Am J Transl Res 2023;15(5):3572-3578
www.ajtr.org /ISSN:1943-8141/AJTR0147993

Original Article

Effect of live combined Bifidobacterium and Lactobacillus tablets combined with Soave radical operation on postoperative intestinal flora and immune function in children with Hirschsprung disease

Tao Cheng, Yuan Yao, Sheng Zhang, Chongzhi Hou

The First Ward of General Surgery, Xi'an Children's Hospital, Xi'an 710002, Shaanxi, China

Received November 24, 2022; Accepted February 5, 2023; Epub May 15, 2023; Published May 30, 2023

Abstract: Objective: To investigate the effects of live combined Bifidobacterium and Lactobacillus tablets combined with Soave radical operation on postoperative intestinal flora and immune function in children with Hirschsprung disease (HD). Methods: A total of 126 cases in the Xi'an Children's Hospital from January 2018-December 2021 were retrospective analyzed. Among them, 60 cases treated with Soave radical operation alone were the control group (CG), and the other 66 cases with live combined Bifidobacterium and Lactobacillus tablets and Soave radical operation were set as the observation group (OG). We compared the treatment efficacy, adverse effects, defecation function, and the number of intestinal flora and IgG and IgA levels at the time of admission and after 3 months of treatment between both groups of children. Results: The efficacy efficiency and excellent defecation function rate were dramatically higher in the OG than in the CG after treatment ($P<0.05$). The bifidobacteria, lactobacilli and enterococcus faecalis were dramatically higher in the OG than in the CG after treatment ($P<0.05$), and the E. coli was dramatically lower than in the CG ($P<0.05$). After treatment, IgA and IgG in the OG were higher than those in the CG ($P<0.05$), and the incidence of postoperative complications was lower than that in the CG ($P<0.05$). Conclusion: Live combined Bifidobacterium and Lactobacillus tablets combined with Soave radical operation can effectively improve intestinal flora dysbiosis and immune function in children with HD. It has a better effect on the ability to defecate and a marked effect on the prevention of complications, which has a high clinical application value.

Keywords: Live combined Bifidobacterium and Lactobacillus tablets, Soave radical operation, HD, intestinal flora, immune function

Introduction

Hirschsprung disease (HD) is a common and representative congenital developmental defect disease in pediatric general surgery, occurring in 1 in 5000 live births. Its incidence varies widely among ethnic groups, with China having one of the highest rates of neonatal HD [1, 2]. The characteristic pathological changes of HD are the absence of ganglion cells in the distal stenotic segment of the intestinal wall, resulting in spastic narrowing of the diseased intestine and inability to move normally. As well as functional intestinal obstruction in the proximal normal intestinal segment because the contents cannot be emptied on time [3]. Over time, the proximal intestinal canal undergoes compensatory and secondary dilatation and thickening, forming megacolon changes with subsequent degenerative degeneration, atrophy, and even reduction or disappearance of its ganglion cells [4]. Clinically, the main manifestations are non-defecation or delayed defecation of meconium, abdominal distension, vomiting, intractable constipation, and malnutrition [5]. The disease not only affects the bowel and normal defecation function of children, but also can cause severe acidosis, dehydration, and shock as the disease progresses, endangering their life and safety [6].

The most clinically recognized and effective treatment is the surgical removal of ganglion cell-free intestinal tubes [7]. Transanal Soave radical operation avoids damage to the rectal wall and peribladderal nerves caused by extensive separation of the pelvis and preserves most of the anal sphincter, protects the defecation reflex and the sexual nerve, and provides better postoperative defecation function [8]. Despite surgical resection of the ganglion cell-free segment, approximately 40% of patients continue to suffer from recurrent life-threatening HD-associated small bowel colitis [9]. Other common postoperative complications include perianal dermatitis and fecal soiling, with an incidence of about 10% [10]. Research has shown that intestinal microbiota and immune changes are also involved in the development of these postoperative complications [11]. Microecological agents have been mentioned in many studies as live microorganisms with beneficial health effects to optimize gastrointestinal function and intestinal flora, and therefore they have also started to be used in the prevention and treatment of perioperative small bowel colitis in HD [12]. However, the specific effects of microbial agents on intestinal flora and immune function are not known, and more clinical studies are needed to verify their specific therapeutic effects.

