Therapeutic effect of *Saccharomyces boulardii* combined with *Bifidobacterium* and on cellular immune function in children with acute diarrhea

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Abstract. Clinical effect of *Saccharomyces boulardii* combined with *bifidobacterium* and its effect on cellular immune function in children with acute diarrhea were studied. In total 116 cases of children with acute diarrhea admitted to Xuzhou Children's Hospital from March 2015 to March 2017 were collected and analyzed retrospectively. There were 59 children treated with *Saccharomyces boulardii* as control group and 57 children treated with *Saccharomyces boulardii* combined with *bifidobacterium* as experimental group. The clinical effect, stool frequency in different time periods, mean antidiarrheal time, mean antipyretic time and length of stay, and immune function of children in the two groups after treatment were analyzed. The cure rate (73.68%) and the total effective rate (87.72%) in the experimental group were significantly higher than those in the control group (47.46 and 71.19%) (P<0.05). The stool frequency in the experimental group was significantly lower than that in the control group 3 days after treatment (P<0.05). The mean antidiarrheal time in the experimental group was significantly shorter than that in the control group (P<0.05). The length of stay in the control group was significantly longer than that in the experimental group (P<0.05). $CD3^+$, $CD4^+$ and $CD4^+/CD8^+$ increased significantly in the experimental group after treatment while $CD8^+$ decreased significantly (P<0.05). After treatment, the ratio of Th1 and Th2 in the two groups decreased significantly compared with before treatment (P<0.05), and the experimental group was significantly lower than the control group (P<0.05). After treatment, Th1/Th2 ratio was significantly higher than that before treatment (P<0.05), and the experimental group was significantly higher than the control group (P<0.05). In conclusion, treatment of acute diarrhea in children with *Saccharomyces boulardii* combined with *bifidobacterium* can effectively shorten the duration of diarrhea and hospital stay, reduce the number of diarrhea and enhance the cellular immune function.

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Introduction

Acute diarrhea is a common intestinal disease in children, and also one of the diseases with high incidence in childhood, ranking second in pediatric diseases in China (1). The clinical manifestations of acute diarrhea in children before the confirmed etiology is the increase of defecation frequency and the change of trait (2). Diet regulation, oral rehydration salt (ORS) and other methods are the routine treatment for controlling the development of the disease (3), which may lead to intestinal dysbacteriosis due to its prolonged effective time of stopping diarrhea. A study (4) has shown that infantile diarrhea not only cause acid-base imbalance in the intestine, but also affect the respiratory system of the children.

Saccharomyces boulardii powder is a biological antidiarrheal agent (5). The study of Micklefield (6) showed that *Saccharomyces boulardii* had a good preventive effect on antibiotic-associated diarrhea, acute infection, irritable bowel syndrome and nonspecific diarrhea. Its main active ingredient is lyophilized and viable *Saccharomyces boulardii* (7), and it is the first choice of drugs in the treatment of infantile diarrhea (8). *Saccharomyces boulardii* powder has nutritive effect on intestinal mucosa (9) and antitoxin effect on microorganism (10), it is effective in treating diarrhea in children and diarrhea caused by intestinal flora disorders and can also prevent and treat diarrhea caused by antibiotics (11). *Saccharomyces boulardii* does not degrade its activity due to gastrointestinal fluids, sulfanilamides or antimicrobial agents and has natural tolerance to antibiotics (12). Studies of O'Mahony *et al* (13) have shown that in animal experiments, *Saccharomyces boulardii* has antimicrobial activity (including albicans Saccharomyces) *in vivo* and *in vitro*. Bifidobacterium triple viable powder is a compound preparation (14), the main components are *bifidobacterium*, lactobacillus and enterococcus viable organism (15). After oral administration, it can directly act on the body to supplement normal human bacteria,

adjust the balance of intestinal flora (16), inhibit the growth of pathogenic bacteria, promote the digestive function of the body, synthesize vitamins needed for normal metabolism and growth (17), prevent the fixed value of pathogenic bacteria on intestinal mucosa and improve the local immunity of intestinal mucosa (18). Moreover, it has small adverse reactions and high application value in clinic. T lymphocyte subsets are important indexes for the detection of cellular immune function, and are of great significance for the diagnosis and observation of therapeutic effects of some diseases (19). $CD4^+$ is an important immune cell in the human immune system, which is mainly responsible for assisting T cells and directing the body against microorganisms. $CD8^+$ cells are inhibitory lymphocytes with the main function of killing the target cells directly. Whether infantile diarrhea affects cellular immune function is also worth clarification. Therefore, its treatment has drawn the attention of the medical field.

