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Effects of a combined perioperative microbiota-targeted intervention on postoperative recovery and gut microbiota in children with Hirschsprung disease

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Abstract

Objective: To evaluate the effects of a combined perioperative microbiota-targeted intervention on short-term clinical outcomes, perioperative immune indices, and gut microbiota changes after definitive surgery in children with Hirschsprung disease (HD).

Methods: Sixty children with HD who underwent Soave pull-through surgery at Shanghai Children's Medical Center Guizhou Hospital between December 2025 and June 2026 were randomly assigned to an intervention group or a control group (30 children per group). All participants had short-segment HD. The control group received routine perioperative treatment. In addition to routine care, the intervention group received oral Bifidobacterium-Lactobacillus triple viable tablets, Clostridium butyricum viable powder, and glutamine during the perioperative period, together with total enteral nutritional support individualized to nutritional status. Clinical recovery and perioperative complications were compared, with structured telephone follow-up at 3 months after surgery. IL-6, TNF-alpha, CRP, IgA, IgG, C3, and C4 were measured 1 day before surgery and on postoperative days 3 and 7. Fecal samples were collected 3 days before surgery and 2 weeks after surgery for analysis of Shannon, Simpson, and Chao1 indices, major genera, principal coordinates analysis (PCoA), and differential genera.

Results: The intervention group had a shorter hospital stay than the control group [15.0 (13.0, 17.8) vs. 18.0 (15.3, 20.8) days; P = 0.005] and earlier first postoperative flatus (47.4 +/- 12.6 vs. 75.4 +/- 14.1 h; P < 0.001). HAEC was numerically lower in the intervention group than in the control group (6.7% vs. 20.0%; RR = 0.33, 95% CI 0.07 to 1.52; absolute risk difference = -13.3 percentage points, 95% CI -31.3 to 4.7; Fisher's exact P = 0.254). Overall perioperative complications were also numerically lower (23.3% vs. 40.0%; RR = 0.58, 95% CI 0.27 to 1.28; absolute risk difference = -16.7 percentage points, 95% CI -37.8 to 6.7; Fisher's exact P = 0.267). Event numbers were small. Postoperative TNF-alpha levels were lower in the intervention group (P < 0.01), and CRP on postoperative day 3 was lower (P = 0.045). Postoperative IgA and C4, C3 on day 3, and IgG on day 7 were higher in the intervention group (P < 0.05). Postoperative Shannon, Simpson, and Chao1 indices were higher in the intervention group (all P < 0.01). The postoperative relative abundances of Bifidobacterium and Lactobacillus increased in the intervention group, and PCoA indicated differences in overall postoperative community structure between groups (P < 0.01). Correlation analyses suggested exploratory trends between selected genera and inflammatory or immune indices.

Conclusion: A combined perioperative microbiota-targeted intervention may promote postoperative gut microbiota recovery, attenuate inflammation, improve selected immune indices, accelerate bowel function recovery, and shorten hospitalization in children with HD. HAEC and overall perioperative complications were numerically lower in the intervention group, although these findings remain inconclusive because events were few. Larger studies with longer follow-up are needed.

Keywords: Hirschsprung disease; microbiota-targeted intervention; immune function; gut microbiota; enterocolitis

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Introduction

Hirschsprung disease (HD) is a common congenital intestinal developmental disorder in pediatric surgery. It is caused by the absence of ganglion cells in the distal bowel, resulting in persistent spasm of the affected segment

and impaired passage of intestinal contents. Children commonly present with difficult defecation, abdominal distension, and intestinal obstruction [1]. Definitive surgery relieves the obstruction, but some children continue to experience delayed recovery of bowel function, recurrent abdominal distension, and intestinal inflammation. Hirschsprung-associated enterocolitis (HAEC) is among the most frequent complications and, in severe cases, can lead to repeated hospitalization or become life-threatening [2,3].

Increasing evidence indicates that gut microbiota dysbiosis contributes to HAEC . 16S rRNA sequencing studies by Chantakhow et al. [4] and Shi et al. [5] found marked alterations in children with HD or HAEC, including reductions in beneficial genera such as Bifidobacterium and Lactobacillus and increases in selected opportunistic bacteria. Arnaud et al. [6] also reported differences in fecal microbial composition between patients with and without HAEC. Dysbiosis may participate directly in HAEC by disrupting the mucosal barrier, abnormally activating intestinal inflammatory pathways, and impairing mucosal immunity [7]. Microbiota-derived short-chain fatty acids may additionally influence epithelial barrier integrity and host inflammatory responses.

Most microbiota-targeted studies in children with HD have been limited to postoperative supplementation with a single probiotic product, and clinical effects have been heterogeneous. The ERNICA guideline concluded that current evidence is insufficient to support routine probiotics for HAEC prevention [8]. Important limitations may include late initiation and reliance on a single intervention. As immunomicroecology has gained recognition [9], it has become clear that successful management may require not only supplementation of beneficial organisms but also a mucosal and nutritional environment that supports their growth. Perioperative care may therefore need to target the microbiota, intestinal barrier, and nutritional status simultaneously. Bifidobacterium-Lactobacillus triple viable tablets provide commensal lactobacilli and bifidobacteria, Clostridium butyricum may support short-chain-fatty-acid-related microbial functions, and glutamine is an important energy substrate for intestinal mucosal cells [10,11].

