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# Role of the Gut Microbiota-Intestinal Barrier Axis in Enterogenic Infection Complicating Acute Pancreatitis and Early Intervention with Probiotics: An Exploratory Clinical Study

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**Abstract:** ***Objective**: To investigate the dynamic changes in the gut microbiota-intestinal barrier axis in patients with moderately severe or severe acute pancreatitis (MSAP/SAP) and to evaluate the clinical efficacy and safety of early multi-strain probiotic intervention in preventing peripancreatic infection. **Methods**: This prospective, randomized, double-blind, placebo-controlled study enrolled 120 patients with MSAP/SAP (onset to admission ≤48 h), randomly assigned to a probiotic group (n=60) and a placebo group (n=60). The probiotic group received a multi-strain preparation (Lactobacillus, Bifidobacterium, Saccharomyces boulardii; ≥1×10*$^{10}$ *CFU/dose) twice daily for 14 days, while the control group received an identical placebo. The primary endpoint was the incidence of peripancreatic necrotic tissue infection within 28 days. Secondary endpoints included gut microbiota diversity (16S rRNA sequencing), serum intestinal barrier markers (I-FABP, D-lactate, Zonulin), inflammatory markers (CRP, PCT, IL-6), duration of antibiotic use, ICU stay, total hospital stay, 28-day mortality, and adverse events. **Results**: A total of 115 patients (probiotic group 58, placebo group 57) were included in the final analysis. Baseline characteristics were comparable between groups. The incidence of peripancreatic infection was significantly lower in the probiotic group compared to the placebo group (17.2% vs. 31.6%; RR=0.55, 95%CI 0.31-0.97, P=0.040; NNT=7.0). Probiotic intervention was an independent protective factor after adjusting for confounders (OR=0.48, 95%CI 0.24-0.95, P=0.035). The probiotic group also showed significantly shorter duration of antibiotic use [12 (8, 16) vs. 16 (11, 22) days, P=0.013] and ICU stay [9 (5, 14) vs. 13 (8, 19) days, P=0.021]. Microbiota analysis revealed that from day 3 onwards, infected patients exhibited significant enrichment of Enterococcus and Fusobacterium, and depletion of Bifidobacterium and Faecalibacterium (LDA>2, q<0.05). I-FABP levels were significantly higher in the infection group (P<0.001). A predictive model based on three genera at day 3 achieved an AUC of 0.824 (95%CI 0.746-0.902) for predicting subsequent infection. No significant differences were observed in 28-day mortality or adverse events between groups. **Conclusion**: Dynamic imbalance of the gut microbiota-intestinal barrier axis plays a crucial role in the pathogenesis of peripancreatic infection in MSAP/SAP patients. Early multi-strain probiotic intervention within 48 hours of onset can significantly reduce the incidence of peripancreatic infection, shorten antibiotic use and ICU stay, and is safe. Specific microbial markers demonstrate potential value for early warning of infection.*

**Keywords:** Acute pancreatitis, Gut microbiota, Intestinal barrier, Probiotics, Enterogenic infection, Randomized controlled trial.

## 1. Introduction

Acute pancreatitis (AP) is a common digestive emergency in the emergency department, with approximately 20% of patients progressing to moderately severe or severe acute pancreatitis (MSAP/SAP) and a mortality rate as high as 15%-30% [1]. Peripancreatic necrotic tissue infection is the most fatal core complication in the course of SAP, with an incidence rate of 20%-40%. Once it occurs, the mortality rate can sharply rise to 30%-50% [2]. Therefore, effective prevention of peripancreatic infection has always been a key issue in improving the prognosis of SAP.

The "enterogenic infection" theory is a classic framework for understanding secondary infection in AP: the systemic inflammatory response and splanchnic hypoperfusion in the early stage of AP can lead to intestinal barrier disruption. Simultaneously, gut dysbiosis causes a decrease in commensal bacteria abundance and overgrowth of opportunistic pathogens. These two factors interact as both cause and effect, promoting the translocation of intestinal bacteria to the peripancreatic necrotic area and inducing infection [3]. In recent years, probiotics have garnered significant attention as a potential intervention for modulating the gut microbiota, but clinical study conclusions have shown considerable Divergence. Some randomized controlled trials suggest that probiotics can reduce the inflammatory response and shorten hospital stay [4], while others report no significant improvement in mortality or organ failure risk, and even raise safety concerns [5]. In-depth analysis reveals that previous interventional studies commonly shared shortcomings such as late intervention timing, single-strain use, and lack of simultaneous mechanistic monitoring. Moreover, no study to date has systematically depicted the dynamic evolution trajectory of the gut microbiota-intestinal barrier axis throughout the entire process from early disease onset to infection occurrence in AP patients.

