan), PT, APTT and INR by haemagglutination analyser (Model: Coag-X2000, Hemotech Corporation, Germany). AST and ALT were determined by chemiluminescence analyser (Model: LIAISON® XL, DiaSorin, Italy). TBIL, ALK-P, Cr, urea and other parameters were measured using a spectrophotometric method with an automatic analyser (Shimadzu, Japan). Observation indicators:

(1) The general clinical data of subjects were collected, including age, gender, smoking history, drinking history, and complications.

(2) The RA, Alpha diversity index, and Beta diversity of intestinal bacterial community were compared before and after treatment, and the structure and diversity of the bacterial community were analysed.

(3) The body temperature, HR, RR, DBP, SBP, CRP, WBC, PLT, PT, APTT, INR, AST, ALT, TBIL, ALK-P, Cr and urea were compared between VCG and nVCG before and after treatment.

Statistical Analysis

All data were presented as mean ± std and analysed by SPSS19.0 (IBM Corporation, USA). The t test and one-way ANOVA test were adopted. A value of $p < 0.05$ was considered statistically meaningful.

Results and Discussion

General clinical data

There were 58 males (60.42%) and 38 females (39.58%) in the control group. The mean age was (62.21 ± 6.89) (50 - 80). Twenty subjects (20.83%) were smokers, and 23 subjects (23.96%) were alcohol drinkers. Alcohol drinkers are considered those with excessive weekly alcohol consumption: for males, drinking more than 14 units of alcohol *per* week; for females, drinking more than 7 units of alcohol *per* week.). There were 30 males (62.5%) and 18 females (37.5%) in the VCG. The mean age was (60.11 ± 6.82) (47 - 74) years. There were 11 subjects (22.92%) who smoked and 13 subjects (27.08%) who drank alcohol. There were 28 males (58.33%) and 20 females (41.67%) in the nVCG. The mean age was (59.99 ± 6.03) (50 - 74) years. There were 13 subjects (27.08%) who smoked and 11 subjects (22.92%) who drank alcohol. Age, the proportion of males, the proportion of females, smokers and drinkers were similar between VCG, nVCG and Ctrl ($p > 0.05$), suggesting comparability (Figure 1).

RA of bacterial communities at the phylum taxonomic level before and after treatment

After species annotation, the Ctrl was examined by high-throughput sequencing technology, and a total of 10 phyla and 154 genera of bacteria were obtained, among which the top 4 RA at the phylum level were: *Firmicutes*, *Bacteroidetes*, *Actinobacteria*, *Proteobacteria*, which represented about 97% of the sequences (Figure 2). *Firmicutes*, *Bacteroidetes*, *Actinobacteria* and *Proteobacteria* were also the dominant phyla in VCG and nVCG before and after treatment. However, the RA composition ratio of each phylum in VCG and nVCG was significantly different.