This study not only verified that the combination of microecological agents can reduce the incidence of complications after radical Soave treatment and improve the defecation function in HD patients, but also analyzed the specific effects of this treatment regimen on the intestinal flora and immune function of the children. Related reports are presented below.

Methods and materials

General data

A total of 126 child patients in the Xi'an Children's Hospital from January 2018-December 2021 were retrospective analyzed. Among them, 60 cases treated with Soave radical operation alone were the control group (CG), including 37 males and 23 females with a mean age of (2.48±0.95) years. The remaining 66 cases with microecological agents and Soave radical operation were the observation group (OG), including 38 males and 28 females with a mean age of (2.53±0.79) years. The parents of the children voluntarily participated in this study and signed the informed consent form. This study was approved by the Medical Ethics Committee of the Xi'an Children's Hospital (Lot No. 20190910).

Inclusion and exclusion criteria

Inclusion criteria: Children aged 6 months to 12 years; The diagnosis of HD was confirmed by rectal manometry, X-ray, anal finger examination and biopsy [13]; A barium enema showed a stenosis of the sigmoid colon and above; The child was eligible for surgery. Children with symptoms such as difficult bowel movements and abdominal distention; The medical records are complete.

Exclusion criteria: Children with co-morbid infections, immune system diseases, hematologic diseases; Persons with digestive system malformations; Intolerance to treatment regimens; Poor adherence to treatment.

Treatment options

Soave radical operation was performed in both groups, and the children were successfully anesthetized and placed in the bladder osteotomy position. A 0.5-cm-long incision was made at the superior umbilical rim, a pneumoperitoneum was established, a 5-mm T-tube was placed, and abdominal pressure was controlled at 8 to 10 mmHg. Two incisions were made at 4 cm on the left and right sides of the umbilicus, and a 5 mm T-tube was placed to pad the child's buttocks and observe the location of the lesion under the laparoscope to determine the extent of resection. The rectal mesentery and collateral ligaments were separated using an ultrasonic knife, the mesentery was clamped, the mesenteric tertiary vessels were coagulated and then freed toward the colon, and the perineum was operated using the transanal Soave radical procedure steps. The anal canal was removed on the 7th postoperative day, and the anal anastomosis and stricture were checked 2 weeks after surgery. If there was anal stricture, anal dilation was required. Starting on the 3rd postoperative day, the children in the OG were combined with microecological preparations on this basis and were given Live Combined Bifidobacterium and Lactobacillus Tablets (Hangzhou Yuanda Biopharmaceutical Co., Ltd); Dose: 3 tablets/time, 3 times/d, for 3 months.

Table 1. Baseline data

	Observation group (n=66)	Control group (n=60)	X^2/t	P
Age (year)	2.53±0.79	2.48±0.95	0.322	0.748
Gender			0.218	0.640
Male	38 (57.58)	37 (61.67)		
Female	28 (42.42)	23 (38.33)		
Body mass (kg)	12.87±1.61	13.26±1.58	1.370	0.173
Preoperative bowel washout time (days)	10.4±3.3	10.07±2.43	0.948	0.345
Type of lesion			0.903	0.637
Short-segment type	13 (19.70)	16 (26.67)		
Long-segment type	23 (34.85)	20 (33.33)		
common-segment type	30 (45.45)	24 (40.00)		
Family history of enterocolitis	10 (15.15)	7 (11.67)	0.327	0.567
Malnutrition	14 (21.21)	15 (25.00)	0.255	0.614
Family history of congenital malformation	8 (12.12)	9 (15.00)	0.223	0.637
History of passive smoking	19 (28.79)	15 (25.00)	0.229	0.632