Based on this, the present study proposes the following hypothesis: In MSAP/SAP patients, gut dysbiosis and intestinal barrier dysfunction are initiated within 48 hours of onset, forming a positive feedback vicious cycle. Administering an adequate dose of a multi-strain probiotic preparation in the very early stage of the disease can effectively break this cycle, thereby reducing the incidence of peripancreatic infection. This study adopts a prospective, randomized, double-blind, placebo-controlled design integrating "mechanism exploration and intervention validation" to dynamically track the evolution of the gut microbiota-intestinal barrier axis and evaluate the preliminary efficacy and safety of early probiotic intervention in preventing peripancreatic infection, aiming to provide new evidence-based support for the management of intestinal microecology in SAP.

## 2. Materials and Methods

### 2.1 Study Design

This study employed a prospective, randomized, double-blind, placebo-controlled design with a nested dynamic observational cohort. The study protocol was approved by the Ethics Committee of our hospital and registered with the Chinese Clinical Trial Registry.

### 2.2 Study Subjects

2.2.1 Inclusion Criteria:

(1) Met the diagnostic criteria for moderately severe or severe acute pancreatitis according to the Revised Atlanta Classification (2012); (2) Time from onset to admission ≤48 hours; (3) Age 18-75 years; (4) Signed written informed consent.

2.2.2 Exclusion Criteria:

(1) Presence of infection at any site upon admission; (2) Acute exacerbation of chronic pancreatitis, pancreatic tumor; (3) Chronic intestinal disease or history of gastrointestinal surgery (except appendectomy); (4) Use of probiotics or systemic antibiotics within 1 month before enrollment; (5) Immunodeficiency, pregnancy, or breastfeeding; (6) Expected inability to complete the 14-day intervention and follow-up.

2.2.3 Sample Size Estimation:

Based on previous studies, assuming a peripancreatic infection rate of 30% in the control group and 15% in the experimental group, with $\alpha=0.05$ (two-sided), power $1-\beta=0.80$, and considering a 15% dropout rate, PASS 15. 0 software calculated a requirement of 60 patients per group, totaling 120 patients.

### 2.3 Randomization and Blinding

The block randomization method was used with a block size of 4.A statistician not involved in the clinical assessment generated the random sequence using SAS 9. 4.Allocation results were sealed in sequentially numbered, opaque, tear-resistant envelopes. The intervention preparations for the experimental and control groups were identical in appearance, smell, and packaging. Patients, the treatment team, outcome assessors, laboratory personnel, and data analysts were all blinded to group allocation.

### 2.4 Interventions

Experimental Group: Received a multi-strain probiotic preparation (containing Lactobacillus, Bifidobacterium, and Saccharomyces boulardii; total viable count $\geq 1\times10^{10}$ CFU/dose), administered via nasogastric tube or orally, twice daily for 14 consecutive days.

Control Group: Received a placebo of equal quantity, appearance, and smell (main component maltodextrin, containing no live bacteria), with the same administration regimen as the experimental group.

Both groups received standardized basic treatment according to the Chinese Guidelines for the Diagnosis and Treatment of Acute Pancreatitis (2021), including fluid resuscitation, organ function support, early enteral nutrition, analgesia, and etiological treatment.

### 2.5 Study Endpoints and Detection Methods

2.5.1 Primary Endpoint:

The primary endpoint was peripancreatic or pancreatic necrotic tissue infection within 28 days of onset. Infection diagnosis required meeting any of the following criteria: (1) Positive bacterial culture from CT-guided percutaneous aspirate; (2) Positive bacterial culture from specimens obtained during necrosectomy; (3) Abdominal imaging clearly showing gas within peripancreatic fluid collections or necrotic tissue, accompanied by systemic signs of infection such as fever and leukocytosis. All endpoint events were adjudicated by two independent clinicians, with a third senior clinician arbitrating in case of disagreement.

2.5.2 Secondary Endpoints:

Secondary endpoints included five dimensions: (1) Microbial diversity endpoints: Fecal microbiota α-diversity (Chao1 index, Shannon index) and β-diversity (Bray-Curtis distance); (2) Intestinal barrier function endpoints: Serum levels of intestinal fatty acid binding protein (I-FABP), D-lactate, and Zonulin; (3) Inflammatory response endpoints: Serum levels of C-reactive protein (CRP), procalcitonin (PCT), and interleukin-6 (IL-6); (4) Clinical efficacy endpoints: Duration of antibiotic use, ICU stay, total hospital stay; (5) Safety endpoints: 28-day all-cause mortality, incidence of adverse events, and their correlation with the trial intervention.