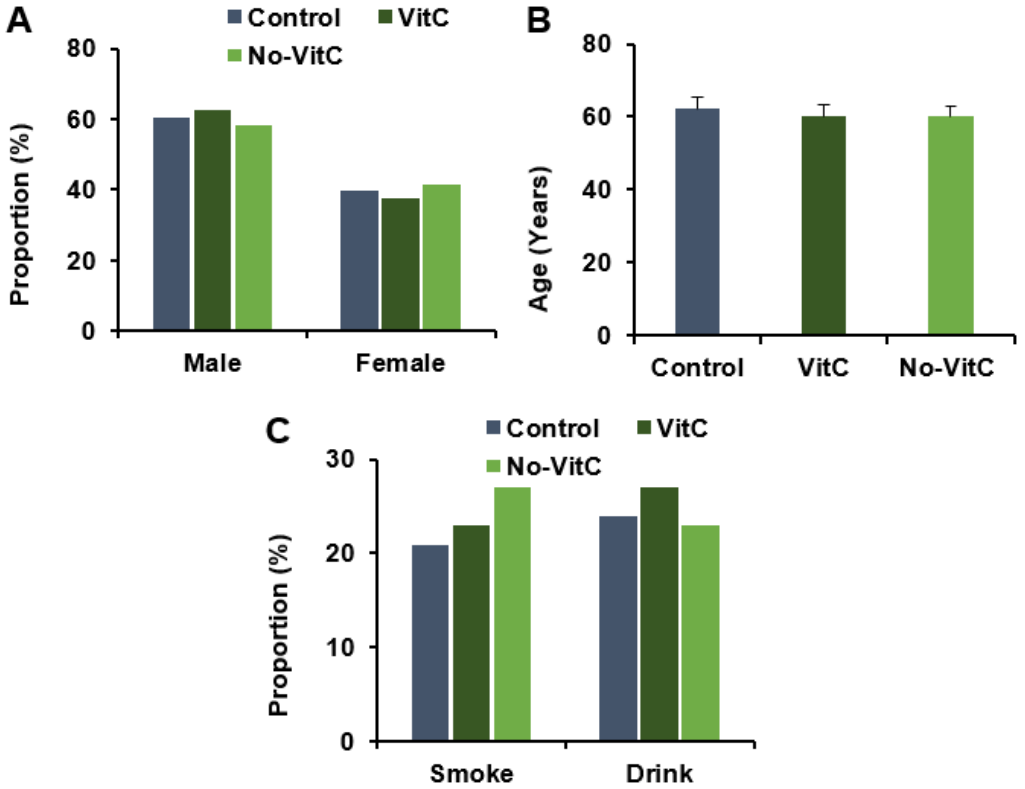

Figure 1.
General clinical data. A: Gender; B: age; C: smoking and drinking

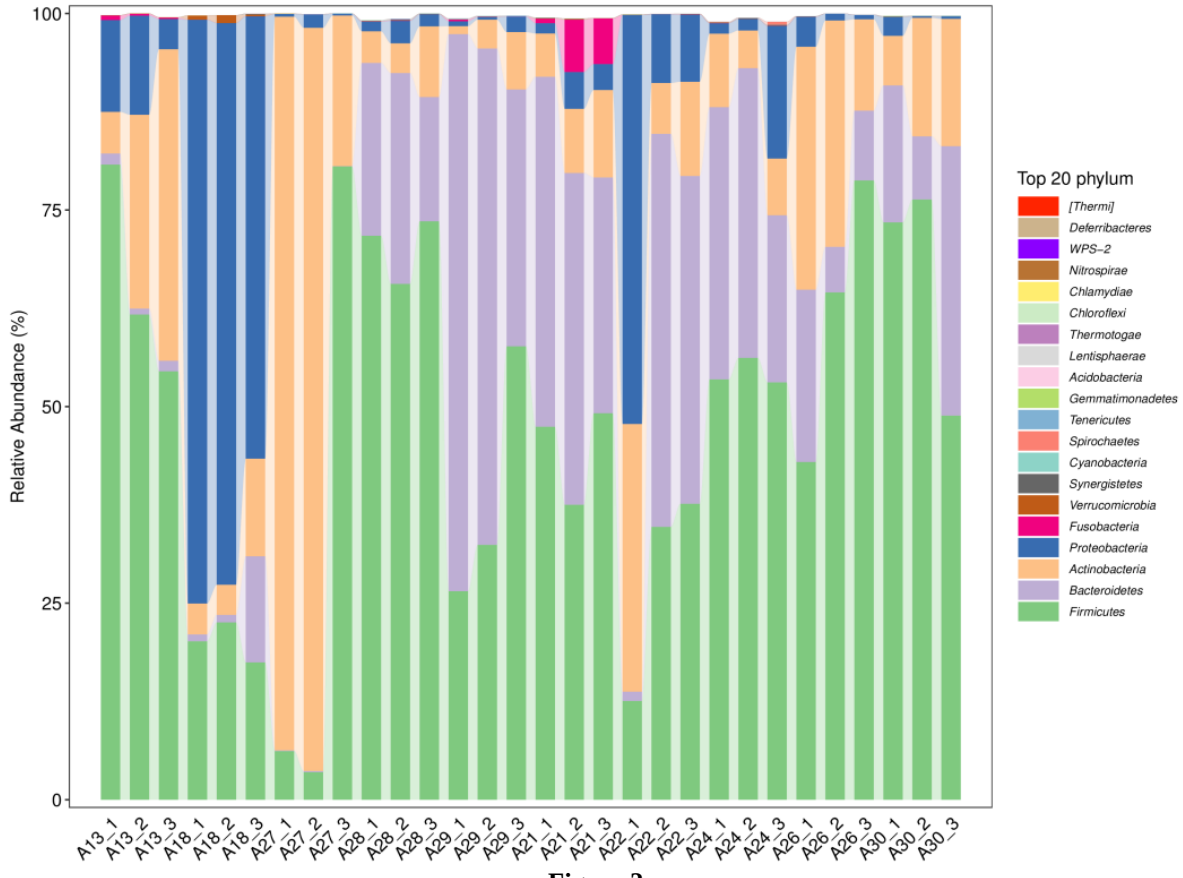

Figure 2.
Contrast of RA at the taxonomic level of the phyla of microflora

RA before treatment

The RA of *Firmicutes*, *Bacteroidetes*, *Actinobacteria* and *Proteobacteria* in the nVCG was 41.08%, 19.04%, 21.51%, and 17.62%, respectively. The RA of each bacterial flora in VCG was 45.97%, 23.93%, 17.22%, and 12.16%, respectively. The RA of *Firmicutes* and *Bacteroidetes* was lower, and the RA of *Actinobacteria* and *Proteobacteria* was higher in the nVCG as against the VCG ($p > 0.05$) (Figure 3).