Accordingly, we initiated a multi-target combined microbiota strategy before surgery and integrated probiotics, mucosal nutritional support, and individualized enteral nutrition. This prospective randomized controlled study evaluated clinical outcomes, perioperative inflammatory and immune indices, and gut microbiota changes to assess the potential value of this approach for refined perioperative management of children with HD.

Methods

1. Patients and eligibility criteria

We enrolled 60 children with HD who underwent their first definitive Soave pull-through operation in the Department of Pediatric Surgery at Shanghai Children's Medical Center Guizhou Hospital between December 2025 and June 2026. All participants had short-segment HD. No child had undergone a prior stoma or staged operation. The study was approved by the Ethics Committee of Shanghai Children's Medical Center Guizhou Hospital [Approval No. (2024) 170], written informed consent was obtained from each child's legal guardian, and the trial was registered with the Chinese Clinical Trial Registry (ChiCTR2600129694). Inclusion criteria were: (1) HD diagnosed preoperatively by barium enema, anorectal manometry, and/or rectal mucosal biopsy; (2) age 3 months to 10 years; (3) first definitive operation with postoperative pathological confirmation of HD; and (4) complete perioperative, laboratory, microbiota, and follow-up data. Exclusion criteria were: (1) severe cardiac, pulmonary, or other major organ disease, or another severe congenital anomaly; (2) preoperative HAEC or confirmed intestinal infection, or use of probiotics, antibiotics, or immunomodulators within 1 month before enrollment; and (3) loss to follow-up or incomplete data.

2. Randomization and intervention

Children were assigned 1:1 by a random-number table to an intervention group or a control group (30 per group). The random sequence was generated and retained by a researcher who was not involved in the study procedures. Because the intervention was readily identifiable, guardians and clinical staff were not blinded. Both groups received the same principles of preoperative cleansing enemas, glycerin enema for defecation, operative technique, anesthesia, intravenous nutritional support, and antibiotic treatment. In addition to routine care, the intervention group began oral Bifidobacterium-Lactobacillus triple viable tablets 3 days before surgery (2 tablets

twice daily), Clostridium butyricum viable powder (1 sachet twice daily), and glutamine (0.3-0.5 g/kg/day). Enteral nutrition was individualized according to nutritional status. After oral intake resumed, probiotics and enteral nutrition were continued through postoperative day 7.

3. Outcomes and sample collection

Perioperative data and postoperative follow-up information were collected for all participants. Baseline and clinical variables included sex, age, body weight, disease duration, operative time, intraoperative blood loss, length of hospital stay, and time to first postoperative flatus. The clinical safety analysis focused on perioperative and early postoperative complications. All children completed a structured telephone follow-up 3 months after surgery to identify HAEC and other complications. HAEC occurring within 3 months after surgery was assessed according to the clinical criteria proposed by Gosain et al. [12], incorporating fever, diarrhea, rectal bleeding, abdominal distension, intestinal obstruction, systemic infection, and available imaging findings. Fever, diarrhea, pulmonary infection, abdominal distension, and anastomotic leakage were also recorded separately. When calculating the overall complication rate, each child was counted once, even if more than one complication occurred. Peripheral venous blood was collected 1 day before surgery and on postoperative days 3 and 7 to measure IL-6, TNF-alpha, CRP, IgA, IgG, C3, and C4. Fecal samples were obtained 3 days before surgery and 14 days after surgery for 16S rRNA sequencing, alpha-diversity analyses, major-genus relative abundance analyses, and visualization of postoperative community structure.

4. 16S rRNA gene sequencing and bioinformatic analysis

Fresh fecal samples (2-3 g) were collected 3 days before surgery and 14 days after surgery, temporarily stored at 4 degrees C, and transferred to -80 degrees C within 2 h. Total microbial DNA was extracted with a commercial kit. The V4 region of the 16S rRNA gene was amplified, sequencing libraries were constructed, and paired-end sequencing was performed on the Illumina NovaSeq 6000 platform. After quality control, operational taxonomic units were clustered and taxonomically annotated. Shannon, Simpson, Chao1, and ACE indices were calculated. Major genera were compared at the genus level, and PCoA was performed using Bray-Curtis and Euclidean distances. Differential genera were assessed with the Wilcoxon rank-sum test, followed by Benjamini-Hochberg false-discovery-rate (FDR) correction; adjusted Q values were used for multiple-comparison inference. In Figure 3, group-specific arithmetic means are shown for descriptive purposes. The plotted intervention-minus-control mean differences and their 95% CIs were calculated by a normal approximation using independent-group standard errors; Wilcoxon tests were used only to obtain P values. Associations between genus abundance and inflammatory or immune indices were evaluated by Spearman rank correlation with the same FDR correction. Sequencing and part of the bioinformatic analysis were performed by Chongqing Life Origin Technology Co., Ltd.