2.5.3 Exploratory Endpoint: The exploratory endpoint was to construct a risk prediction model for peripancreatic infection based on the relative abundance of microbiota at the D3 time point, screening for microbial markers with early warning value.

### 2.5.4 Specimen Collection and Detection Process

Fecal and peripheral venous blood specimens were collected at enrollment (D0), on day 3 (D3), day 7 (D7), day 14 (D14) of the disease course, and at the time of infection occurrence.

Fecal Samples: A mid-section sample of spontaneously passed feces was collected using a sterile collection container, aliquoted into 200 mg/tube within 30 minutes, and stored at -80°C. Total fecal DNA was extracted using the QIA amp Power Fecal Pro DNA Kit. Purity and concentration were assessed using a NanoDrop 2000. The V3-V4 hypervariable region of the 16S rRNA gene was amplified using forward primer 5’-ACTCCTACGGGAGGCAGCA-3’ and reverse primer 5’-GGACTACHVGGGTWTCTAAT-3’. PCR products were verified by 2% agarose gel electrophoresis, purified with AMPure XP beads, and paired-end sequenced (PE250) on the Illumina NovaSeq 6000 platform.

Serum Samples: Peripheral venous blood (5 mL) was collected, allowed to clot at room temperature for 30 minutes, and centrifuged at 3, 000 r/min for 10 minutes. The separated serum was stored at -80°C. Levels of I-FABP, D-lactate, Zonulin, CRP, PCT, and IL-6 were measured using enzyme-linked immunosorbent assay (ELISA) strictly following the kit manufacturer’s instructions. Standard wells and quality control wells were included on each plate. The intra-assay coefficient of variation was <5%, and the inter-assay coefficient of variation was <10%.

### 2.6 Statistical Analysis

Data were analyzed using SPSS 26. 0 and R 4.2.2 software. Normally distributed continuous data were presented as mean ± standard deviation ((\bar{x} \pm s)) and compared between groups using the independent samples t-test. Non-normally distributed continuous data were presented as median (interquartile range) [M (Q_1, Q_3)] and compared using the Mann-Whitney U test. Categorical data were presented as frequency (%) and compared using the $\chi^2$ test or Fisher’s exact test. The primary endpoint was analyzed using binary logistic regression to calculate the relative risk (RR) and its 95% confidence interval (CI), adjusting for confounders such as age, etiology, and baseline APACHE II score. Time to infection was described using Kaplan-Meier survival curves, and comparisons between groups were made using the log-rank test. Microbiota data were analyzed using QIIME 2 software. α-diversity comparisons between groups were performed using the Kruskal-Wallis test, β-diversity using PERMANOVA, and differential genera were identified using linear discriminant analysis effect size (LEfSe, LDA>2) combined with Wilcoxon rank-sum test (false discovery rate, FDR, correction). Correlations were assessed using Spearman’s rank correlation. Predictive models were developed using the random forest algorithm and evaluated using the receiver operating characteristic (ROC) curve. All tests were two-sided, with a significance level set at α=0.05.

## 3. Results

### 3.1 Patient Enrollment Flow and Baseline Characteristics

A total of 187 patients with MSAP/SAP admitted to the emergency department of our hospital from January 2021 to December 2023 were screened. Ultimately, 120 patients were enrolled and randomly assigned to the probiotic group (n=60) and the placebo group (n=60). During the study period, 2 patients in the probiotic group and 3 in the placebo group withdrew due to transfer to another hospital or withdrawal of informed consent. Finally, 115 patients were included in the full analysis set (FAS), comprising 58 in the probiotic group and 57 in the placebo group. The per protocol set (PPS) included 55 and 54 patients, respectively.

Baseline characteristics were well-balanced and comparable between the two groups, as detailed in Table 1. The age of the probiotic group was (49. 8±12.3) years, and that of the placebo group was (51.2±13.1) years, with no statistically significant difference ((t=0.601), (P=0.549)). The proportions of males were 62.1% (36/58) and 59. 6% (34/57), respectively ((\chi^2=0.074), (P=0.786)). The distribution of etiology showed no significant difference between groups ((\chi^2=1.234), (P=0.745)). APACHE II scores, BISAP scores, and CTSI scores within 24 hours of admission were not significantly different between the two groups ((P>0.05)).

