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Comparison of safety, efficacy and serum immune indexes of *Clostridium butyricum* Enterococcus triple viable vs Bifidobacterium triple viable in the treatment of bronchial asthma in children

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Abstract: The principal objective of our study was to investigate the safety and efficacy of *Clostridium butyricum* enterococci triple viable vs Bifidobacterium triple viable in the cure of bronchial asthma in children, and to compare the serum immune indexes. We retrospectively investigated 180 children with BA treated in Lianshui County People's Hospital from June 2019 to June 2021. These children were divided into three groups in accordance with the cure methods: The control group (group A) gave consent to routine drug treatment, the children in the *Clostridium butyricum* enterococci triple viable group (group B) received routine treatment, combined with *Clostridium butyricum* enterococci triple viable tablets, and the children in the Bifidobacterium Lactobacillus triple viable group (group C) received routine treatment, combined with Bifidobacterium Lactobacillus triple viable powder. Combined with the treatment results, the effect of combined probiotics on BA in children is more significant than that of routine treatment, which can effectively enhance the immune function, lung function and inflammation of children. The effect of *Clostridium butyricum* enterococci triple viable bacteria is better than that of Bifidobacterium triple viable bacteria.

Keywords: Bronchial asthma, children, *Clostridium butyricum* Enterococcus triple viable, bifidobacterium triple viable.

INTRODUCTION

Bronchial asthma (BA) is very common in children, it is a common chronic respiratory disease. The main manifestations were recurrent wheezing, breathe hard, oppression in the chest and cough. Its prevalence rate is increasing with each passing year. The results of the third urban Ba epidemiological survey in China show that (Liu *et al*., 2013) the prevalence rate of childhood asthma in 2010 has reached 3.01%, significantly higher than 1.97% in 2000 and 1.08% in 1990 and the involved prevalence rate has increased by 53.2%. The prevalence rate has increased by 50.6% in recent two years, 61.4% of children have acute asthma attack in recent one year, and 32.1% of children go to emergency treatment due to wheezing, 16.3% of the children were hospitalized. The data showed that the prevalence of preschool and school-age children increased significantly compared with infants. Most children's asthma symptoms can be relieved by treatment or by themselves, but once there is a serious acute attack, it may be fatal (Ebmeier *et al*., 2017). The specific pathogenesis of asthma is unknown. At present, the targeted treatment of asthma is mainly heteropathy, emphasizing long-term comprehensive management. The main objectives of treatment emphasize that patients have no shortness of breath or dyspnea, minimize daytime symptoms, no nocturnal awakening caused by asthma and no restriction of normal activity. In addition, it also includes the prevention of future risk of asthma and acute attack. Controlling asthma is to eliminate or reduce the degree of clinical manifestations of asthma through treatment, so that patients can live a completely normal life without being affected by the disease (Panek *et al*., 2016). At present, the treatment of asthma is mainly inhaled cortisol and the preventive drugs mainly rely on some leukotriene receptor modulators, which can temporarily control the clinical symptoms of asthma. Although the hormone content in therapeutic and preventive drugs is not high, there is still no research to prove that taking drugs for a long time will not influence the growth, life and learning of children, and even, After stopping the drug, the condition may be repeated, so that it is impossible to get rid of the use of the drug (Stanford *et al*., 2012). The most important thing is that long-term repeated treatment will not only cause the family economic burden, but also bring a certain psychological burden to a family.

Studies have shown that changes in intestinal microorganisms can lead to allergic diseases and induce the onset of asthma (Noverr and Huffnagle 2004; Kranich *et al*., 2011; Kalliomäki *et al*., 2001a; Dewan and Goldman 2020 and Gill *et al*., 2010). Compared with healthy children, the intestinal flora of asthmatic children was significantly different. The results showed that the colonization of pathogenic bacteria such as Clostridium difficile in the intestinal tract of asthmatic children raised obviously, and the amount of non pathogenic bacteria such as bifidobacteria decreased significantly (Kalliomäki *et al*., 2001ab). Probiotics are living bacteria colonized in the gastrointestinal tract. It could induce tolerance in allegro-inflammatory reactions and alter immune