5. Statistical analysis

Statistical analyses were performed with SPSS version 26.0. Normally distributed continuous variables are presented as mean +/- SD, non-normally distributed variables as median (25th percentile, 75th percentile), and categorical variables as counts and percentages. Between-group comparisons used the independent-samples t test, Mann-Whitney U test, chi-square test, or Fisher exact test, as appropriate. For HAEC and overall perioperative complications, relative risks (RRs) and absolute risk differences (intervention minus control) are reported with 95% CIs. RR intervals were calculated on the log scale, and risk-difference intervals used the Newcombe-Wilson method. Because of the broad age range, age was included as a covariate in analyses of microbial diversity and major genera. Two-sided $P < 0.05$ was considered statistically significant.

Results

1. Clinical characteristics and perioperative outcomes

All 60 participants had short-segment HD. The groups did not differ significantly in sex, age, body weight, operative time, or intraoperative blood loss (all $P > 0.05$). The intervention group had a shorter hospital stay [15.0 (13.0, 17.8) vs. 18.0 (15.3, 20.8) days; $P = 0.005$] and earlier first postoperative flatus (47.4 +/- 12.6 vs. 75.4 +/-

14.1 h; P < 0.001) (Table 1). HAEC was numerically lower in the intervention group than in the control group (2/30 [6.7%] vs. 6/30 [20.0%]; RR = 0.33, 95% CI 0.07 to 1.52; absolute risk difference = -13.3 percentage points, 95% CI -31.3 to 4.7; Fisher's exact P = 0.254). Overall perioperative complications were also numerically lower (7/30 [23.3%] vs. 12/30 [40.0%]; RR = 0.58, 95% CI 0.27 to 1.28; absolute risk difference = -16.7 percentage points, 95% CI -37.8 to 6.7; Fisher's exact P = 0.267). Each child contributed only once to the overall complication count. Neither comparison was statistically significant, and the low event counts limited precision (Table 2).

Table 1. Comparison of perioperative characteristics between groups

Variable	Intervention group (n = 30)	Control group (n = 30)	t/chi-square/Z	P value
Sex (male/female)	21/9	15/15	1.74	0.188
Age (months)	29.0 +/- 33.7	34.1 +/- 35.4	-0.57	0.569
Body weight (kg)	11.8 +/- 6.3	12.3 +/- 5.7	-0.59	0.561
Operative time (h)	2.78 +/- 0.54	2.97 +/- 0.50	-1.39	0.171
Intraoperative blood loss (mL)	24.33 +/- 6.53	23.83 +/- 6.39	0.30	0.765
Length of hospital stay (days)	15.0 (13.0, 17.8)	18.0 (15.3, 20.8)	-2.84	0.005
Time to first postoperative flatus (h)	47.35 +/- 12.61	75.39 +/- 14.09	-8.12	<0.001

Note: Continuous variables are presented as mean +/- SD or median (25th percentile, 75th percentile) and were compared by the independent-samples t test or Mann-Whitney U test. Categorical variables are presented as n (%) and were compared by the chi-square test or Fisher exact test. Test statistics are shown to two decimal places and P values to three decimal places.

Table 2. Comparison of perioperative and early postoperative complications between groups

Complication	Intervention group (n = 30)	Control group (n = 30)	P value
Hirschsprung-associated enterocolitis (HAEC)	2 (6.7%)	6 (20.0%)	0.254
Fever	1 (3.3%)	4 (13.3%)	0.353
Diarrhea	1 (3.3%)	1 (3.3%)	1.000
Pulmonary infection	1 (3.3%)	1 (3.3%)	1.000
Abdominal distension	2 (6.7%)	2 (6.7%)	1.000
Anastomotic leakage	0 (0.0%)	1 (3.3%)	1.000
Any perioperative complication	7 (23.3%)	12 (40.0%)	0.267

Note: Data are presented as n (%). A child with more than one complication was counted once in the overall complication rate. All comparisons used Fisher's exact test.

2. Perioperative changes in inflammatory and immune indices

TNF-alpha was lower in the intervention group on postoperative days 3 and 7 (P = 0.009 and P < 0.001, respectively). CRP was lower in the intervention group on postoperative day 3 (P = 0.045). IgA was higher in the intervention group on postoperative days 3 and 7 (both P < 0.01), C3 was higher on postoperative day 3 (P = 0.034), and C4 was higher on postoperative days 3 and 7 (P < 0.001 and P = 0.032, respectively). IL-6 did not differ significantly between groups at any time point (all P > 0.05) (Table 3).