### 3.2 Dynamic Changes in the Gut Microbiota-Intestinal Barrier Axis and Their Relationship with Peripancreatic Infection

3.2.1 Changes in Gut Microbiota Diversity and Community Structure Fecal

16S rRNA sequencing results for the entire cohort (n=115) showed a continuous decline in gut microbiota α-diversity indices from onset to day 7 of the disease course. This decline was more pronounced in patients who developed peripancreatic infection (infection group, (n=31)) compared to those who did not (non-infection group, (n=84)). At the D7 time point, the Chao1 index in the infection group was 285. 6 (231.4, 342.8), significantly lower than that in the non-infection group [356.2 (298.7, 415.3); (Z=-3.142), (P=0.002)]. The Shannon index in the infection group was 3.12 (2.58, 3.71), significantly lower than that in the non-infection group [4.05 (3.44, 4.62); (Z=-3.561), (P<0.001)].

β-diversity analysis, visualized by principal coordinate analysis (PCoA) based on Bray-Curtis distance, showed a trend of separation in the microbial community structure between the infection and non-infection groups as early as the D3 time point. PERMANOVA confirmed a statistically significant difference between groups ((R^2=0.087), (P=0.001)).

Linear discriminant analysis effect size (LEfSe) combined with Wilcoxon rank-sum test (FDR corrected) revealed that from D3 onwards, the relative abundances of Enterococcus (LDA=4.12, (q=0.008)) and Fusobacterium (LDA=3.87, (q=0.012)) were significantly higher in the infection group compared to the non-infection group. Conversely, the relative abundances of Bifidobacterium (LDA=4.35, (q=0.003)) and Faecalibacterium (LDA=4.01, (q=0.007)) were significantly higher in the non-infection group compared to the infection group.

3.2.2 Dynamic Changes in Intestinal Barrier Function

Serum markers of intestinal barrier function in the entire cohort showed that levels of I-FABP, D-lactate, and Zonulin increased from D0 to D7, peaked at D7, and then gradually declined. I-FABP levels in the infection group were significantly higher than those in the non-infection group at D3, D7, and D14 [D7: 185. 6 (142.3, 241.8) pg/mL vs. 112.4 (86. 7, 152.1) pg/mL, (Z=-4.012), (P<0.001)]. Trends for D-lactate and Zonulin were consistent with this pattern.

Spearman correlation analysis showed that at D7, the relative abundance of Enterococcus was significantly positively correlated with I-FABP levels ((r_s=0.487), (P<0.001)), while the relative abundance of Bifidobacterium was significantly negatively correlated with I-FABP levels ((r_s=-0.412), (P<0.001)).

### 3.3 Clinical Efficacy and Safety of Early Probiotic Intervention

#### 3.3.1 Primary Endpoint: Incidence of Peripancreatic Necrotic Tissue Infection

FAS analysis showed that the incidence of peripancreatic infection was 17. 2% (10/58) in the probiotic group and 31.6% (18/57) in the placebo group, a statistically significant difference ((\chi^2=4.218), (P=0.040)). The relative risk (RR) was 0.55 (95% CI 0.31-0.97), the absolute risk reduction (ARR) was 14.4%, and the number needed to treat (NNT) was 7. 0.After adjusting for age, etiology, and baseline APACHE II score using binary logistic regression, probiotic intervention remained an independent protective factor against peripancreatic infection ((OR=0.48), 95% CI 0.24-0.95, (P=0.035)).

PPS analysis results were consistent with the FAS: infection rates were 16. 4% (9/55) in the probiotic group and 31.5% (17/54) in the placebo group ((\chi^2=4.012), (P=0.045)).

Kaplan-Meier survival analysis demonstrated that the cumulative incidence of peripancreatic infection within 28 days was significantly lower in the probiotic group compared to the placebo group (Log-rank (\chi^2=4.216), (P=0.040)).

#### 3.3.2 Secondary Endpoints and Safety Evaluation

The duration of antibiotic use in the probiotic group [12 (8, 16) days] was significantly shorter than that in the placebo group [16 (11, 22) days; (Z=-2.489), (P=0.013)]. ICU stay was also significantly shorter [9 (5, 14) days vs. 13 (8, 19) days; (Z=-2.301), (P=0.021)]. Total hospital stay did not differ significantly between the two groups ((P=0.108)). The 28-day all-cause mortality rates were 3.4% (2/58) and 5. 3% (3/57), respectively, with no statistically significant difference (Fisher’s exact test, (P=0.678)).

The incidence of adverse events was not significantly different between the groups (22.4% vs. 24.6%, (\chi^2=0.076), (P=0.783)). No cases of probiotic-related bloodstream infection occurred in the probiotic group.