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responses in allergic conditions. Probiotics could also modulate cellular and humoral immune responses and prevent allergic disorders (Eslami *et al*., 2020; Mennini *et al*., 2017 and Wu *et al*., 2022). In addition, probiotics can also effectively treat BA in children (Butov *et al*., 2019; Moura *et al*., 2019). It can reduce asthma exacerbations in children provides a potential complementary therapy (Drago *et al*., 2022). Bifidobacterium Lactobacillus triple viable tablet is one of the common probiotic preparations in clinic. After oral administration, it can directly supplement probiotics in the body, regulate intestinal flora and eliminate and inhibit potential pathogenic bacteria in the intestine. At the same time, it can also regulate the host intestinal micro ecosystem by adjusting the intestinal micro ecosystem, and finally achieve the purpose of preventing and treating allergic diseases. *Clostridium butyricum* is a Gram-positive anaerobic bacillus, which has remarkable characteristics compared with Bifidobacterium, Lactobacillus and other lactic acid bacteria. Firstly, butyric acid is the main metabolic end product, the energy source of intestinal mucosal epithelial cells and an important immune regulator, which can induce the regeneration, repair, proliferation and differentiation of intestinal mucosal epithelium; Secondly, *Clostridium butyricum* exists in the form of spores, has good stability, is not disturbed by gastric acid and bile in the gastrointestinal tract, and can maintain strong activity after oral administration (Ji *et al*., 2015 and Liu *et al*., 2013). Finally, as a microecological agent, *Clostridium butyricum* is widely used in clinic. It can not only regulate human gastrointestinal function, but also has significant curative effects on *Pseudomembranous enteritis*, antibiotic associated enteritis, diarrhea and constipation caused by flora imbalance (Li *et al*., 2013). And researches indicate that administration with *Clostridium butyricum* enforces the effect of specific immunotherapy on asthma (Liao *et al*., 2016 and Juan *et al*., 2017). The principal objective of our research was to investigate the efficacy and safety of *Clostridium butyricum* enterococci triple viable vs Bifidobacterium triple viable in the treatment of BA in children, and to compare the serum immune indexes.

MATERIALS AND METHODS

Patient selection

We retrospectively collected the medical records of children with BA in Lianshui County People's Hospital during June 2019 to June 2021 (fig. 1).

Inclusion criteria: (I) meet the diagnosis criteria of BA in children laid down by the respiratory branch of the scientific branch of the Chinese Medical Association in 2016 (Bao *et al*., 2016) and 2021 Global Initiative for Asthma; (II) age ≤ 14 years;(III) no probiotic treatment was given 14 days before enrollment. Exclusion criteria: (I) Suffering from cardiac insufficiency, tracheal tumor and other diseases that may cause wheezing and immune system diseases; (II) taking immunomodulators or glucocorticoids in recent 1 month; (III) patients with congenital diseases or immune diseases; (IV) general clinical data are incomplete.

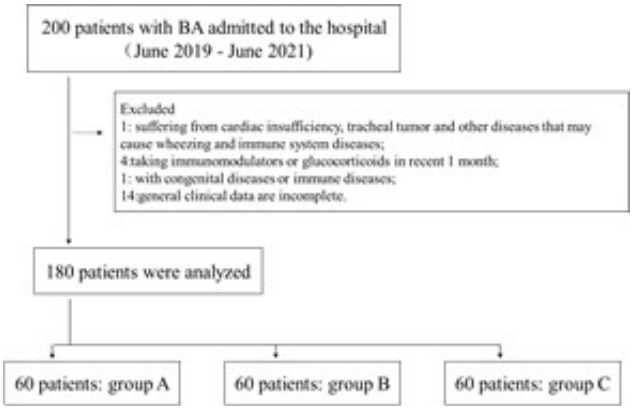

Fig. 1: Flowchart of participants.

The collected cases meeting the inclusion criteria were studied retrospectively. Divide patients into three groups based on treatment. Group A was only treated with conventional medication; Group B was treated with conventional medication and *Clostridium butyricum* enterococci triple viable tablets; Group C was treated with conventional medication and Bifidobacterium Lactobacillus triple viable powder. The observation indexes before and after 30 days of treatment were compared.