Outcome measures

Clinical outcomes were evaluated after 3 months of treatment in both groups of children. Markedly effective: Disappearance of symptoms and absence of abnormal bowel movements. Effective: Slight improvement in symptoms and improvement in stool properties and frequency. Ineffective: Symptoms were not transformed. Total response rate = (effective + effective) number of cases/total number of cases × 100%. (2) Defecation function was assessed after 3 months of treatment in both groups, and the Krickenbeck score was used to evaluate the defecation function, including constipation, fecal soiling, and voluntary bowel motility function. The total score is 9, and the higher the score, the more obvious the improvement of the function: 9-6 is excellent, 4-5 is good, 3 and below is poor. Total excellent rate = number of (excellent + good) cases/total number of cases × 100%. (3) The immune function and the number of intestinal florae of children were assessed after 3 months of treatment. Fresh stool specimens were collected from the children, and the number of E. coli, Lactobacillus and Bifidobacterium were detected in both groups by using the live plate count method. Before and after treatment, 4 mL of venous blood was collected from the children in a procoagulant tube, and immunoglobulin G (IgG) and immunoglobulin A (IgA) levels were abbreviated using an immunoturbidimetric method, and the kit was purchased from Beckman Coulter. (4) Patients were counted for postoperative complications, including small bowel colitis, constipation, perianal dermatitis, and foul feces.

Statistical methods

SPSS 21.0 statistical software was applied to analyze the data. The measurement data were expressed as mean ± standard deviation, and the counting data were percentages. When two groups of measurement data were compared, they conformed to normal distribution and independent t-test was used. While paired t-test was conducted for comparison of two times in the same group and chi-square test for that of rates. P<0.05 signifies a statistical significance.

Results

Baseline data

It was found that there was no statistical difference (P>0.05) between both groups in terms of age, gender, body mass, preoperative bowel washout time, type of lesion, family history of enterocolitis, malnutrition, family history of congenital malformation, and history of passive smoking (**Table 1**).

Treatment efficacy of both groups of children

The treatment efficacy of children in the two groups was observed, and the total response rate in the OG was 93.94%, which was higher

Table 2. Treatment efficacy table

	Observation group (n=66)	Control group (n=60)	X^2	P
Markedly effective	34 (51.52)	27 (45.00)		
Effective	28 (42.42)	22 (36.67)		
Ineffective	4 (6.06)	11 (18.33)		
Total response	62 (93.94)	49 (81.67)	4.514	0.034

Table 3. Defecation function table

	Observation group (n=66)	Control group (n=60)	X^2	P
Excellent	31 (46.97)	22 (36.67)		
Good	33 (50.00)	28 (46.67)		
Poor	2 (3.03)	9 (15.00)		
Excellent rate	64 (96.97)	51 (85.00)	5.651	0.017

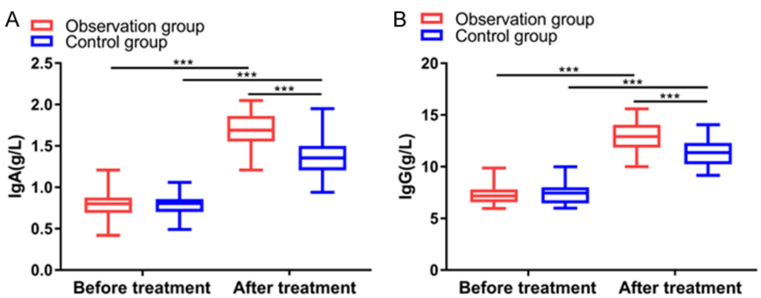

Figure 1. Changes in immune function before and after treatment in both groups. A. IgA is higher in both groups after treatment, and the IgA in the observation group is higher than that in the control group after treatment. B. IgG is higher in both groups after treatment, and the IgG in the observation group is higher than that in the control group after treatment. IgG: immunoglobulin G, IgA: immunoglobulin A.

than that of the CG (81.67%) (P=0.034), see **Table 2**.

Comparison of defecation function between both groups after treatment

After comparing the defecation function of the children in both groups after treatment, the excellent rate in the OG was 96.97%, which was higher than the 85.00% in the CG (P=0.017), as shown in **Table 3**.