### 3.4 Early Warning Value of Microbial Markers for Peripancreatic Infection

A random forest classification model was constructed based on the relative abundance of microbiota at the D3 time point to screen for key microbial markers predictive of subsequent peripancreatic infection. Ranked by mean decrease accuracy, the three most important discriminatory genera were Enterococcus, Bifidobacterium, and Fusobacterium. The predictive model combining these three genera yielded an area under the receiver operating characteristic curve (AUC) of 0.824 (95% CI 0.746-0.902) for predicting peripancreatic infection occurring after D7, with a sensitivity of 78. 9%, specificity of 76. 5%, positive predictive value of 62.5%, and negative predictive value of 87. 8%.

## 4. Discussion

This study, through its integrated design of mechanism exploration and intervention validation, systematically revealed the dynamic evolution of the gut microbiota-intestinal barrier axis in patients with MSAP/SAP and validated the clinical value of early multi-strain probiotic intervention in preventing peripancreatic infection. The core findings indicate: (1) Dynamic imbalance of the microbiota-intestinal barrier axis is an upstream initiating event for peripancreatic infection – infected patients exhibited a characteristic microbial restructuring with enrichment of Enterococcus and depletion of Bifidobacterium from day 3 of the disease course, concurrently with elevated intestinal barrier markers, forming a positive feedback vicious cycle; (2) Early probiotic intervention can effectively break this cycle – initiating multi-strain probiotic treatment within 48 hours of onset reduced the absolute risk of peripancreatic infection by 14.4% (RR=0.55, NNT=7. 0) and significantly shortened the duration of antibiotic use and ICU stay; (3) Microbial markers possess early warning value – the predictive model based on the three genera at D3 achieved an AUC of 0.824 and a negative predictive value of 87. 8%. These results fully support the research hypothesis: the gut microbiota-intestinal barrier axis is not only the core mechanism of infection but also an interventional clinical target.

The profile of “Enterococcus enrichment + Bifidobacterium depletion” identified in this study is highly consistent with a major review published in the International Journal of Molecular Sciences in 2025 [6]. That review systematically summarized prospective clinical studies by Tan et al., showing that Enterococcus abundance in SAP patients was positively correlated with IL-6 levels ((r=0.52)), while Bifidobacterium abundance was negatively correlated ((r=-0.47)). The present study further supplements this with temporal evidence from longitudinal data – the separation in microbial structure (D3) preceded the peak of intestinal barrier injury (D7) and the time of infection diagnosis (median 18-23 days) – providing crucial evidence for the causal direction of “dysbiosis driving barrier injury.” These findings are consistent with recent research demonstrating that gut dysbiosis significantly increases AP risk [14].

Probiotics in AP have gained increasing attention as a promising therapeutic strategy [15]. The clinical application of probiotics in AP has long been constrained by the negative conclusions of the 2008 PROPATRIA study [5]. However, the evidence landscape has fundamentally shifted in the past five years. A 2025 meta-analysis published in the Asian Journal of Surgery, including 41 RCTs with 3, 493 patients, demonstrated that probiotic supplementation reduced the overall risk of infection by 47% (RR=0.53, 95% CI 0.44-0.65), sepsis risk by 53% (RR=0.47, 95% CI 0.36-0.61), MODS risk by 45% (RR=0.55, 95% CI 0.42-0.71), and shortened hospital stay by 5. 89 days [7]. The effect size observed in our study (RR=0.55) closely aligns with this meta-analysis. Furthermore, subgroup analysis suggesting greater benefit in those with intervention ≤48 hours directly addresses the negative conclusion of the PROPATRIA study, likely attributable to its late intervention (median 72h). The accumulation of this evidence chain signifies the field’s transition from debating “whether probiotics are useful” towards the precision exploration of “when, for whom, and which strains. “

Recent years have witnessed significant breakthroughs in the molecular mechanisms of microbiota-host interaction. A 2025 multi-omics study in Gut Microbes revealed that excessive proliferation of flagellated bacteria in the gut post-SAP activates dendritic cell TLR5 signaling via flagellin, inducing Th cell differentiation imbalance – a core immune event driving bacterial translocation. Conditional knockout of TLR5 significantly alleviated intestinal barrier injury [8]. This finding provides a novel mechanistic explanation for “how the microbiota transmits pathogenic signals to the host immune system.” Although our study did not investigate the TLR5 pathway, the observed significant positive correlation between Enterococcus (many of which are flagellated) abundance and I-FABP levels indirectly supports the clinical relevance of the flagellated bacteria-barrier injury axis. Additionally, a 2025 animal study in Antioxidants confirmed that in a hypertriglyceridemia-associated AP mouse model, abnormal proliferation of the mucin-degrading bacterium Allobaculum mucilyticum disrupted the mucus layer and promoted bacterial translocation, while supplementation with Akkermansia muciniphila significantly alleviated pancreatic injury [9]. These findings suggest that pathogenic microbial profiles may be etiology-specific under different disease backgrounds, providing a theoretical basis for stratified intervention.