Table 3. Perioperative inflammatory and immune indices (mean +/- SD)

Variable	Time point	Intervention group	Control group	P value
IL-6 (pg/mL)	Preoperative	4.76 +/- 3.92	4.09 +/- 3.31	0.475
IL-6 (pg/mL)	POD 3	10.02 +/- 7.79	12.18 +/- 7.47	0.278
IL-6 (pg/mL)	POD 7	6.29 +/- 5.11	7.56 +/- 5.48	0.357
TNF-alpha (pg/mL)	Preoperative	7.03 +/- 1.17	7.57 +/- 0.88	0.051
TNF-alpha (pg/mL)	POD 3	8.69 +/- 2.34	10.61 +/- 3.13	0.009
TNF-alpha (pg/mL)	POD 7	7.03 +/- 0.88	9.05 +/- 1.67	<0.001
CRP (mg/L)	Preoperative	5.71 +/- 11.05	6.40 +/- 14.63	0.838
CRP (mg/L)	POD 3	21.06 +/- 16.96	35.85 +/- 35.78	0.045
CRP (mg/L)	POD 7	5.12 +/- 6.03	6.87 +/- 8.10	0.348
IgA (g/L)	Preoperative	1.88 +/- 1.02	1.87 +/- 0.98	0.970
IgA (g/L)	POD 3	10.30 +/- 4.61	7.45 +/- 3.18	0.007
IgA (g/L)	POD 7	6.79 +/- 2.54	5.02 +/- 2.60	0.010
IgG (g/L)	Preoperative	8.96 +/- 0.93	8.58 +/- 0.60	0.075
IgG (g/L)	POD 3	6.95 +/- 0.66	6.74 +/- 0.51	0.174
IgG (g/L)	POD 7	9.96 +/- 0.89	8.58 +/- 0.54	<0.001
C3 (g/L)	Preoperative	1.28 +/- 0.26	1.22 +/- 0.30	0.346
C3 (g/L)	POD 3	1.26 +/- 0.24	1.11 +/- 0.29	0.034
C3 (g/L)	POD 7	1.23 +/- 0.28	1.14 +/- 0.27	0.191
C4 (g/L)	Preoperative	0.23 +/- 0.04	0.22 +/- 0.06	0.653
C4 (g/L)	POD 3	0.24 +/- 0.06	0.19 +/- 0.05	<0.001
C4 (g/L)	POD 7	0.24 +/- 0.07	0.20 +/- 0.06	0.032

3. Changes in gut microbiota alpha diversity and microecological reconstruction

Preoperative Shannon, Simpson, and Chao1 indices did not differ significantly between groups (all $P > 0.05$). Postoperatively, all three indices were higher in the intervention group (all $P < 0.001$) (Figure 1). In general linear models adjusted for age in months and the corresponding preoperative alpha-diversity index, the postoperative Shannon, Simpson, and Chao1 indices remained higher in the intervention group. The adjusted group effects were beta = 0.580, 0.047, and 20.564, respectively ($t = 6.67$, 4.24, and 4.53; all $P < 0.001$) (Table 4). Representative-genus analysis showed higher postoperative abundances of Bifidobacterium and Lactobacillus in the intervention group (both $P < 0.001$) (Figure 2). After excluding Bifidobacterium, an exploratory Wilcoxon rank-sum analysis with FDR correction displayed the 15 most abundant differential genera. Lactobacillus, Akkermansia, Blautia, Eubacterium, and Sharpea were relatively more abundant in the intervention group, whereas Streptococcus, Staphylococcus, and Veillonella were relatively more abundant in the control group (Figure 3).

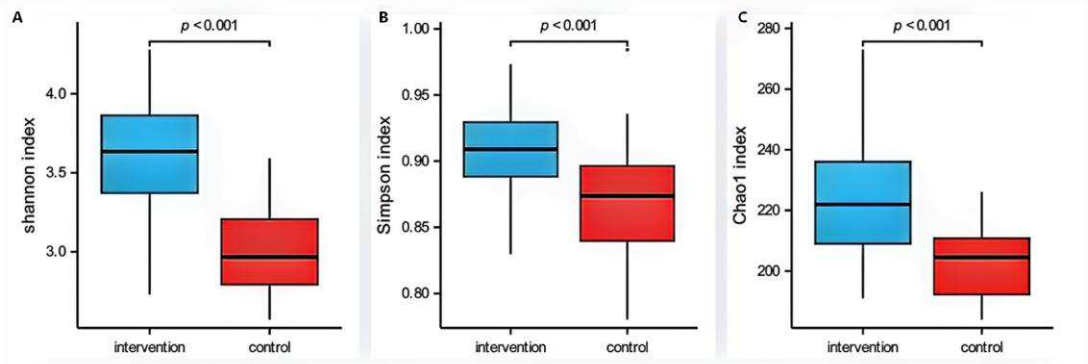


Figure 1. Postoperative alpha-diversity indices of the gut microbiota. (A) Shannon index; (B) Simpson index; (C) Chao1 index. All three indices were higher in the intervention group than in the control group.

Table 4. Postoperative alpha diversity after adjustment for age and preoperative alpha diversity

Outcome	Covariates	Group effect, beta	t value	P value
Shannon index	Age (months), preoperative Shannon index	0.580	6.67	<0.001
Simpson index	Age (months), preoperative Simpson index	0.047	4.24	<0.001
Chao1 index	Age (months), preoperative Chao1 index	20.564	4.53	<0.001

Note: General linear models used the postoperative alpha-diversity index as the dependent variable, study group as the main independent variable, and age in months plus the corresponding preoperative index as covariates. Beta represents the adjusted difference for the intervention group relative to the control group.