Changes in immune function before and after treatment in both groups

The IgA and IgG levels of the children in the two groups before and after treatment were statistically not different (P>0.05). The levels in both groups were higher after treatment (P<0.05), and that in the OG were higher than those of the CG after treatment (P<0.05) (**Figure 1**).

Changes in the number of intestinal flora before and after treatment in both groups

The bifidobacteria, lactobacilli, E. coli and enterococcus faecalis of children in both groups were compared before and after treatment, and there was no statistical difference before treatment (P>0.05). The bifidobacteria, lactobacilli and enterococcus faecalis in the OG were higher than those in the CG after treatment (P<0.05), and E. coli was lower than that in the CG (P<0.05) (**Figure 2**).

Comparison of complication rates between both groups

Small bowel colitis, constipation, perianal dermatitis, and foul feces were observed in both yellow liability groups, while the overall complication rate was lower in the children in the OG than in the CG (P=0.017), as shown in **Table 4**.

Discussion

HD is a common developmental malformation of the gastrointestinal tract characterized by the absence of ganglion cells in the terminal segment of the intestinal wall, which is associated with recurrent constipation and small bowel obstruction and other clinical symptoms [14]. Therefore, timely and effective treatment is essential. Currently, most children with HD are treated clinically by surgery, but some children still have incisional infections and small intestinal colitis after surgery, resulting in the disruption of normal intestinal microecology, intestinal dysfunction, flora translocation, dysbiosis, and enterogenic infections, which seriously affect the prognosis of children [15]. Microecological agents can re-establish the microecological balance of the gastrointestinal tract, effectively reduce the translocation of flora, enhance the barrier function of the gas-

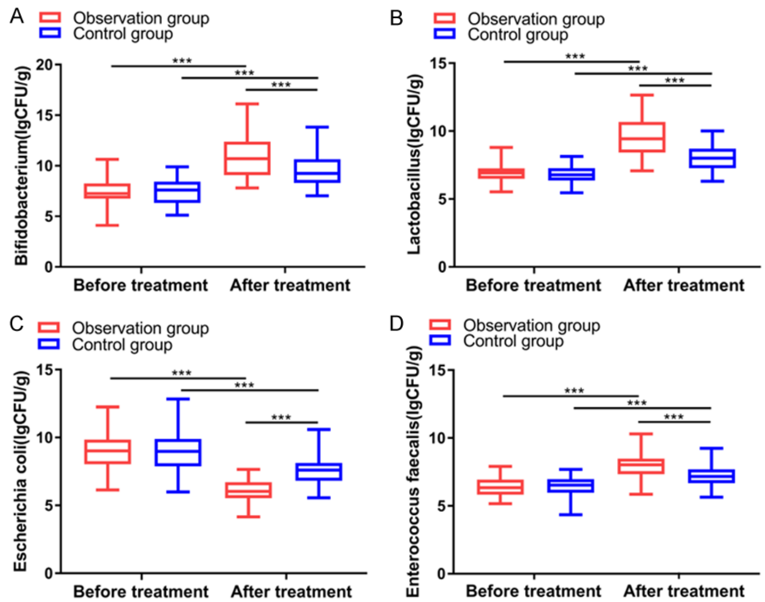

Figure 2. Changes in the number of intestinal florae before and after treatment in both groups. A. Bifidobacteria are higher in both groups after treatment, and the observation group was higher than the control group after treatment. B. Lactobacillus is higher in both groups after treatment, and the observation group was higher than the control group after treatment. C. E. coli is reduced in both groups after treatment, and E. coli is lower in the observation group than in the control group after treatment. D. Enterococcus faecalis are higher in both groups after treatment, and the observation group was higher than the control group after treatment.

Table 4. Comparison of complication rates

	Observation group (n=66)	Control group (n=60)	X^2	P
Small bowel colitis	2 (3.03)	6 (10.00)	2.568	0.109
Constipation	1 (1.52)	4 (6.67)	2.189	0.139
Perianal dermatitis	2 (3.03)	2 (3.33)	0.009	0.923
Foul feces	1 (1.52)	3 (5.00)	1.242	0.265
Overall complication rate	6 (9.09)	15 (25.00)	5.727	0.017

trointestinal mucosa in children, and increase their tolerance [16].