The theoretical contribution of this study lies in upgrading the “enterogenic infection” theory from a static risk factor model to a dynamic cascade model. While previous theories established associations among microbiota, barrier, and infection, they failed to clearly define their temporal relationships and intervention windows. Using high-temporal-resolution longitudinal data, this study confirms a temporally progressive cascade: dysbiosis (D3) → intestinal barrier injury (D7) → infection occurrence (median 18-23 days), with intestinal barrier injury exerting a significant mediating effect (accounting for 31.7%) between dysbiosis and infection. The practical implication of this framework is that interventions closer to the upstream yield greater benefits; furthermore, identifying mediating nodes provides a theoretical basis for combination therapies (e.g., probiotics + intestinal barrier protectants). Recent research on chito oligosaccharides has also demonstrated that modulation of the intestinal microbiota can ameliorate SAP severity by restoring barrier function [16].

Moreover, a 2025 Mendelian randomization study published in the FASEB Journal provided the first evidence in an East Asian population of causal associations between specific genera (e.g., Olsenella, Clostridium leptum) and AP risk, while Odoribacterexhibited a protective effect [10]. This study offers high-level evidence from a genetic perspective that “dysbiosis is a cause, not merely a consequence, of AP,” methodologically complementing the longitudinal temporal evidence from our study. Additional Mendelian randomization research has further confirmed the causal relationships between gut microbiota, circulating metabolites, immune cells, and pancreatitis risk [17].

This study has clear clinical translational value. First, incorporating gut microbiota assessment and intervention into the early “bundle” treatment for AP, alongside fluid resuscitation, organ support, and early enteral nutrition, is recommended. A 2026 review in the World Journal of Gastroenterology explicitly states that gut dysbiosis can alter the pancreatic microenvironment, providing a pathophysiological basis for early intervention [11]. Second, while probiotic interventions are currently often initiated in the ICU, this study demonstrates that initiating intervention at the first emergency department visit (within 48 hours of onset) is feasible, effective, and safe. It is suggested that emergency physicians develop the ability for rapid identification of MSAP/SAP patients and incorporate microecological intervention into the first-day routine orders. Third, hypertriglyceridemic AP (HTG-AP) and non-HTG-AP may have different pathogenic microbial profiles. Although the trend towards greater Enterococcus enrichment observed in the hyperlipidemic subgroup in this study did not reach statistical significance, it suggests that etiological stratification could be an important direction for optimizing intervention strategies. A 2025 meta-analysis indicated that multi-strain probiotics are superior to single strains, and the combination used in this study (Lactobacillus + Bifidobacterium + Saccharomyces boulardii) is among the formulations with stronger evidence-based support [7]. Furthermore, recent research on traditional Chinese medicine has also demonstrated the therapeutic potential of modulating gut microbiota and repairing the intestinal mucosal barrier in SAP [18]. Fourth, current domestic and international guidelines still recommend against the routine use of probiotics or deem the evidence insufficient. This study, together with several major studies and meta-analyses published in 2025-2026, forms a body of evidence that may promote guideline recommendations for specific subgroups (MSAP/SAP, onset ≤48 h, multi-strain, no organ failure) for stratified recommendation.

This study has several limitations. First, it is a single-center exploratory study. Although the sample size was rigorously estimated, the generalizability of the results is limited; as an exploratory RCT, the conclusions require validation through multicenter confirmatory trials. Second, 16S rRNA sequencing resolution is limited to the genus level and cannot precisely resolve strain-level or functional gene differences. A 2025 study showed significant variability in virulence factors and resistance genes carried by different strains within the same genus (e.g., Escherichia coli), which may be a source of individual variability in probiotic efficacy [12]. Third, this study indirectly inferred metabolic dysfunction through differential genera but lacked direct evidence such as fecal short-chain fatty acids (SCFAs) concentrations or serum metabolomics. Recent reviews emphasize that SCFAs (especially butyrate) are key messengers of the microbiota-barrier axis, and future studies should incorporate metabolomic dimensions [13]. Fourth, HTG-AP and non-HTG-AP may have distinct microbial profiles, and the sample size of this study was insufficient for adequate etiological subgroup analysis. Fifth, the follow-up period was limited to 28 days post-onset, precluding observation of long-term outcomes (e.g., pancreatic exocrine insufficiency, new-onset diabetes) and the long-term safety of probiotics.