Therapeutic method

The children of the control group (group A) received regular medication. The main therapeutic drugs were anti-inflammatory drugs, antiasthmatic drugs and hormone drugs. The anti-inflammatory drugs are beclomethasone propionate (gyzz: h20084607), salbutamol sulfate tablets (gyzz: hb2024535), and the hormone drug is Seretide (salmeterol ticasone powder inhaler, gyzz: h20140164).

The children of *Clostridium butyricum* enterococci triple viable group (group B) were given conventional medication, combined with *Clostridium butyricum* enterococci triple viable tablets (Japan East Asia Pharmaceutical Industry Co., Ltd., specification: 100 mg / tablet, gyzz j20110038, the main components are lactic acid bacteria, *Clostridium butyricum* and saccharifying bacteria), orally, washed with warm boiled water or milk (≤ 3 years old, 1 tablet each time, 2 times / D; older than 3 years old, 1 tablet each time, 2 tablets each time, 3 times / D). 30 d.

The children in Bifidobacterium Lactobacillus triple viable group (Group C) were given conventional medication, use in combination with Bifidobacterium Lactobacillus triple viable powder (Inner Mongolia

Shuangqi Pharmaceutical Co., Ltd., specification: 500 mg/tablet, gyzz s19980004, the main components are Bifidobacterium longum, Lactobacillus bulgaricus and Streptococcus thermophilus), oral, warm boiled water or milk (≤ 3 years old, 2 tablets each time), 2 times / day; ＞ 3 years old, 4 tablets each time, 4 tablets once, 3 times / D). 30 d. The dose of conventional medication is determined according to the physical condition of each child.

Observation index

Pulmonary function detection

The pulmonary function of children was measured by masterscreen pulmonary function instrument provided by American viaon medical devices. The forced expiratory volume in one second (FEV1), forced vital capacity (FVC) and peak expiratory flow (PEF) were detected, and the operation was carried out in strict accordance with the instructions.

Inflammatory factor detection

Before and 30 days after treatment, 3ml fasting venous blood of all children in the morning was taken, 2000r / min, centrifuged for 15min, and took the supernatant and placed in the fridge at -80^{o}C for testing. Detected serum IL-2, IL-4, IL-6 and TNF-α by enzyme-linked immunosorbent assay (ELISA). The detection kits were from Wuhan amejie Technology Co., Ltd. and strictly follow the kit instructions.

Immune function index detection

Before and 30 days after treatment, took 1 ~3ml of fasting venous blood from all children. The T cell subsets CD3 +, CD4 + and CD4 + / CD8 + were detected by bdaccuric6 flow cytometry provided by Jiangsu Zhonghong bioengineering innovation and Drug Research Institute Co., Ltd.

Clinical efficacy

Efficacy criteria: (I) significant effect: the symptoms of cough and breathe hard were completely improved; (II) Effective: The clinical symptoms of patients have improved and recovered well; (III) Ineffective: the patient's clinical symptoms show no signs of improvement or even aggravation. The treatment effects of the three groups were evaluated by asthma control test scale formulated by American Thoracic Society in 2005. Total effective rate = (Number of markedly effective cases+ Number of effective cases) / 60 × 100%.

Adverse reaction

The adverse reactions of the two groups were observed and recorded.

Ethical approval

The study was approved by the Ethics Committee of Lianshui County People's Hospital (2019132) and was conducted in accordance with the Declaration of Helsinki (as revised in 2013). All participants provided informed consent.

STATISTICAL ANALYSIS

After organizing the collected data, applied SPSS 24.0 statistical software (SPSS Inc., Chicago, IL, USA) for statistical analysis. The measured data of normal distribution is expressed as mean ± standard deviation (SD). One way ANOVA was applied for intergroup comparison, and paired sample t-test was applied for intra group comparison; the chi-square ($\chi 2$) test was applied for count data. When $P<0.05$, the difference was considered to be statistically significant.