The therapeutic effect of *Saccharomyces boulardii* combined with *bifidobacterium* and its effect on cellular immune function in children with acute diarrhea were analyzed in this study.

Patients and methods

General information. In total, 116 cases of children with acute diarrhea admitted to Xuzhou Children's Hospital (Xuzhou, China) from March 2015 to March 2017 were collected, 59 children treated with *Saccharomyces boulardii* were the control group and 57 children treated with *Saccharomyces boulardii* combined with *bifidobacterium* were the experimental group. In the control group, there were 28 males and 31 females, aged 1-11 years, with an average age of 5.2 years. In the experimental group, there were 30 males and 27 females, aged 4-12 years, with an average age of 6.8 years.

This study was approved by the Ethics Committee of Xuzhou Children's Hospital. The signed informed consents were obtained from the patients or the guardians.

Inclusion criteria: Patients with no contraindications, with treatment for more than 3 days and patinets with positive bacterial culture.

Exclusion criteria: Patients with antibiotics before treatment, with severe malnutrition, with digestive system disease, congenital heart disease, respiratory disease and patients with moderate dehydration, hypovolemic shock or other critical disease.

Administration methods. The control group was treated with *Saccharomyces boulardii*, 0.25 g *Saccharomyces boulardii* powder (Laboratories Biocodex; SFDA approval no. H20046379) was given orally, children over 3 years old were given 0.25 g each time, once every 12 h, children under 3 years old were given 0.25 g each time, once per day. The experimental group was treated with 0.1 g *bifidobacterium* triple viable powder (Shanghai Shangmiao Xinyi Pharmaceutical Co., Ltd.; SFDA approval no. S10970105) on the basis of the control group, children aged 0-1 years old were given 0.05 g each time, 3 times per day, children aged 1-5 years were given 0.1 g each time, 3 times per day, children over 6 years old were given 0.2 g each time, 3 times per day. The course of treatment in the two groups was one week.

Observation indicators and judgement criteria. The judgment criteria for therapeutic effect (20) are shown in Table I.

Standard for the detection of cellular immunological indexes. The cellular immune indexes $CD3^+$, $CD4^+$, $CD8^+$ in Xuzhou Children's Hospital at the time of visit and one month after treatment were detected by flow cytometry, and the changes of $CD4^+/CD8^+$ were measured and calculated.

Main observation indicators and secondary observation indicators of infantile diarrhea. Main observation indicators: Stool frequency in different time periods, mean antidiarrheal time, mean antipyretic time and length of stay in the two groups. Fasting venous blood samples were collected before and after treatment. The ratio of Th1 and Th2 in peripheral blood was detected by flow cytometry, and the ratio of Th1/Th2 was calculated. Cellular immunological indexes were examined before and one month after treatment in both groups. Secondary observation indicators: clinical efficacy (apparent, effective, non-effective).

Statistical analysis. The data were analyzed by SPSS19.0 (SPSS, Inc.). The enumeration data were expressed as n (%). The comparison method was Chi-square test. t-test was used for comparison between the experimental and the control group. $P<0.05$ was statistically significant.

Results

Comparison of basic data between the two groups. There was no significant difference in sex, age, height, weight, fever, vomiting, abdominal pain, fecal routine, crying and loss of appetite between the two groups ($P>0.05$) (Table II).

Comparison of clinical efficacy. The cure rate (73.68%) and the total effective rate (87.72%) in the experimental group were significantly higher than those in the control group (47.46 and 71.19%) and there was a statistical difference between the two groups ($P<0.05$) (Table III).

Stool frequency in different time period in the two groups. There was no significant difference in the average stool frequency before treatment between the experimental group and the control group ($P>0.05$), the stool frequency in the two groups was significantly decreased after treatment, and the experimental group was significantly lower than the control group 3 days after treatment ($P<0.05$). There was no significant difference in average stool frequency between the two groups after 6 days of treatment ($P>0.05$). The stool frequency in different time periods in the two groups are shown in Fig. 1.