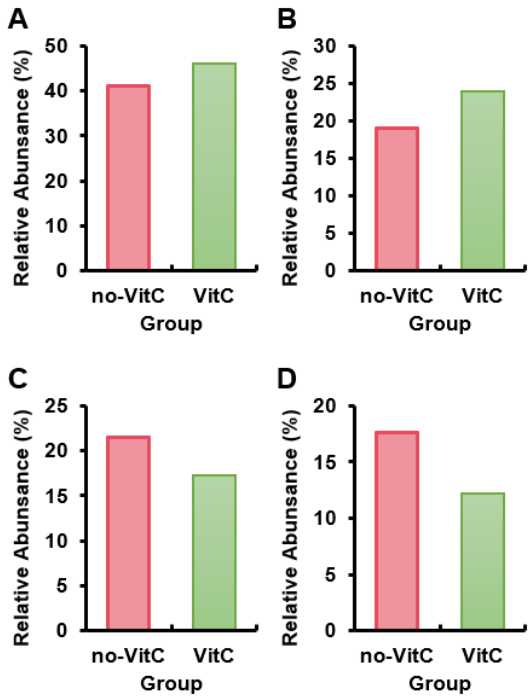

Figure 3.
RA at the phylum level
A: *Firmicutes*, B: *Bacteroidetes*. C: *Actinobacteria*, D: *Proteobacteria*

RA after treatment

The RA of *Firmicutes*, *Bacteroidetes*, *Actinobacteria* and *Proteobacteria* in the nVCG was 37.16%, 18.36%, 26.09%, and 17.78%, respectively; The RA of each bacterial flora in VCG was 53.86%, 28.57%, 12.66%, and 3.18%, respectively. By comparison, the RA of *Firmicutes* and *Bacteroidetes* was lower, and the RA of *Actinobacteria* and *Proteobacteria* was higher in the nVCG as against the VCG ($p < 0.05$) (Figure 4).

Contrast of the changing trend of RA before and after treatment

After VitC treatment, the RA of *Firmicutes* and *Bacteroidetes* decreased by 3.92% and 0.68%, while the RA of *Actinobacteria* and *Proteobacteria* increased by 4.58% and 0.16%, respectively. The RA of *Firmicutes* increased by 7.89%, *Bacteroidetes* increased by 4.64%, *Actinobacteria* decreased by 4.56%, and *Proteobacteria* decreased by 8.98% (Figure 5).

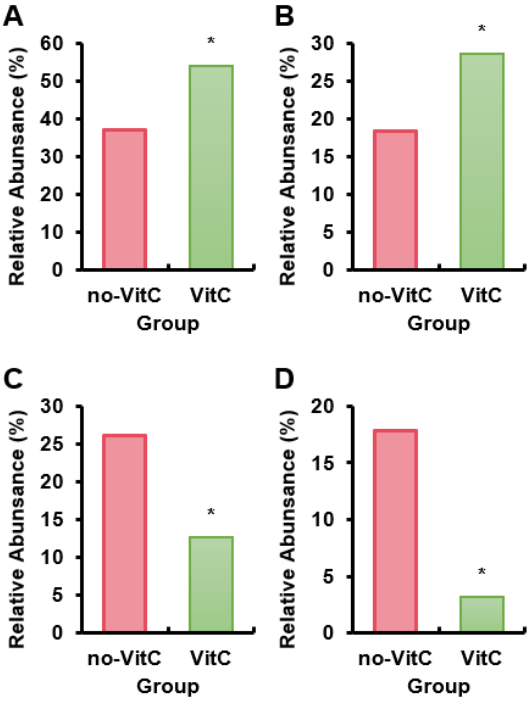

Figure 4.
RA of flora at the phylum level
A: *Firmicutes*; B: *Bacteroidetes*; C: *Actinobacteria*; D: *Proteobacteria*; * $p < 0.05$ as against nVCG.