RESULTS

General information

The study included 180 children. There were 60 cases in each of the three groups. Group A had 39 males and 21 females, aged 5-13 years, with an average of (8.23±2.09) years old and an average weight of (30.48±9.76) kg, 45 of them living in city and 14 in villages, 3 of them are newly renovated, 32 of them with allergen avoidance and 32 of them are passive smokers. There were 60 cases in group B, 36 males and 24 females, aged from 4 to 14 years, with an average of (8.85±2.41) years and an average weight of (31.91±7.60) kg, 38 of them living in city and 22 in villages, 4 of them are newly renovated, 26 of them with allergen avoidance and 35 of them are passive smokers. There were 60 cases in group C, 34 males and 26 females, aged from 2 to 14 years, with an average of (8.89±2.37) years and an average weight of (29.94±9.67) kg, 40 of them living in city and 20 in villages, 5 of them are newly renovated, 30 of them with allergen avoidance and 28 of them are passive smokers. There was no statistical difference in gender, age and weight among the three groups ($P>0.05$). As shown table 1.

Comparison of pulmonary function detection

In group A, FVC was (1.36±0.28) L, FEV1 was (1.38± 0.17) L and PEF was (80.29±4.52) L before treatment, and (1.66±0.33) L, (1.69±0.22) L, (92.8±3.95) L respectively after 30 days of treatment; In group B, FVC was (1.42±0.32) L, FEV1 was (1.38±0.06) L and PEF was (80.45±3.84) L before treatment and (1.88±0.51) L, (1.67 ±0.09) L, (94.11±3.29) L respectively after 30 days of treatment; In group C, FVC was (1.41±0.30) L, FEV1 was (1.39±0.07) L and PEF was (79.78±3.86) L before treatment, and (1.76±0.51) L, (1.66±0.09) L, (94.10± 3.58) L respectively after 30 days of treatment. There was no statistical difference in the three indexes among the three groups before treatment ($P>0.05$). While after 30 days of treatment, the three indexes in the three groups were remarkable higher than those before treatment ($P< 0.05$); FVC and FEV1 in group B and group C were

Table 1: General information of the 3 participant groups

Group	Group A	Group B	Group C	F /χ2	P
n	60	60	60	-	-
Gender (male / female, n)	39/21	36/24	34/26	1.705	0.426
Age (years)	8.23±2.09	8.85±2.41	8.89±2.37	1.532	0.219
Weight (kg)	30.48±9.76	31.91±7.60	29.94±9.67	0.756	0.471
Residence (Urban /Rural, n)	45/14	38/22	40/20	0.996	0.372
Newly renovated (Yes / No, n)	3/57	4/56	5/55	0.264	0.768
Allergen avoidance (Yes / No, n)	32/28	26/34	30/30	0.616	0.541
Passive smoking (Yes / No, n)	32/28	35/25	28/32	0.819	0.443

Table 2: Pulmonary function indexes of the three groups

Group	N	Time	FVC (L)	FEV1 (L)	PEF (L/min)
Group A	60	Before treatment	1.36±0.28	1.38±0.17	80.29±4.52
		30 days after treatment	1.66±0.33	1.69±0.22	92.80±3.95
t			2.615	2.753	16.127
P			0.010	0.012	0.000
Group B	60	Before treatment	1.42±0.32	1.38±0.06	80.45±3.84
		30 days after treatment	1.88±0.51△	1.67±0.09△	94.11±3.29
t			3.713	20.174	20.917
P			0.000	0.000	0.000
Group C	60	Before treatment	1.41±0.30	1.39±0.07	79.78±3.86
		30 days after treatment	1.76±0.51△*	1.66±0.09△	94.10±3.58
t			2.835	18.192	21.095
P			0.005	0.000	0.000

Table 3: Inflammatory factors of the three groups

Group	N	Time	IL-2(pg/ml)	IL-4(pg/ml)	IL-6(pg/ml)	TNF-α (pg/ml)
Group A	60	Before treatment	104.90±10.89	110.62±20.35	100.43±10.50	156.37±33.63
		30 days after treatment	47.58±7.04	56.62±10.31	43.65±8.07	75.40±9.04
t			34.23	18.336	33.215	18.007
P			0.000	0.000	0.000	0.000
Group B	60	Before treatment	103.87±12.36	110.48±22.70	101.82±13.73	160.28±29.23
		30 days after treatment	39.05±5.88△	33.97±9.43△	31.95±4.96△	40.05±6.08△
t			36.690	24.109	37.062	31.195
P			0.000	0.000	0.000	0.000
Group C	60	Before treatment	106.03±13.37	113.52±19.43	99.73±13.15	156.22±26.68
		30 days after treatment	45.42±7.82*	41.83±8.23△	40.25±7.06*	38.93±6.13△
t			30.312	26.313	30.864	33.185
P			0.000	0.000	0.000	0.000

remarkable higher than those in group A after 30 days of treatment (P<0.05); FVC in group B was higher than that in group C, and the difference was statistically significant (P<0.05) (table 2).