Comparison of mean antidiarrheal time, mean antipyretic time and mean length of stay between the two groups. The mean antidiarrheal time in the experimental group (3.7±0.3 h) was significantly shorter than that in the control group (6.1±0.5 h), and there was a statistical difference ($P<0.05$) (Fig. 2). The antipyretic time in the control group (3.5±0.3 h) was longer than that in the experimental group (3.4±0.4 h), and there was no statistical significance ($P>0.05$) (Fig. 3). The length of stay in the control group (6.9±0.7 h) was significantly longer than that in the experimental group (5.2±0.5 h), with a statistical significance ($P<0.05$) (Fig. 4).

Table I. Criteria for therapeutic effect of diarrhea.

Therapeutic effect	Medication duration	Stool character	Stool frequency	Clinical symptoms
Apparent	24-48 h	Return to normal	≤ Twice daily	Complete disappearance
Effective	48-72 h	Improvement and water reduction	≤ Twice daily	Basic disappearance
Non-effective	72 h	No obvious improvement	> Twice daily	Exacerbation of illness

Table II. Basic data of 116 children with acute diarrhea [n (%)].

Basic data	Control group (n=59)	Experimental group (n=57)	χ^2 value	P-value
Sex				
Male	28 (47.46)	30 (52.63)	0310	0.577
Female	31 (52.54)	27 (47.37)		
Age (years)				
<6	33 (55.93)	31 (54.39)	0.028	0.867
≥6	26 (44.07)	26 (45.61)		
Weight (kg)				
<15	34 (57.63)	38 (66.67)	1.006	0.316
≥15	25 (42.37)	19 (33.33)		
Height (m)				
<1	36 (61.02)	34 (59.65)	0.023	0.880
≥1	23 (38.98)	23 (40.35)		
Fever				
Yes	48 (81.36)	45 (78.95)	0.106	0.745
No	11 (18.64)	12 (21.05)		
Vomiting				
Yes	45 (76.27)	49 (85.96)	1.773	0.183
No	14 (23.73)	8 (14.04)		
Abdominal pain				
Yes	47 (79.66)	47 (82.46)	0.147	0.701
No	12 (20.34)	10 (17.54)		
Fecal routine				
Normal	49 (83.05)	51 (89.47)	1.006	0.316
Abnormal	10 (16.95)	6 (10.53)		
Crying				
Yes	47 (79.66)	49 (85.96)	0.807	0.369
No	12 (20.34)	8 (14.04)		
Loss of appetite				
Yes	48 (81.36)	48 (84.21)	0.166	0.684
No	11 (18.64)	9 (15.79)		

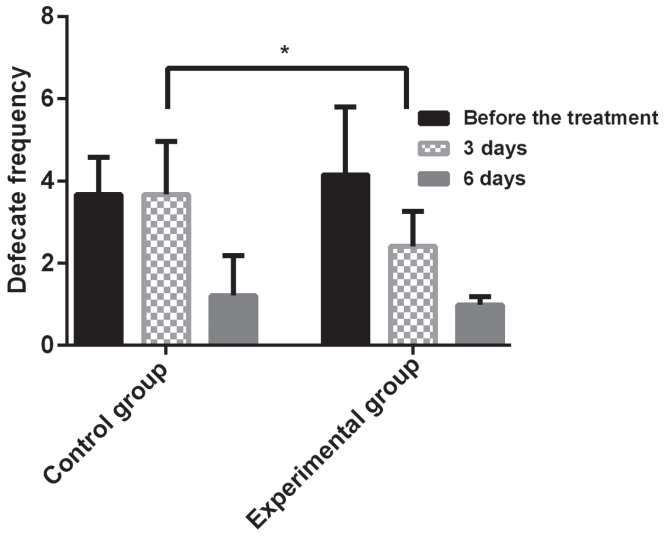

Figure 1. Average stool frequency before treatment of the experimental group and control group. The stool frequency in the two groups was significantly decreased after treatment, and the experimental group was significantly lower than the control group 3 days after treatment (*P<0.05). There was no significant difference in average stool frequency between the two groups after 6 days of treatment (P>0.05).

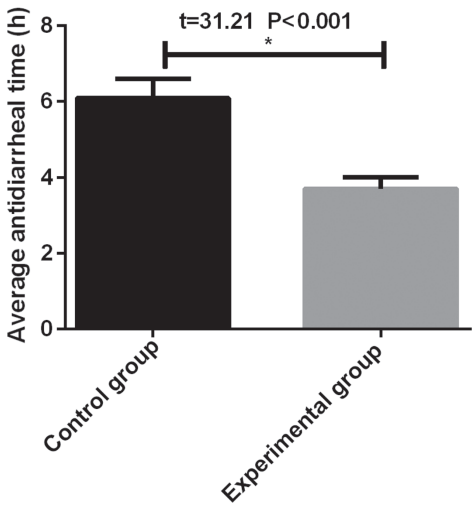

Figure 2. The mean antidiarrheal time in both groups. The mean antidiarrheal time in the experimental group was significantly shorter than that in the control group (t=31.21, *P<0.001).