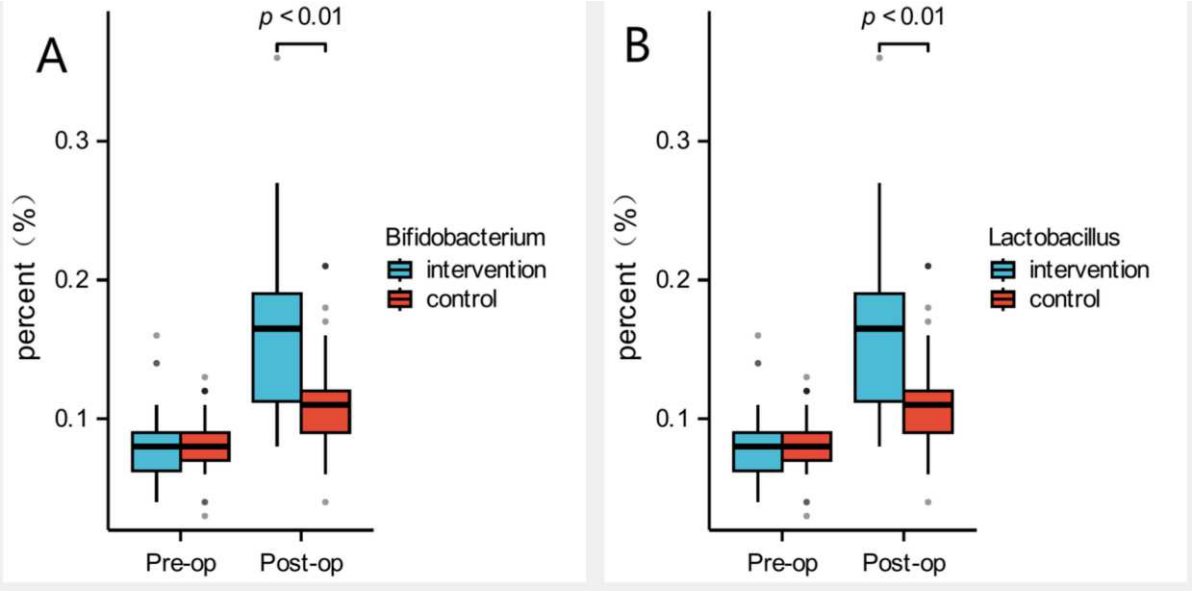


Figure 2. Relative abundances of Bifidobacterium and Lactobacillus before and after surgery. Postoperative abundances were higher in the intervention group.

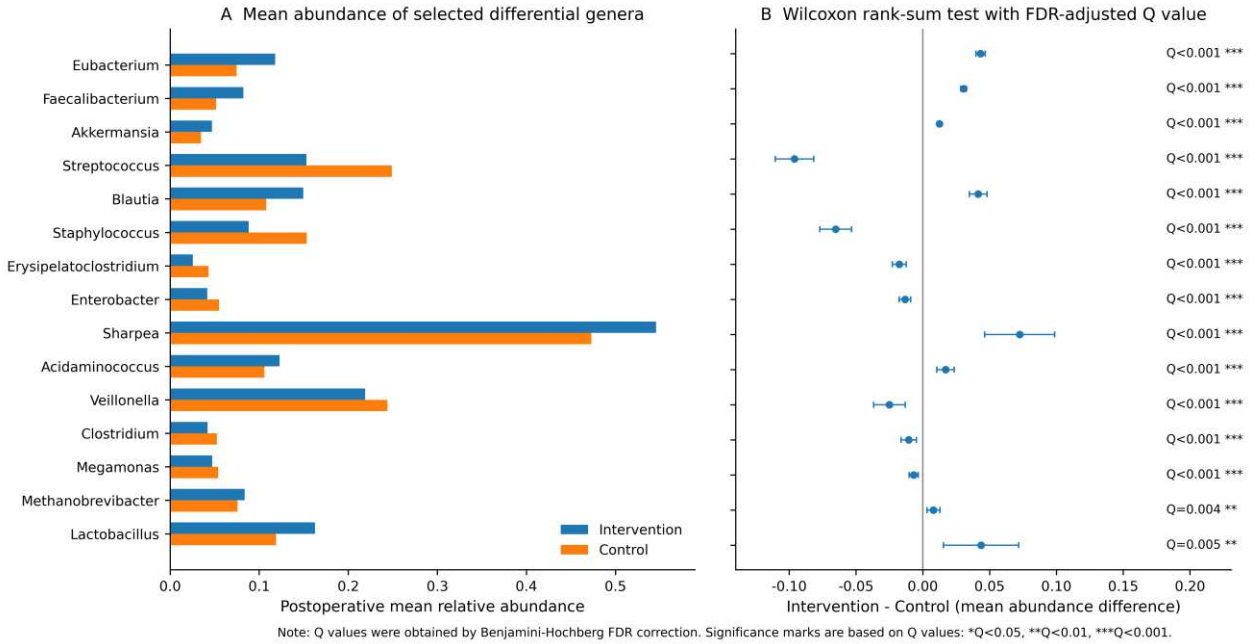


Figure 3. Postoperative differential genera after exclusion of Bifidobacterium. (A) Mean postoperative relative abundance of selected differential genera. (B) Unadjusted intervention-minus-control arithmetic mean differences with 95% confidence

intervals calculated by a normal approximation using independent-group standard errors. Wilcoxon rank-sum tests were used to obtain P values, which were adjusted with the Benjamini-Hochberg procedure; Q values are shown. *Q < 0.05, **Q < 0.01, ***Q < 0.001.