Comparison of Inflammatory factor detection level

In group A, the levels of four inflammatory factors (IL-2, IL-4, IL-6 and TNF-α) were (104.90±10.89) pg/ml, (110.62±20.35)pg/ml, (100.43±10.50)pg/ml and (156.37± 33.63)pg/ml respectively before treatment and (47.58± 7.04) pg/ml, (56.62±10.31)pg/ml, (43.65±8.07) pg/ml, (75.40±9.04)pg/ml respectively after 30 days of treatment; In group B, the levels of four inflammatory factors were (103.87±12.36)pg/ml, (110.48±22.70)pg/ml, (101.82±13.73) pg/ml and (160.28±29.23) pg/ml respectively before treatment and (39.05±5.88)pg/ml, (33.97±9.43)pg/ml, (31.95±4.96)pg/ml, (40.05±6.08) pg/ml respectively after 30 days of treatment; In group C, the levels of four inflammatory factors were (106.03± 13.37)pg/ml, (113.52±19.43)pg/ml, (99.73±13.15)pg/ml and (156.22±26.68)pg/ml respectively before treatment, and(45.42±7.82)pg/ml, (41.83±8.23)pg/ml, (40.25±7.06) pg/ml, (38.93±6.13)pg/ml respectively after 30 days of treatment. The levels of four inflammatory factors in the three groups before treatment were not statistically significant (P>0.05). After 30 days of treatment, the levels of four inflammatory factors in the three groups were remarkable lower than those before treatment (P<0.05). After 30 days of treatment, the levels of four inflammatory factors in group B were remarkable lower

Table 4: Immune function indexes of the three groups

Group	N	Time	CD3+(%)	CD4+(%)	CD4+/CD8+
Group A	60	Before treatment	59.91±4.59	36.77±5.80	0.98±0.20
		30 days after treatment	66.40±6.17	43.97±4.83	1.19±0.22
t			7.387	6.531	6.072
P			0.000	0.000	0.000
Group B	60	Before treatment	60.21±4.57	37.88±5.85	1.04±0.21
		30 days after treatment	77.55±6.12Δ	51.41±3.73Δ	1.44±0.25Δ
t			17.576	15.097	5.705
P			0.000	0.000	0.000
Group C	60	Before treatment	29.95±2.53	36.80±4.69	1.00±0.18
		30 days after treatment	77.65±5.28Δ	51.83±3.95Δ	1.45±0.25Δ
t			19.001	15.232	6.375
P			0.000	0.000	0.000

△ P<0.05 vs. Group A, *P<0.05 vs. Group B

Table 5: Clinical effect of the three groups n (%)

Group	N	Remarkable effect	Effective	invalid	Total effective rate
Group A	60	14	22	24	46(76.7)
Group B	60	4	22	34	56(93.3)
Group C	60	5	20	35	55(91.7)
χ^2		10.425			9.072
P		0.034			0.01

than those in group A (P<0.05); IL-4 and TNF-α in group C were remarkable lower than those in group A (P<0.05); IL-2 and IL-6 in group B were remarkable lower than those in group C (P<0.05) (table 3).

Comparison of immune function indicators

In group A, CD3 + was (59.91±4.59)%, CD4+ was (36.77±5.80)% and CD4 + / CD8 +) was (0.98±0.20) before treatment and (66.40±6.17)%, (43.97±4.83)%, (1.19±0.22) respectively after 30 days of treatment; In group B, CD3 + was (60.21±4.57)%, CD4 + was (37.88±5.85)% and CD4 + / CD8 +) was (1.04±0.21) before treatment, while (77.55±6.12)%, (51.41±3.73)%, (1.44±0.25) respectively after 30 days of treatment; In group C, CD3 + was (29.95±2.53)%, CD4 + was (37.88±5.85)% and CD4 + / CD8 +) was (36.80±4.69)% and (1.00±0.18) before treatment, while (77.65±5.28)%, (51.83±3.95)%, (1.45±0.25) respectively after 30 days of treatment. There was no significant difference in immune function indicators among the three groups before treatment (P>0.05). After 30 days of treatment, immune function indicators of the three groups were higher than those before treatment (P<0.05). And after 30 days of treatment, the three index in group B and group C were remarkable higher than those in group A (P<0.05). As shown in table 4.