In this study, the total response rate of the treatment of children in the OG was higher than that of the CG, and the rate of good defecation function in the OG was higher after treatment, indicating that the combination of microecological preparations can dramatically improve the recovery of intestinal function of children. Numerous studies have confirmed that the cellular immune function of postoperative children is suppressed, as well as anemia and growth retardation due to chronic abdominal distension and constipation caused by HD, which severely affects the absorption of nutrients and leads to immune deficiency [17]. Several studies have shown that the combined application after colorectal cancer as well as biliary tract cancer has been shown to enhance the immune function of patients [18, 19]. The present study revealed that all immune indexes of the children in the OG were higher than those in the CG after surgery, suggesting that microecological agents combined with Soave radical operation had less impact on the immune function of the children and helped them recover after surgery.

Intestinal infections are the result of mutual transformation of intestinal microecological balance and dysbiosis, and the microorganisms that cause infections are not necessarily pathogenic or pathogens, but the result of translocation or translocation of the normal microbiota, of which the translocation of the normal intestinal flora leading to infection has attracted widespread attention [20, 21]. The main causes of intestinal bacterial translocation are dysbiosis of the intestinal microecology, disruption of the balance of the bacterial flora, resulting in disruption of the bacterial film structure of the intestine, reduced intestinal colonization resistance, increased intestinal mucosal barrier permeability and decreased host immune function, and breakthrough of the intestinal mucosal barrier by bacteria or their products (endotoxins) into the mesenteric lymph nodes or portal system, further reaching other organs far from the intestine [22]. Shen et al. [23] found that bifidobacteria and lactobacilli in the stool of children with congenital megacolon associated small bowel colitis were significantly lower than in normal children. In this study, the sick children in the OG were given oral micro-ecological preparation after surgery. The micro-ecological preparation (Live Combined Bifidobacterium and Lactobacillus Tablets) consisted of Lactobacillus, Bifidobacterium, and Streptococcus thermophilus together, all of which are normal strains of bacteria in the human intestine, which not only helped the sick children improve their intestinal flora but also constituted a definite resistance. Bifidobacterium inhibits the multiplication of pathogens, promotes the recovery of gastrointestinal function, reduces the inflammation, improves the immune function of the intestine, and prevents bacterial displacement [24]. Tang et al. [25] mentioned that breastfeeding improved the intestinal flora composition of children with HD, which prevented the development of megacolon associated small bowel colitis with a higher mortality. In our study, we found higher E. coli and lower Bifidobacterium, Lactobacillus, and Enterococcus faecalis in both groups before treatment, and lower E. coli and higher Bifidobacterium, Lactobacillus, and Enterococcus faecalis in both groups after treatment. The level of E. coli of children in the OG after treatment was lower than that of the CG, and Bifidobacterium, Lactobacillus and Enterococcus faecalis were higher than that of the CG, indicating that the use of microecological agents can stabilize the intestinal flora and reduce the risk of lesions. In comparison, Sireekarn et al. [26] mentioned that E. coli increases, and Bifidobacterium decreases in affected children. By altering this phenomenon we were able to prevent the development of small intestinal colitis, making it similar to our study. Finally, we compared the complications of both groups, and the overall complication rate of the OG, including small bowel colitis, was lower than that of the CG, indicating that the combination of microecological agents with Soave radical operation is safe and reliable, and can ensure a therapeutic effect while avoiding serious complications.

There are also some shortcomings in the current study. Firstly, the treatment of HD varies depending on the age of children at presentation, the length of the ganglion-free bowel, nutritional status, and general condition, so more studies are needed to compare and investigate the findings. Secondly, we do not know the effect of the treatment on the subsequent development and growth of children, which will require more long-term follow-up. Finally, more animal experiments are needed to explore the specific mechanisms of the effects of microecological agents.