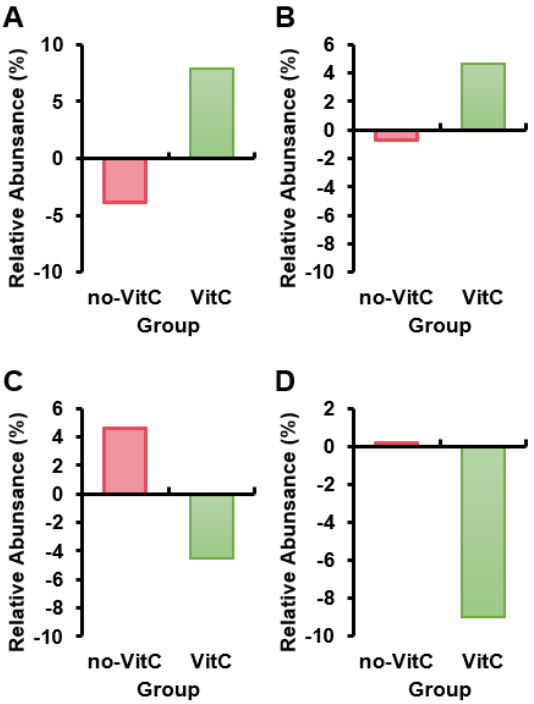

Figure 5.
RA changes at the phylum level
A: *Firmicutes*; B: *Bacteroidetes*; C: *Actinobacteria*; D: *Proteobacteria*

Alpha diversity analysis of intestinal microecology

Alpha diversity was evaluated using Chao1, Simpson diversity index, Shannon diversity index, Pielou e, Observed species, Faith pd, and Goods coverage.

The distribution of data based on Alpha diversity did not conform to the normal distribution, so the Kruskal-Wallis rank sum test was adopted.

Alpha diversity before treatment. Figure 6 shows that Chao1, Simpson, Shannon, observed species and Faith pd were lower and Pielou e and Goods coverage were higher in the nVCG than in the VCG ($p > 0.05$).

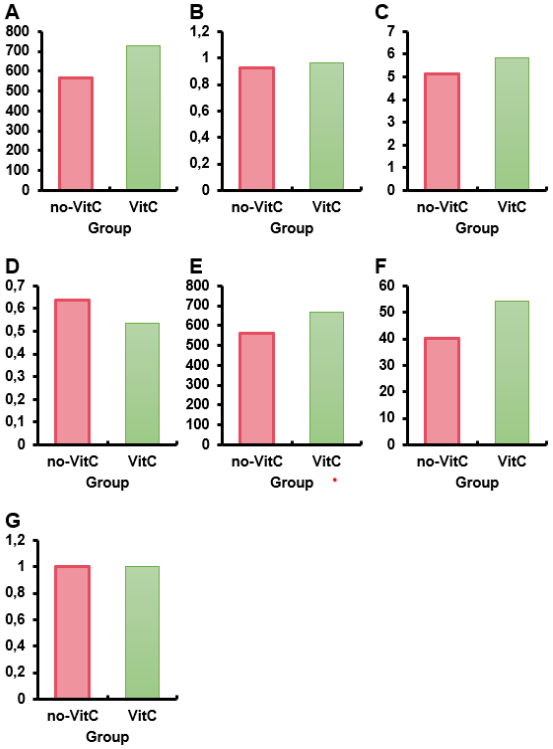

Figure 6.
Alpha diversity
A: Chao1, B: Simpson, C: Shannon, D: Pielou e, E: Observed species, F: Faith pd, G: Goods coverage

Alpha diversity after treatment

Figure 7 shows that Chao1, Simpson, Shannon, Pielou e, observed species, belief pd and goods coverage were higher in the VCG than in the nVCG.

Alpha diversity before and following treatment

Figure 8 shows that Chao1 and Faith pd were decreased in the VCG and nVCG, and the decrease in Chao1 was smaller and the decrease in Faith pd was smaller in the VCG compared to the nVCG ($p < 0.05$). Simpson and Shannon were decreased in the VCG and increased in the nVCG. Pielou e and observed species increased in the VCG and decreased in the nVCG. Product coverage of the VCG and nVCG increased, but the degree of increase of the VCG was lower than that of the nVCG ($p > 0.05$).

Beta diversity analysis of intestinal microecology

Figure 9-A shows that before the treatment, the distribution distance of the gut flora in the VCG and the nVCG overlapped. Figure 9-B illustrates that after treatment, the distribution distance of the gut flora colony in the VCG and the nVCG also overlapped. Figure 9-C illustrates that the difference in beta diversity between the VCG and the nVCG before and after treatment was relatively small.