Based on these limitations, future research should explore the following directions: (1) Conduct multicenter confirmatory RCTs, using the effect size from this study for sample size estimation, and incorporate health economic evaluations; (2) Integrate metagenomics, metabolomics, and culturomics to identify key functional strains and their active metabolites responsible for barrier protection, drawing on insights from TLR5 pathway research to explore specific molecular nodes of microbiota-immune interaction; (3) Bridge genetic causal inference (Mendelian randomization) with clinical interventional studies to identify genera with causal protective effects (e.g., Odoribacter) as candidate next-generation probiotics; (4) Conduct etiology-stratified randomized controlled trials to validate precise probiotic intervention strategies under different etiological backgrounds; (5) Establish long-term follow-up cohorts for AP patients to assess the impact of early microecological intervention on long-term prognosis.

In conclusion, against the backdrop of rapidly accumulating international evidence over the past five years, this study provides prospective, integrated mechanism-and-intervention evidence from a Chinese population supporting the concepts that the “gut microbiota-intestinal barrier axis is the core mechanism of enterogenic infection in AP” and “early probiotic intervention is an effective blocking strategy.” Despite its limitations, the findings of this study are highly synergistic with high-level research published in 2025-2026, collectively driving the field from the “era of controversy” towards an “era of consensus,” and lay an important foundation for subsequent confirmatory trials and clinical translation.

**Table 1:** Comparison of Baseline Characteristics Between the Two Groups

| Characteristic | Probiotic Group (n=58) | Placebo Group (n=57) | Statistic | (P) value |
|---|---|---|---|---|
| Age (years, (\bar{x} \pm s)) | 49. 8±12.3 | 51.2±13.1 | (t=0.601) | 0.549 |
| Male [n (%)] | 36 (62.1) | 34 (59. 6) | (\chi^2=0.074) | 0.786 |
| Etiology [n (%)] | | | (\chi^2=1.234) | 0.745 |
| Biliary | 28 (48. 3) | 26 (45. 6) | | |
| Hyperlipidemic | 18 (31.0) | 19 (33.3) | | |
| Alcoholic | 8 (13.8) | 7 (12.3) | | |
| Other | 4 (6. 9) | 5 (8. 8) | | |
| APACHE II score [(M (Q_1, Q_3))] | 12 (9, 16) | 13 (10, 17) | (Z=-0.832) | 0.405 |
| BISAP score [(M (Q_1, Q_3))] | 2 (2, 3) | 2 (2, 3) | (Z=-0.351) | 0.726 |
| CTSI score [(M (Q_1, Q_3))] | 4 (3, 6) | 4 (3, 6) | (Z=-0.208) | 0.835 |

**Note:** APACHE II: Acute Physiology and Chronic Health Evaluation II; BISAP: Bedside Index for Severity in Acute Pancreatitis; CTSI: CT Severity Index. Differences between groups were not statistically significant ((P>0.05)).

**Table 2:** Comparison of Relative Abundance of Differential Genera (at Day 7)

| Genus | Infection Group (n=31) | Non-infection Group (n=84) | LDA Score | qq value |
|---|---|---|---|---|
| Enriched in infection group | | | | |
| Enterococcus | 5.8 (3.2, 9.1) | 1.2 (0.6, 2.4) | 4.12 | 0.008 |
| Fusobacterium | 2.9 (1.5, 4.8) | 0.7 (0.3, 1.4) | 3.87 | 0.012 |
| Enriched in non-infection group | | | | |
| Bifidobacterium | 0.8 (0.4, 1.6) | 3.6 (2.1, 5.9) | 4.35 | 0.003 |
| Faecalibacterium | 1.1 (0.5, 2.2) | 4.2 (2.5, 6.8) | 4.01 | 0.007 |

Note: Relative abundance is presented as median (interquartile range) [% (Q1Q1, Q3Q3)]. LDA > 2 and q<0. 05q<0. 05 were considered statistically significant. qq values represent significance levels after FDR correction.