In conclusion, microecological agents combined with Soave radical operation can effectively improve intestinal flora dysbiosis and immune function in children with HD. It has a better effect on the ability to defecate and an obvious effect on the prevention of complications, which has a high clinical application value.

Disclosure of conflict of interest

None.

Address correspondence to: Chongzhi Hou, The First Ward of General Surgery, Xi'an Children's Hospital, No. 69 Beimadao Lane, Lianhu District, Xi'an 710002, Shaanxi, China. E-mail: houchongzhi1976@126.com

References

[1] Wang Y, He Q, Zhang R, Zhong W, Zhu D, Zhang Y and Xia H. Association between DSCAM polymorphisms and non-syndromic Hirschsprung disease in Chinese population. BMC Med Genet 2018; 19: 116.

[2] Jensen AR and Frischer JS. Surgical history of Hirschsprung disease. Semin Pediatr Surg 2022; 31: 151174.

[3] Ma S, Yu Y, Pan A, Gong H, Lou Z, Liu L, Hao L, Meng R, Sui J and Zhang W. The classification and surgical treatments in adult Hirschsprung's disease: a retrospective study. Front Med (Lausanne) 2022; 9: 870342.

[4] Gandhi A, Bapaye J and Bapaye A. Per Rectal Endoscopic Myotomy (PREM) for Hirschsprung's disease and megacolon. Minerva Gastroenterol (Torino) 2022; [Epub ahead of print].

[5] Rodas A, Barillas S and Ardebol J. Ultrashort-segment Hirschsprung disease in a 4-year-old female. J Surg Case Rep 2020; 2020: rjaa320.

[6] Ali A, Haider F and Alhindi S. The prevalence and clinical profile of Hirschsprung's disease at a tertiary hospital in Bahrain. Cureus 2021; 13: e12480.

[7] Handaya AY, Fauzi AR, Andrew J and Hanif AS. Modified Duhamel with lateral anal sphincterotomy and coloanal stump for adult Hirschsprung's disease: a case series. Int J Surg Case Rep 2020; 77: 174-177.

[8] Lukac M, Antunovic SS, Vujovic D, Petronic I, Nikolic D, Radlovic V, Krstajic T and Krstic Z. Effectiveness of various surgical methods in