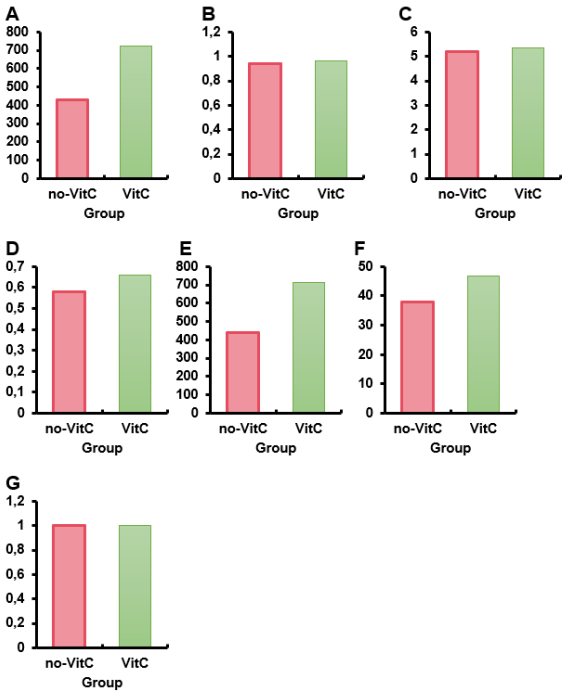

Figure 7.
Alpha diversity
A: Chao1, B: Simpson, C: Shannon, D: Pielou e, E: Observed species, F: Faith pd, G: Goods coverage

Analysis of the distinction of specific genus of intestinal flora prior to and following treatment

LEfSe software was used to analyse the distinctions of specific genera of the gut flora. The LDA value was set to 10 and those greater than the set value were considered as statistically different species to find possible biomarker distinctions of bacteria. The results indicated that ph2, *Varibaculum*, *Peptococcus* and *Peptococcaceae* were enriched in VCG at the genus level before treatment. After treatment, *Pseudomonadales* and *Pseudomonadaceae* under *Proteobacteria* were enriched in nVCG. In VCG, *Bacteroidaceae*, *Bacteroides*, *Butyricimonas* under *Bacteroidetes*, and *Actinomycetaceae* under *Actinomycetes* were enriched. At the genus level, the gut microbiota of each group differed in specific genera (Figure 10).

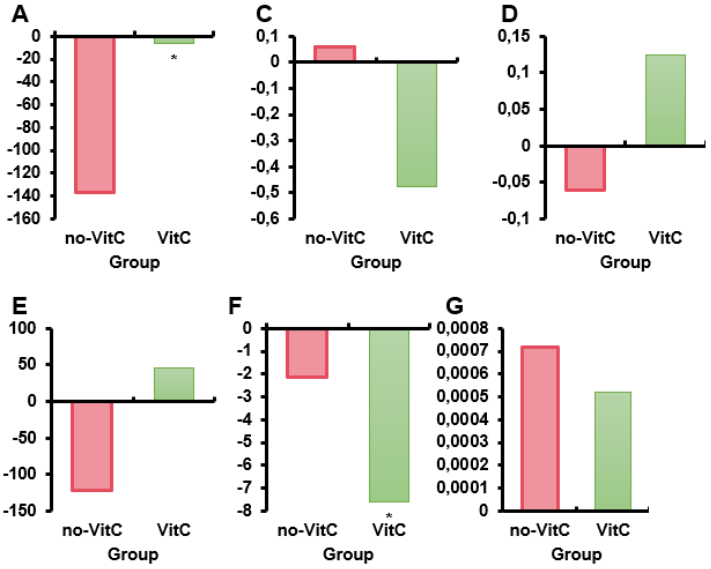

Figure 8.
Alpha diversity
A: Chao1, B: Simpson, C: Shannon, D: Pielou e, E: Observed species, F: Faith pd, G: Goods coverage
* $p < 0.05$ compared to nVCG