Comparison of the results of cellular immunologic indexes before treatment and one month after treatment between the two groups. There was no significant difference in $CD3^+$, $CD4^+$, $CD8^+$ and $CD4^+/CD8^+$ between the two groups before treatment (P>0.05). The results of review conducted one month after treatment showed that $CD3^+$, $CD4^+$ and $CD4^+/CD8^+$ increased significantly in the experimental group while $CD8^+$ decreased significantly (P<0.05), which indicated that the cellular immune function of the children in the experimental group was significantly improved. Compared with those before treatment, $CD3^+$, $CD4^+$ and $CD4^+/CD8^+$ increased slightly

Table III. Comparison of clinical efficacy between the two groups [n (%)].

Groups	Cure (%)	Effective (%)	Non-effective (%)	Total effective rate (%)
Control (n=59)	28 (47.46)	14 (23.73)	17 (28.81)	42 (71.19)
Experimental (n=57)	42 (73.68)	8 (14.04)	7 (12.28)	50 (87.72)
χ^2 value	8.333	1.773	4.829	4.829
P-value	0.004	0.183	0.030	0.030

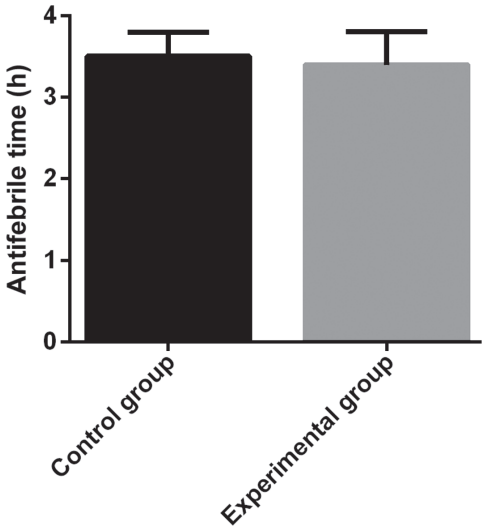

Figure 3. The antipyretic time in both groups. There was no statistical significance of the antipyretic time between the control group and the experimental group. (t=1.527, P>0.05).

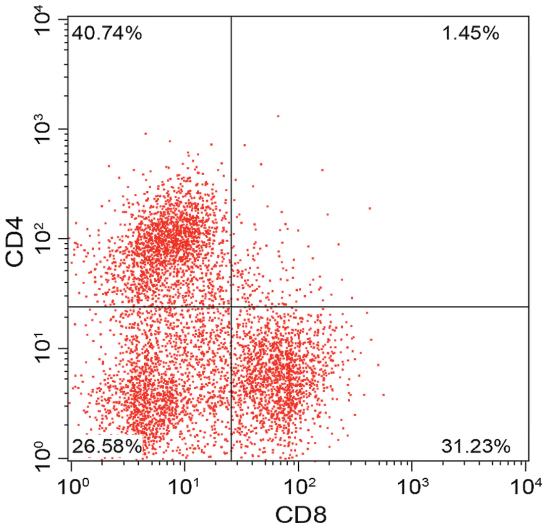

Figure 5. Flow cytometry dot plots in control group after treatment. CD4 cells accounted for 40.74%. CD4/CD8 cells accounted for 1.45%. CD8 cells accounted for 31.23%, and non-expressed cells accounted for 26.58%.

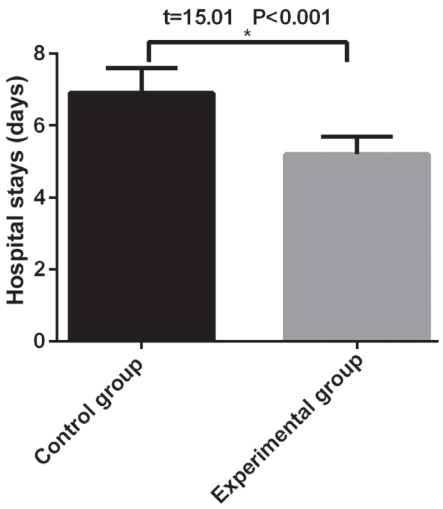

Figure 4. The length of stay in both groups. The length of stay in the control group was significantly longer than that in the experimental group (t=15.01, *P<0.001).