4. Gut microbiota beta diversity and overall between-group structural differences

PCoA of postoperative samples based on Bray-Curtis distance showed a significant difference in overall community composition between groups (PERMANOVA: F = 10.041, R2 = 0.148, P < 0.001). ANOSIM results were R = 0.167, P = 0.001 for Bray-Curtis distance and R = 0.121, P = 0.002 for Euclidean distance. Both R values were below 0.2, indicating substantial overlap. Thus, the observed differences were driven mainly by relative-abundance changes in selected genera rather than complete separation of the microbial communities (Figure 4).

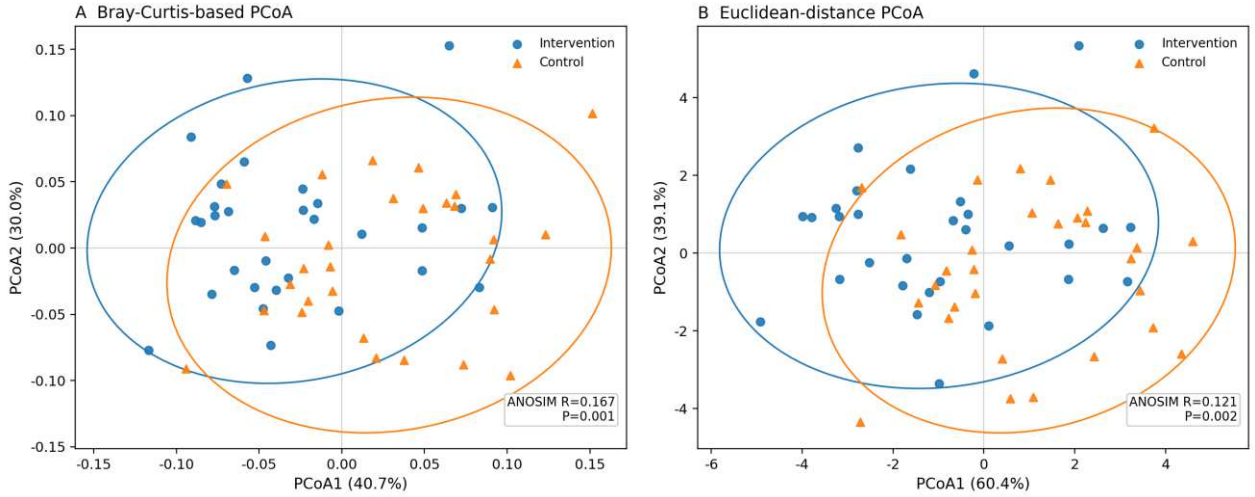


Figure 4. Overall perioperative gut microbiota structure and postoperative beta diversity. (A) Bray-Curtis-based PCoA. (B) Euclidean-distance PCoA. ANOSIM results were R = 0.167, P = 0.001 and R = 0.121, P = 0.002, respectively.

5. Associations between gut microbial abundance and immune indices

The stacked-area plot of the major genera showed higher postoperative relative abundances of beneficial genera, including Bifidobacterium and Lactobacillus, and lower abundance of opportunistic pathogen-related genera such as Enterobacter in the intervention group (Figure 5). After FDR correction, no association between postoperative genera and inflammatory or immune indices simultaneously met a strict significance threshold and a correlation magnitude of at least moderate strength. Several exploratory trends were present at the raw-P level: Eubacterium was negatively associated with C3 on postoperative day 3 (rho = -0.485, raw P = 0.007, Q = 0.779); Clostridium was negatively associated with IL-6 (rho = -0.470, raw P = 0.009, Q = 0.779) and C3 (rho = -0.465, raw P = 0.010, Q = 0.779) on postoperative day 7; and Enterobacter was positively associated with IL-6 on postoperative day 7 (rho = 0.440, raw P = 0.015, Q = 0.779) (Figure 6). Because none of these Q values was significant and all absolute rho values were below 0.50, they are presented only as exploratory signals.

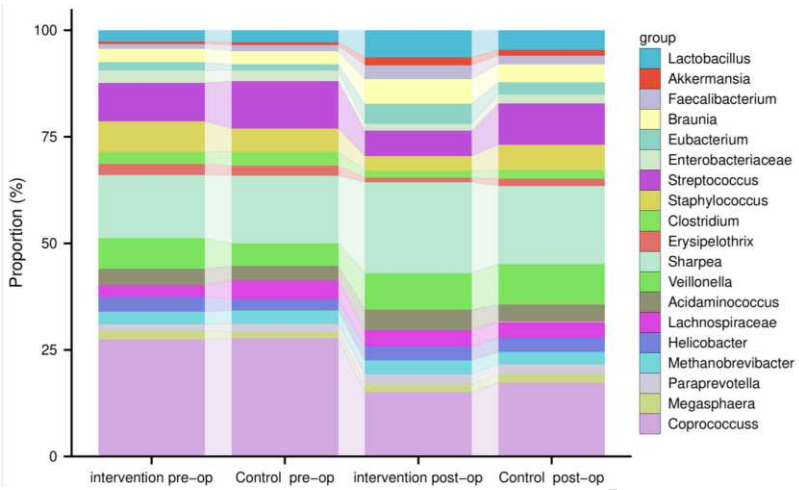


Figure 5. Perioperative changes in the relative abundance of major gut microbial genera in the two groups.