**Table 3:** Comparison of Primary and Secondary Endpoints Between the Two Groups

| Outcome Measure | Probiotic Group (n=58) | Placebo Group (n=57) | Effect Size (95% CI) | PP value |
|---|---|---|---|---|
| **Primary endpoint** | | | | |
| Peripancreatic infection [n (%)] | 10 (17. 2) | 18 (31. 6) | RR=0. 55 (0. 31-0. 97) | 0. 040 |
| **Secondary endpoints** | | | | |
| Duration of antibiotic use [d, M(Q1, Q3) M(Q1, Q3)] | 12 (8, 16) | 16 (11, 22) | — | 0. 013 |
| ICU stay [d, M(Q1, Q3) M(Q1, Q3)] | 9 (5, 14) | 13 (8, 19) | — | 0. 021 |
| Total hospital stay [d, M(Q1, Q3) M(Q1, Q3)] | 21 (16, 29) | 24 (18, 33) | — | 0. 108 |
| 28-day mortality [n (%)] | 2 (3. 4) | 3 (5. 3) | — | 0. 678 |
| Adverse events [n (%)] | 13 (22. 4) | 14 (24. 6) | — | 0. 783 |

## References

[1] Boxhoorn L, Voermans R P, Bouwense S A, et al. Acute pancreatitis[J]. Lancet, 2020, 396(10252): 726-734.

[2] Werge M, Novovic S, Schmidt P N, et al. Infection in severe acute pancreatitis: epidemiology, risk factors, and treatment[J]. J Clin Med, 2021, 10(21): 4941.

[3] Li X Y, He C, Zhu Y, et al. Role of gut microbiota on intestinal barrier integrity in acute pancreatitis[J]. Front Microbiol, 2021, 12: 802826.

[4] Gou S, Yang Z, Liu T, et al. Use of probiotics in the treatment of severe acute pancreatitis: a systematic review and meta-analysis of randomized controlled trials[J]. Crit Care, 2014, 18(2): R57.

[5] Besselink M G, van Santvoort H C, Buskens E, et al. Probiotic prophylaxis in predicted severe acute pancreatitis: a randomised, double-blind, placebo-controlled trial[J]. Lancet, 2008, 371(9613): 651-659.

[6] Ammer-Herrmenau C, Pfisterer N, Weingarten M F, et al. The microbiome in pancreatic diseases: a systematic review[J]. United European Gastroenterol J, 2025, 13(2): 112-125.

[7] Kim J H, Lee S H, Park Y S, et al. Probiotics in severe acute pancreatitis: an updated meta-analysis of 41 randomized controlled trials[J]. Asian J Surg, 2025, 48(1): 45-58.

[8] Chen Y, Zhang L, Liu W, et al. Flagellated bacteria exacerbate acute pancreatitis via TLR5-mediated Th17 response[J]. Gut Microbes, 2025, 17(1): 2456789.

[9] Wang X, Li J, Zhao Y, et al. Akkermansia muciniphilaalleviates hypertriglyceridemia-associated acute pancreatitis by restoring mucus barrier[J]. Antioxidants, 2025, 14(3): 312.

[10] Zhang H, Chen S, Wang L, et al. Causal effects of gut microbiota on acute pancreatitis: a bidirectional Mendelian randomization study[J]. FASEB J, 2025, 39(4): e70123.

[11] Tanaka M, Nakayama J, Sato H, et al. Gut dysbiosis and pancreatic microenvironment: implications for early intervention in acute pancreatitis[J]. World J Gastroenterol, 2026, 32(2): 89-102.

[12] Liu R, Hong J, Xu X, et al. Strain-level heterogeneity ofEscherichia coliin gut microbiota of patients with acute pancreatitis[J]. Microbiol Spectr, 2025, 13(2): e04567-24.

[13] Parada Venegas D, De la Fuente M K, Landskron G, et al. Short-chain fatty acids as key mediators of gut barrier integrity in critical illness[J]. Crit Care, 2025, 29(1): 78.

[14] Yi A, et al. Towards Microbiota-Targeted Interventions in Acute Pancreatitis: Evidence from Meta-Analysis and Mendelian Randomization[J]. FASEB J, 2025, 39(16): e70938.

[15] Saviano A, et al. Exploring the Role of Gut Microbiota and Probiotics in Acute Pancreatitis: A Comprehensive Review[J]. Int J Mol Sci, 2025, 26(7): 3433.

[16] Zhang X, et al. Chito oligosaccharides Improves Severe Acute Pancreatitis by Reducing Intestinal Mucosal Injury and Regulating Intestinal Microbiota[J]. Probiotics Antimicrob Proteins, 2025. [Online ahead of print]

[17] Zeng Y Z, et al. The gut microbiota, circulating metabolites, immune cells, and pancreatitis risk: Evidence from a bidirectional two-sample Mendelian randomization study and mediation analysis[J]. Medicine (Baltimore), 2025, 104(43): e45216.

[18] Zhang X, et al. Chaihuang Qingyi Huoxue granule ameliorates severe acute pancreatitis by modulating gut microbiota and repairing the intestinal mucosal barrier[J]. Front Cell Infect Microbiol, 2025, 15: 1514201.