treatment of Hirschsprung's disease in children. Vojnosanit Pregl 2016; 73: 246-250.
[9] Gosain A, Frykman PK, Cowles RA, Horton J, Levitt M, Rothstein DH, Langer JC and Goldstein AM; American Pediatric Surgical Association Hirschsprung Disease Interest Group. Guidelines for the diagnosis and management of Hirschsprung-associated enterocolitis. Pediatr Surg Int 2017; 33: 517-521.
[10] Wang YJ, He YB, Chen L, Lin Y, Liu MK and Zhou CM. Laparoscopic-assisted Soave procedure for Hirschsprung disease: 10-year experience with 106 cases. BMC Surg 2022; 22: 72.
[11] Pierre JF, Barlow-Anacker AJ, Erickson CS, Heneghan AF, Leverson GE, Dowd SE, Epstein ML, Kudsk KA and Gosain A. Intestinal dysbiosis and bacterial enteroinvasion in a murine model of Hirschsprung's disease. J Pediatr Surg 2014; 49: 1242-1251.
[12] Alam Z, Shang X, Effat K, Kanwal F, He X, Li Y, Xu C, Niu W, War AR and Zhang Y. The potential role of prebiotics, probiotics, and synbiotics in adjuvant cancer therapy especially colorectal cancer. J Food Biochem 2022; 46: e14302.
[13] Limousin IT, Valdes JA, Jimenez MJ, Cendon RG, Fernandez Pineda I, Garcia RC and Alonso ML. Neonatal diagnostic protocol of the Hirschsprung's disease. Cir Pediatr 2008; 21: 232-234.
[14] Slavikova T, Zabojnikova L, Babala J and Varga I. An embryological point of view on associated congenital anomalies of children with Hirschsprung disease. Bratisl Lek Listy 2015; 116: 640-647.
[15] Frykman PK, Nordenskjold A, Kawaguchi A, Hui TT, Granstrom AL, Cheng Z, Tang J, Underhill DM, Iliev I, Funari VA and Wester T; HAEC Collaborative Research Group (HCRG). Characterization of bacterial and fungal microbiome in children with Hirschsprung disease with and without a history of enterocolitis: a multicenter study. PLoS One 2015; 10: e0124172.
[16] Feng Y, Gao SJ, Wei RD, Liu T, Fan XP, Han YD and Zhu N. Effects of probiotics on intestinal flora, inflammation and degree of liver cirrhosis in rats with liver cirrhosis by regulating Wnt/beta-catenin signaling pathway. J Biol Regul Homeost Agents 2021; 35: 25-33.
[17] Bao MDL, Ge L, Su H, Luo S, Xu Z, Wang XW, Liu Q, Wang ZX, Zhou XS and Zhou HT. Analysis of short-term efficacy of overlapping delta-shaped anastomosis in totally laparoscopic left hemicolectomy for digestive tract reconstruction. Zhonghua Wei Chang Wai Ke Za Zhi 2021; 24: 433-439.
[18] Xie X, He Y, Li H, Yu D, Na L, Sun T, Zhang D, Shi X, Xia Y, Jiang T, Rong S, Yang S, Ma X and Xu G. Effects of prebiotics on immunologic indicators and intestinal microbiota structure in perioperative colorectal cancer patients. Nutrition 2019; 61: 132-142.
[19] Sugawara G, Nagino M, Nishio H, Ebata T, Takagi K, Asahara T, Nomoto K and Nimura Y. Perioperative synbiotic treatment to prevent postoperative infectious complications in biliary cancer surgery: a randomized controlled trial. Ann Surg 2006; 244: 706-714.
[20] Shi S, Liu J, Dong J, Hu J, Liu Y, Feng J and Zhou D. Research progress on the regulation mechanism of probiotics on the microecological flora of infected intestines in livestock and poultry. Lett Appl Microbiol 2022; 74: 647-655.
[21] Fan S, Zhang Z, Zhong Y, Li C, Huang X, Geng F and Nie S. Microbiota-related effects of prebiotic fibres in lipopolysaccharide-induced endotoxemic mice: short chain fatty acid production and gut commensal translocation. Food Funct 2021; 12: 7343-7357.
[22] Liu X, Li H, Lu A, Zhong Y, Hou X, Wang N, Jia D, Zan J, Zhao H, Xu J and Liu F. Reduction of intestinal mucosal immune function in heat-stressed rats and bacterial translocation. Int J Hyperthermia 2012; 28: 756-765.
[23] Shen DH, Shi CR, Chen JJ, Yu SY, Wu Y and Yan WB. Detection of intestinal bifidobacteria and lactobacilli in patients with Hirschsprung's disease associated enterocolitis. World J Pediatr 2009; 5: 201-205.
[24] Wu Y, Li S, Tao Y, Li D, Han Y, Show PL, Wen G and Zhou J. Fermentation of blueberry and blackberry juices using Lactobacillus plantarum, Streptococcus thermophilus and Bifidobacterium bifidum: growth of probiotics, metabolism of phenolics, antioxidant capacity in vitro and sensory evaluation. Food Chem 2021; 348: 129083.
[25] Tang W, Su Y, Yuan C, Zhang Y, Zhou L, Peng L, Wang P, Chen G, Li Y, Li H, Zhi Z, Chang H, Hang B, Mao JH, Snijders AM and Xia Y. Prospective study reveals a microbiome signature that predicts the occurrence of post-operative enterocolitis in Hirschsprung disease (HSCR) patients. Gut Microbes 2020; 11: 842-854.
[26] Chantakhow S, Khorana J, Tepmalai K, Boonchooduang N, Chattipakorn N and Chattipakorn SC. Alterations of gut bacteria in Hirschsprung disease and Hirschsprung-associated enterocolitis. Microorganisms 2021; 9: 2241.