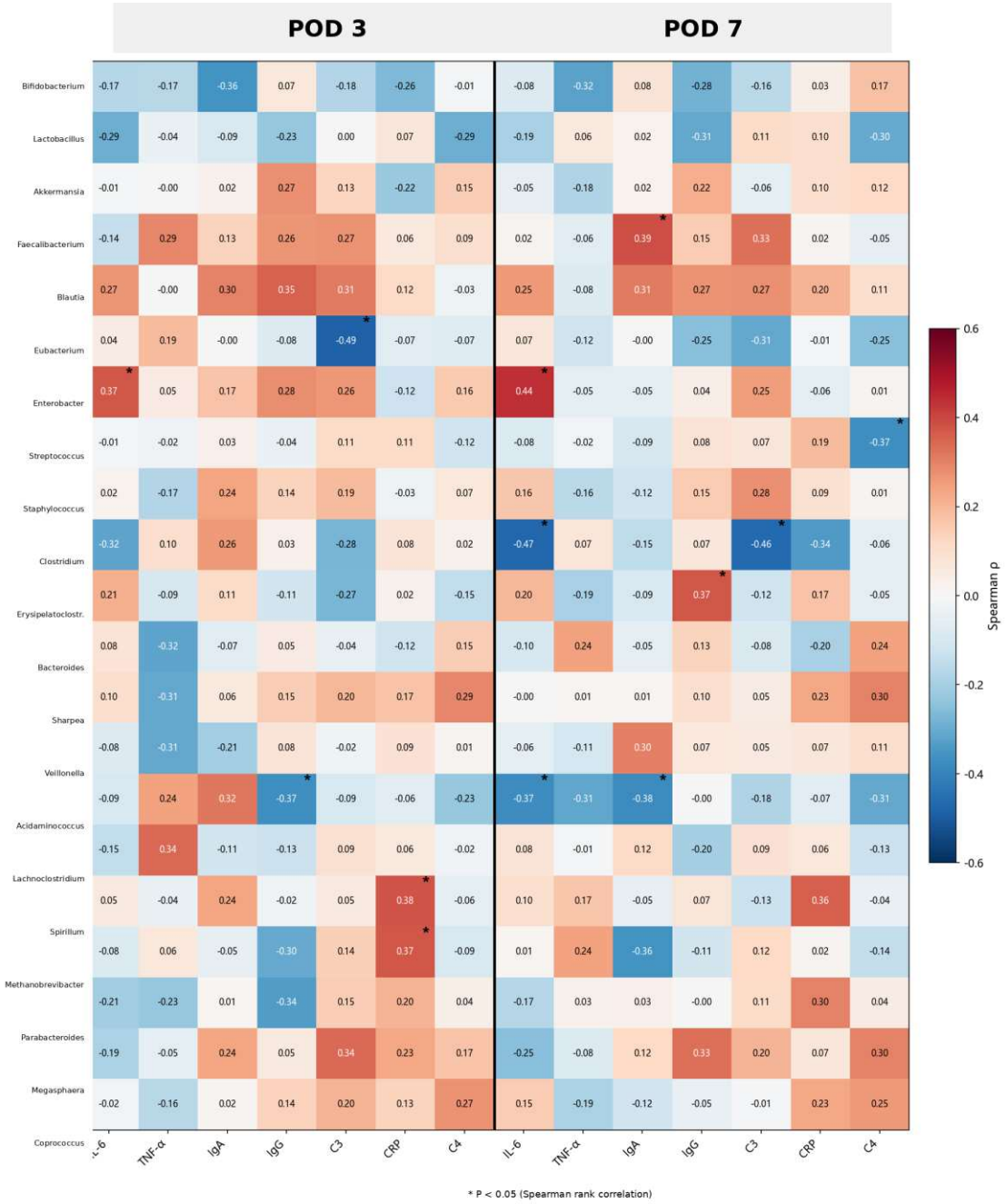


Figure 6. Exploratory Spearman correlation heatmap between postoperative gut microbial genera and inflammatory or immune indices in the intervention group. Raw correlations are displayed; no association remained significant after Benjamini-Hochberg FDR correction.