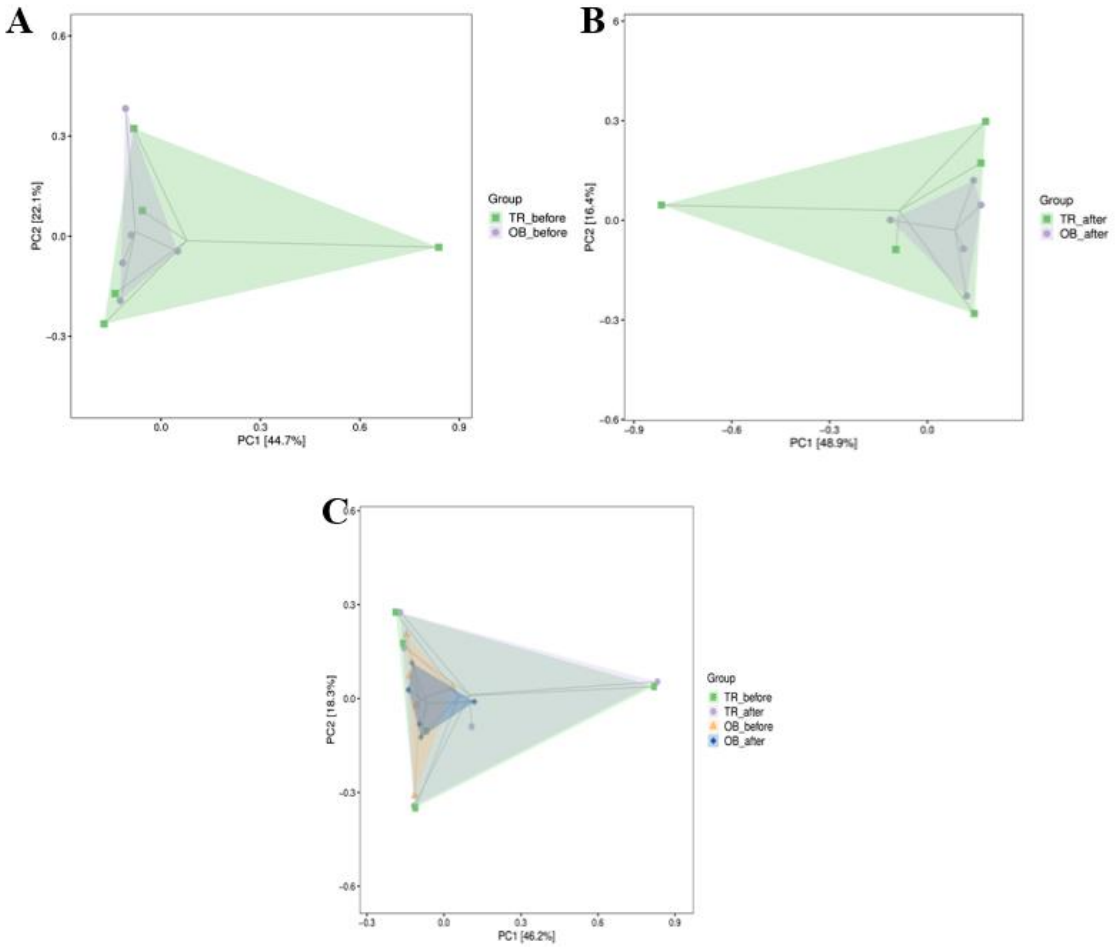

Figure 9.
Beta diversity analysis of intestinal microflora
A: prior to remedy B: following remedy C: prior to and following remedy; TR-Prior to: nVCG prior to remedy, TR-After: nVCG following remedy; OB-Prior to: VCG prior to remedy, OB-After: VCG following remedy

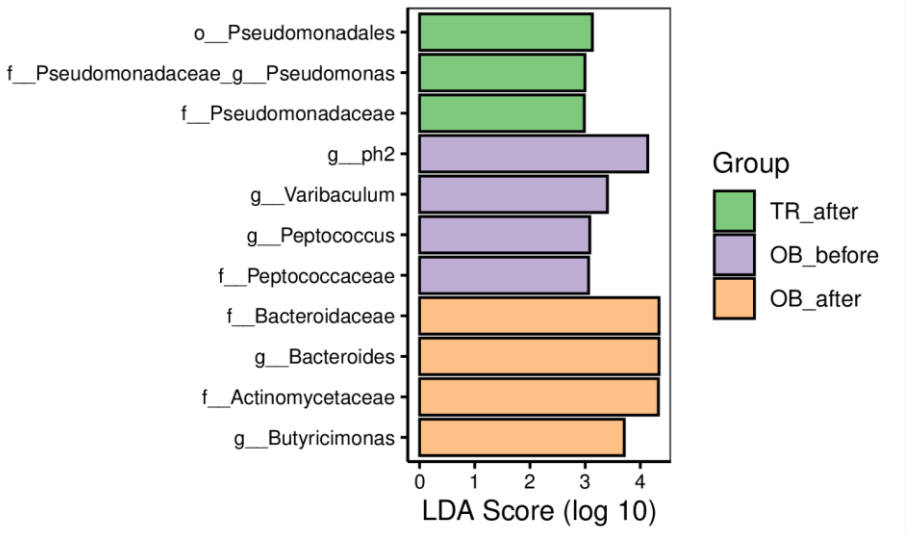

Figure 10.

Analysis of genus-specific distinctions in gut microbiota

TR-After: nVCG following treatment; OB-Prior to: VCG prior to treatment, OB-After: VCG following treatment

Changes in sepsis-related indicators

By comparing the changes in body temperature, HR, RR, DBP, SBP, CRP, WBC, PLT, PT, APTT, INR, AST, ALT, TBIL, ALK-P, Cr and urea nitrogen before and after treatment in VCG and nVCG, the changes in sepsis status were evaluated (Table I). After the treatment, the pulse rate, CRP, TBIL and Cr levels of both groups were better than before the treatment. The TBIL level was higher, and the Cr level was lower in the VCG compared to the nVCG. The PLT in the nVCG was significantly increased and was higher than in the VCG. The PT and urea levels in the VCG were significantly lower than before the treatment, while the ALK-P level was significantly higher in the VCG compared to the nVCG (all $p < 0.05$).