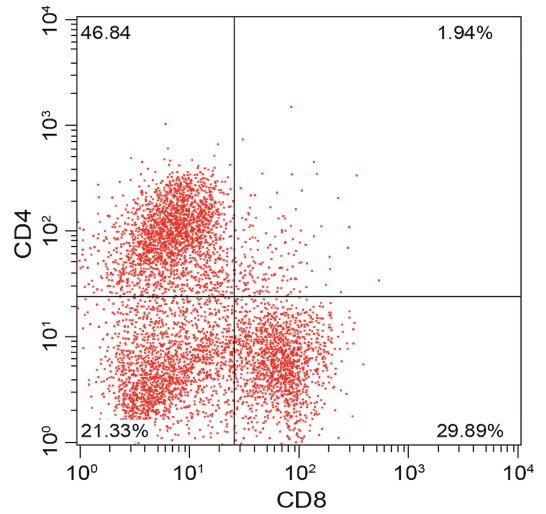

Figure 6. Flow cytometry dot plots in experimental group after treatment. CD4 cells accounted for 46.84%. CD4/CD8 cells accounted for 1.94%. CD8 cells accounted for 29.89%, and non-expressed cells accounted for 21.33%.

in the control group while $CD8^+$ decreased slightly, and the difference was statistically significant (P<0.05) (Figs. 5, 6 and Table IV).

Comparison of T lymphocyte levels between the two groups. After treatment, the ratio of Th1 and Th2 in the two groups decreased significantly compared with before treatment, with a statistical significance (P<0.05), and the experimental group was significantly lower than the control group, with a statistical significance (P<0.05). After treatment, Th1/Th2 ratio was significantly higher than that before treatment, with a statistical significance (P<0.05), and the experimental group was

Table IV. Comparison of the results of cellular immunologic indexes before and after treatment between the two groups.

Groups	Time	$CD3^+$ (%)	$CD4^+$ (%)	$CD8^+$ (%)	$CD4/CD8^+$ (%)
Control (n=59)	Before treatment	57.28±1.54	37.24±7.12	28.52±1.68	1.19±0.57
	One month after treatment	61.84±2.95[a]	40.74±6.31[a]	31.23±1.50[a]	1.45±0.61[a]
Experimental (n=57)	Before treatment	62.54±3.54	41.23±6.87	32.14±1.94	1.59±0.65
	One month after treatment	65.48±2.51[a,b]	46.84±4.54[a,b]	29.89±2.14[a,b]	1.94±0.51[a,b]
t value		1.172	1.250	1.159	0.964
P-value		0.362	0.338	0.366	0.437

[a]P<0.05 compared with the control group before treatment; [b]P<0.01 compared with the experimental group before treatment.

Table V. Comparison of T lymphocyte levels between the two groups.

	Th1 (%)		Th2 (%)		Th1/Th2	
Groups	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
Experimental (n=57)	19.37±2.47	11.72±2.01[a]	28.37±3.26	13.47±3.17[a]	0.63±0.12	0.89±0.13[a]
Control (n=59)	19.28±2.51	15.63±2.27[a]	28.38±3.12	19.37±3.38[a]	0.61±0.16	0.81±0.11[a]
t value	0.195	9.809	0.017	9.690	0.760	0.005
P-value	0.846	<0.001	0.987	<0.001	0.449	3.582

[a]P<0.05 compared with the same group before treatment.

significantly higher than the control group, with a statistical significance (P<0.05) (Table V).

Discussion

As a common disease in children with a high incidence in children at 6 months to 2 years of age (21), the main clinical symptoms of infantile diarrhea are thin stool, a small amount of gas and sometimes mucus discharging, increased defecation and often accompanied by copracrasia and shortness of defecation and other symptoms (22). Attention should be paid to the disease once it is contracted, the exacerbation can cause severe dehydration, leading to shock and even be life-threatening. Its influencing factors are complex, mainly divided into infectious and non-infectious categories (23). Infectious diarrhea is caused by pathogenic organisms entering the digestive tract with contaminated food or drinking water (24). The ability of pathogenic organisms to cause intestinal infection also determines the strength of their defense and the size of infected pathogenic organisms (25). Most of the cases are viral infectious diarrhea in clinic because children's immune system is not mature, the immune function is not perfect and the pathogen can easily invade (26). Infectious diarrhea is mainly caused by improper diet. For example, improper composition and food intolerance can cause malfunction of digestion, making food difficult to digest and absorb, thus leading to food stagnation in the upper small intestine and decreased acidity of the intestinal cavity, which is more favorable for bacteria reproduction and upward migration in the lower part of the small intestine (27).