Discussion

Although definitive surgery relieves mechanical obstruction in children with HD, operative trauma, perioperative fasting, antibiotic exposure, and dysmotility may aggravate pre-existing dysbiosis [7]. This imbalance is a recognized trigger of HAEC and may delay postoperative recovery by disrupting intestinal inflammatory responses and immune barrier function [13]. Previous microbiota interventions in HD have largely been limited to postoperative probiotic supplementation, with uncertain clinical benefit. A Cochrane review concluded that current evidence does not support routine postoperative probiotics for HAEC prevention [14]. The delayed timing and single-component design of prior interventions may have limited their effectiveness. In the present study, a

multimodal microbiota-targeted strategy was initiated before surgery and was associated with earlier first flatus and a shorter hospital stay.

Earlier flatus provides a direct clinical indication of improved intestinal motility. Potential mechanisms include short-chain fatty acids related to Clostridium butyricum metabolism, which can provide energy to epithelial cells, promote mucosal repair, and support smooth-muscle function [15]. Beneficial bacteria such as Bifidobacterium and Lactobacillus may competitively inhibit opportunistic pathogens, reduce Toll-like-receptor-mediated NF-kappaB activation, limit inflammatory injury to the enteric neuromuscular system, and facilitate recovery of motility. HAEC and overall perioperative complications were numerically lower in the intervention group (6.7% vs. 20.0% and 23.3% vs. 40.0%, respectively), but neither comparison was statistically significant and the estimates were imprecise. The direction of these findings is consistent with reports linking HD and HAEC to characteristic microbiota alterations [16], but a preventive effect cannot be established without larger studies and longer follow-up.

Excessive perioperative inflammation accompanied by impaired immunity contributes to postoperative complications and delayed recovery in HD [17]. Lower TNF-alpha on postoperative days 3 and 7 and lower CRP on day 3 suggested attenuation of the early systemic inflammatory response. This is biologically plausible because pro-inflammatory macrophage activation and increased TNF-alpha in HAEC-affected bowel can aggravate dysmotility by altering the phenotype of interstitial cells of Cajal [18]. Higher postoperative IgA, IgG, C3, and C4 suggested improvement in selected humoral and complement indices. IgA is central to the intestinal mucosal barrier and neutralizes pathogens and their toxins, whereas commensal bacteria can influence B-cell differentiation. The complement system is also an important component of innate mucosal immunity and may contribute to antimicrobial defense and microecological homeostasis [19].

Microbiota reconstruction was central to the intervention and offers a potential link between the clinical and immunological findings. Alpha diversity improved after surgery in both groups, possibly because mechanical obstruction was relieved and intestinal transit resumed, but the increase was greater in the intervention group. This suggests that perioperative microbiota support may mitigate diversity loss related to surgery and antibiotic exposure [20]. The intervention group had higher postoperative abundances of Bifidobacterium and Lactobacillus. Even after Bifidobacterium was excluded from the differential analysis, Lactobacillus, Akkermansia, Blautia, and Eubacterium remained more abundant, whereas Streptococcus and Staphylococcus were less abundant [21]. PCoA also showed a significant, though modest, difference in overall postoperative community structure. Akkermansia may support mucous-layer and barrier integrity, while Blautia and Eubacterium include important short-chain-fatty-acid-producing organisms. These patterns are consistent with the observed reductions in inflammatory indices and improvements in selected immune markers, although functional effects cannot be inferred from genus-level profiles alone.

The correlation analysis suggested possible links between selected genera and inflammatory or complement indices, but none remained significant after FDR correction and all absolute rho values were below 0.50. The directions of the exploratory correlations were also not uniform. These findings do not establish causality and should be viewed as hypothesis-generating. Larger cohorts and functional experiments are needed to define the mechanisms of microbiota-immune interaction.

Several limitations should be considered. This was a single-center study of 60 children, all of whom had short-segment HD. Perioperative complications, particularly HAEC, were uncommon, which limited the power of these comparisons. The findings may not be generalizable to children with long-segment or total colonic aganglionosis.

The participants also covered a wide age range, from 3 months to 10 years. Differences in diet, complementary feeding, and normal age-related microbial development may have affected the baseline microbiota. Age was included in the adjusted analyses, but some residual confounding is still possible.The microbiota analysis was based on 16S rRNA sequencing and therefore provided information mainly at the genus level. Metabolomic data were not available, so the functional links between microbial changes, metabolism, and immune responses could not be examined [23].The intervention combined probiotics, glutamine, and individualized enteral nutrition. Because these components were administered together, their individual contributions could not be determined. Future studies could assess the components separately.

In conclusion, the combined perioperative microbiota-targeted intervention was associated with earlier bowel recovery, a shorter hospital stay, lower postoperative inflammatory activity, improvement in selected immune

indices, and more favorable postoperative microbiota profiles. HAEC and overall perioperative complications were numerically lower in the intervention group, but the estimates were imprecise because events were few. Confirmation will require a larger cohort and longer follow-up.

Author Contributions

Yize Zhuang was responsible for study design, data analysis, and manuscript drafting. Zhijie Qin, Zhuo Zhou, and Yuanhui Chen contributed to case collection and follow-up. Yingming Tang contributed to data organization and statistical analysis. Miao Luo supervised the study, revised the manuscript, and performed quality control. All authors read and approved the final manuscript.

Conflict of Interest

The authors declare no conflicts of interest.

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