Table I

Changes in sepsis-related indicators

	Before treatment		**After treatment**	
	nVCG (n = 48)	VCG (n = 48)	nVCG (n = 48)	VCG (n = 48)
Body temperature (°C)	37.7 ± 0.32	37.04 ± 0.33	36.86 ± 1.21	36.56 ± 1.09
HR (beats/min)	92.2 ± 3.09	105.75 ± 10.83	77.6 ± 7.22#	85.8 ± 5.73#
RR (breaths/min)	21.8 ± 1.21	20 ± 1.92	18.8 ± 8	19.2 ± 2.54
DBP (mmHg)	74.4 ± 1.20	75.4 ± 4.93	74 ± 9	71.2 ± 1.77
SBP (mmHg)	122 ± 3.21	120.6 ± 11.82	133 ± 22	133.2 ± 10.29
Pulse (beats/min)	92.2 ± 4.09	112 ± 20	74.6 ± 21.32#	83.8 ± 4.82#
CRP (mg/L)	185.56 ± 10.32	174.44 ± 9	44.14 ± 17#	53.04 ± 3.22#
WBC (10^9/L)	8.93 ± 1.22	6.80 ± 1.20	8.82 ± 3.21	7.35 ± 1.21
PLT (10^9/L)	176.6 ± 19.22	206 ± 8.21	412 ± 38.21#	276.4 ± 20.09*
PT (s)	15.82 ± 1.22	17.62 ± 1.38	14.64 ± 1.21	14.58 ± 1.03#
APTT	1.18 ± 0.28	1.18 ± 0.03	1.2 ± 0.22	1.25 ± 0.33
INR	1.25 ± 0.21	1.45 ± 0.09	1.13 ± 0.71	1.13 ± 0.10
AST (U/L)	30.2 ± 3.02	25.2 ± 1.22	41.8 ± 7.21	23.8 ± 3.21
ALT (U/L)	26.6 ± 1.22	20.4 ± 3.22	28.4 ± 3.21	18.2 ± 1.28
TBIL (µmol/L)	18.6 ± 1.29	15.8 ± 2.21	14.4 ± 1.28#	20.2 ± 4.09#*
ALK-P (U/L)	70.4 ± 2.81	59.6 ± 4.32	77.33 ± 6.72	107.25 ± 3.33#*
Cr (µmol/L)	86.8 ± 2.37	107.2 ± 9.22	62.8 ± 2.39#	50.2 ± 3.22#*
Urea (mmol/L)	6.7 ± 1.00	11.32 ± 1.21	4.38 ± 1.00	5.4 ± 1.11#

Note: "#" $p < 0.05$ compared to prior to treatment, "*" $p < 0.05$ compared to nVCG

The normal functioning of the gastrointestinal tract in patients with sepsis is closely related to the homeostasis of intestinal microorganisms. For example, *Bacteroides* are involved in many important metabolic activities in the human colon, including carbohydrate fermentation, nitrogen utilization and the biotransformation of bile acids and other steroids, and have a complex and generally beneficial relationship with the host [20, 21]. *Firmicutes* is the largest group of bacteria, most of which are Gram-positive, and many of its members are beneficial bacteria [22]. Some species of these phyla are generally thought to play a beneficial role

in maintaining gut health and preventing the overgrowth of pathogenic bacteria. However, it has been found that the abundance of *Firmicutes* and *Bacteroidetes* bacteria decreases in patients with sepsis, and on the contrary, an overgrowth of harmful bacteria such as *Actinobacteria* and *Proteobacteria* can occur in the gut of patients with sepsis [23, 24]. The RA of *Actinobacteria* and *Proteobacteria* was found to be increased in patients with sepsis. Excessive growth of these bacteria can lead to an increased inflammatory response and have a negative impact on the host. In sepsis, the systemic inflammatory response can cause damage to the intestinal mucosa, making the intestine more susceptible to external pathogens. In this study, the RA of *Firmicutes*, *Bacteroidetes*, *Actinobacteria* and *Proteobacteria* in VCG and nVCG changed significantly before and after treatment. The RA of *Firmicutes* and *Bacteroidetes* were higher, while the RA of *Actinobacteria* and *Proteobacteria* in VCG were lower in VCG compared to nVCG. In conclusion, VitC can adjust the RA of intestinal microorganisms in patients with sepsis and improve the number of different intestinal microorganisms.