Results in this study showed that the cure rate (73.68%) and the total effective rate (87.72%) in the experimental group were significantly higher than those in the control group (47.46 and 71.19%), and there was statistical difference between the two groups (P<0.05), which suggested that therapeutic effect of *Saccharomyces boulardii* combined with *bifidobacterium* on infantile diarrhea is better than that of single use of *Saccharomyces boulardii*. It is inferred that when the *Saccharomyces boulardii* and *bifidobacterium* triple therapy are used together, the *Saccharomyces boulardii* may proliferate rapidly in the intestine, decompose peroxide and consume oxygen to produce anaerobic environment, which is more beneficial to the growth of *bifidobacterium*. Bifidobacterium triple viable powder can directly supplement human normal flora, regulate intestinal balance and eliminate harmful bacteria. Bifidobacterium can also promote intestinal absorption, reduce the absorption and production of toxic substances, and maintain ecological balance. Therefore, the combined use of the two drugs may be compatible with each other. The results of Martins *et al* (28) showed that the combined use of *Saccharomyces boulardii* and *bifidobacterium* triple therapy was more effective than that of Bifidobacterium triple therapy alone, which gives support to our results. However, the data analysis of this experiment was more comprehensive, and the experimental results were more convincing. Then we analyzed the mean antipyretic time, mean antidiarrheal time, and the length of stay. The results showed that there was no difference in the mean antipyretic time, and the mean antidiarrheal time, and length

of stay in the experimental group was significantly shorter than that in the control group. These results indicated that *Saccharomyces boulardii* combined with *bifidobacterium* could shorten the mean antidiarrheal time and length of stay. In the study of El-Soud *et al* (29), a randomized controlled trial showed that probiotics of *bifidobacterium* significantly decreased frequency, duration of antidiarrheal and length of stay. The results of the detection of cellular immunological indexes before and after treatment and the level of T lymphocyte showed that there was no significant difference in $CD3^+$, $CD4^+$, $CD8^+$ and $CD4^+/CD8^+$ between the two groups before treatment. The results of review conducted one month after treatment showed that $CD3^+$, $CD4^+$ and $CD4^+/CD8^+$ increased significantly in the experimental group, while $CD8^+$ decreased significantly. After treatment, the ratio of Th1 and Th2 in the two groups decreased significantly compared with before treatment, and the experimental group was significantly lower than the control group. After treatment, Th1/Th2 ratio was significantly higher than that before treatment and the experimental group was significantly higher than the control group. Thus, our study indicated that the cellular immune function of the children in the experimental group was significantly improved. The results suggest that infantile diarrhea is closely related to cellular immunity, so attention should not be paid only to clinical efficacy, but also to the prevention and treatment of cellular immune dysfunction in the course of treatment, so as to achieve better clinical effect. The observation of intestinal immunology found that the distribution of memory T cells and macrophages changed obviously. After early infantile treatment, macrophages were concentrated in the local intestine to activate the natural immunity. In the study of Akatsu *et al* (30), the elderly were studied. The results showed that long-term *bifidobacterium* intake had potential in increasing the number of *bifidobacterium* cells in intestinal microflora and regulating the immune function of the elderly.

There are some defects in this study. All the children with acute diarrhea included in the study were treated, and untreated children were not included. The cellular immunological indicators and T lymphocyte levels were not observed in the untreated patients. The number of patients included is small.

In conclusion, treatment of acute diarrhea in children with *Saccharomyces boulardii* combined with *bifidobacterium* can effectively improve the clinical efficacy, shorten antidiarrheal time and hospital stay, and promote the improvement of immune function in children.

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Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

GW recorded and analyzed observation indicators. GW and DF collected, analyzed and interpreted general information of patients. All authors read and approved the final manuscript.

Ethics approval and consent to participate

This study was approved by the Ethics Committee of Xuzhou Children's Hospital (Xuzhou, China). The signed informed consents were obtained from the patients or the guardians.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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