Gut microbial diversity was also analysed. In the alpha diversity analysis indicators, higher Chao1, Faith's PD, goods coverage, Shannon and observed species indicated more species and greater diversity. The lower Simpson value indicated higher species diversity. The closer Pielou e is to 1, the more uniform the distribution of species abundance [25]. Combined with the results of this study, Chao1 was not significantly decreased, Simpson was decreased, and Pielou e and observed species were significantly increased in patients injected with VitC. VitC has a certain effect on the structure and diversity of the intestinal microbial community, which is helpful in increasing species diversity. VitC could improve the evenness of species abundance and increase the actual number of species observed. However, there was a visible decrease in Shannon and Faith pd. The reason for the decrease in Shannon could be the increase in RA of some species or the loss of some species leading to a decrease in total diversity. The reason for the decrease in Faith pd may be the loss or reduction of species with relatively long evolutionary branches, leading to a decrease in overall evolutionary diversity. Beta diversity analysis is a method used to distinguish between different samples or communities, focusing on similarities and differences in species composition between samples. In this article, PCoA suggested that the distribution distances of the gut microbiota were all overlapping. This result suggests that the overall effect of VitC supplementation on the beta diversity of the gut microbiota is relatively small, which may be related to the observation time, sample size and insufficient diversity.

The analysis of the specific genus differentiation of the gut microbiota suggested that there were differences in the specific genus of the gut microbiota between VCG and nVCG before and after treatment. *Pseudomonas* is a Gram-negative bacterium that is widely distributed in different environments such as soil, water, plants and animals [26, 27]. These bacteria exhibit a high degree of metabolic adaptability and tolerance, allowing them to thrive in a wide range of environmental conditions. Although *Pseudomonas* has many functions in its natural environment, it is also considered to be a member of a number of pathogenic strains capable of causing infections in humans and other organisms [28, 29]. *Bacteroidetes* is a common phylum of the gut microbiome and is primarily found in the gut of humans and animals. It plays crucial physiological and ecological roles in digestion and metabolism, regulation of the immune system, prevention of pathogen invasion and promotion of intestinal barrier integrity [30, 31]. SCFAs, such as butyric acid, are organic acids produced by bacterial fermentation of indigestible dietary fiber [32]. *Butyricimonas* and its butyric acid production affect the ecological balance of other intestinal microflora, mainly by supporting the growth of beneficial bacteria and inhibiting the proliferation of harmful bacteria [33]. Bacteria of the *Actinomycetes* family are commonly found in soil and water, and some members of the *Actinomycetes* family are found in the human body, including the oral cavity, skin and gastrointestinal tract, where they play an important role in host health. Therefore, it is clear that VitC can selectively stimulate the growth and proliferation of normal gut microbiota by influencing various aspects of metabolism, immunity and inflammation between bacterial and host factors [34]. Based on the above-combined findings, it can be concluded that VitC has the potential to improve RA levels and gut microbiota diversity in sepsis patients with GIP. This improvement promotes an increase in beneficial bacteria, thereby improving the stability of the GI microbiota. Li *et al*. (2021) [35] demonstrated that both exercise training and VitC supplementation can alter the composition of the gut microbiota. Huang *et al*. (2023) [36] proposed that VitC can effectively alleviate the effects of fecal microbiota transplantation, thereby regulating the balance of the immune system. These provide valuable support for the present results.

The results indicated that TBIL and ALK-P levels were higher, and PLT and Cr levels were lower in VCG after treatment compared to nVCG and PT and urea levels in VCG were significantly lower than before treatment (all $p < 0.05$). The decrease in Cr levels in VCG after treatment suggests a possible beneficial effect on renal function. In contrast, increased TBIL and ALK-P levels reflected some

effects on liver function. VitC acts as an antioxidant to help neutralize free radicals and reduce oxidative stress in the liver and kidneys. Its anti-inflammatory properties may also have a protective effect on renal function [37]. Vitamin C supplementation may help reduce the severity of fatty liver and improve liver function [38, 39]. The reduction in PT levels after treatment in the VCG suggests that clotting function is affected, as clotting factors are mainly synthesized in the liver. Thus, improved liver function could lead to increased synthesis and release of clotting factors, which could reduce PT levels. It was also suggested that there were no visible differences in inflammatory markers between VCG and nVCG before and after treatment. However, PLA levels were significantly higher in nVCG than in VCG ($p < 0.05$), suggesting a possible effect of VitC in maintaining normal PLA levels. However, further studies are needed to confirm these observations.

Conclusions

The use of VitC in the treatment of sepsis complicated by GIP was used to evaluate changes in gastrointestinal microbial homeostasis and sepsis indicators before and after treatment. VitC could improve the number and diversity of gastrointestinal microorganisms, regulate the intestinal microecological homeostasis of patients, and have a certain ameliorative effect on sepsis. However, due to the lack of sample size and diversity, there are some limitations to the results. A large sample size, multi-centre and long-term follow-up can be used to increase the reliability and generalizability of the results.

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Conflict of interest

The authors declare no conflict of interest.

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