Comparison of clinical effect

The overall effective rates of group B and group C were 93.3% and 91.7% respectively, which was Significantly higher than 76.7% in group A (P<0.05) (table 5).

Comparison of untoward reaction

There was no obvious untoward reaction in the three groups.

DISCUSSION

BA is a chronic airway inflammation characterized disease, which is involved in a variety of cells and cell components such as neutrophils, eosinophils, T lymphocytes, macrophages and mast cells. It is characterized by Airway Hyper Reactivity, Mucus Hypersecretion and airway remodeling (Wegmann, 2009). IL-2, IL-4, IL-6 and TNF-α are inflammatory factors secreted by peripheral blood T lymphocytes. Under normal circumstances, the content of the above indicators in peripheral blood is low.

Once infection occurs, it can activate T lymphocytes and monocyte macrophages, increasing the synthesis and release of these inflammatory factors (Deftereos *et al*., 2020; Nakagome *et al*., 2012 and Barnes, 2009). T lymphocytes are the most important cell group of human immune function. The morbidity of bronchial asthma are closely related to the abnormalities of T lymphocytes. In the classification of T lymphocytes, CD3 + stands for total T lymphocytes, CD4 + stands for T helper cells and CD8 + stands for T killer cells and T suppressor cells, in which CD4 + cells can synthesize Release a variety of inflammatory cytokines to improve the body's immune response, while CD8 + on the contrary can inhibit the body's immune response. Therefore, the stability of CD4

+ / CD8 + level has become a key to maintain the body's normal immune level. Once the above indicators change, it can be determined that there is a problem with immune function. The above indicators can objectively reflect the immune function of children with bronchial asthma and control the disease earlier (Segovia *et al*., 2018). Children with bronchial asthma will cause serious damage to lung function. The lung function indexes FVC, FEV1 and PEF of children show low levels. The above lung function indexes can be used as indexes for the detection of lung function.

Intestinal flora is different between children with allergic constitution such as bronchial asthma and healthy children. The imbalance of intestinal flora will affect the regulation of inflammation, directly affect the immune system, and increase the risk of allergy and asthma (Ruiz-Calderon *et al*., 2016). Probiotics may function by regulating biochemical biomarkers (such as miRNA) to change the composition of flora in a certain part of the host, so as to improve the micro ecological balance of the host (Davoodvandi *et al*., 2021). The reconstruction of healthy intestinal flora can prevent allergy and asthma. Bifidobacterium Lactobacillus triple viable tablet is one of the common probiotic preparations in clinic. After oral administration, it can directly supplement probiotics in the body, regulate intestinal flora and eliminate and inhibit potential pathogenic bacteria in the intestine; meanwhile, it can also regulate the host systemic immune system by adjusting the intestinal micro ecosystem and finally achieve the purpose of preventing and treating allergic diseases. *Clostridium butyricum* enterococci triple viable tablets are composed of lactic acid bacteria, *Clostridium butyricum* and saccharifying bacteria, which can improve various symptoms caused by intestinal flora imbalance. *Clostridium butyricum* belongs to temporary probiotics, which usually acts on the large intestine and cecum. It can not only effectively inhibit the activity of pathogenic bacteria, but also promote the growth and proliferation of bifidobacteria (Yuan and Cheng, 2017). Moreover, CB can not only adjust and supplement the normal flora, but also promote the proliferation and development of BB after mixing with BB, and inhibit the reproduction and growth of pathogens and spoilage bacteria (Ling *et al*., 2015) the combined application of the two can promote each other.

In our study, the total effective rate of group B and C is higher than that of the group A, suggesting that compared with group A of conventional treatment, the effect of combined use of probiotics is more significant, which can effectively improve the treatment effect and improve the clinical symptoms of children. This study showed that FVC and FEV1 increased more in children treated with *Clostridium butyricum* enterococci triple viable bacteria, suggesting that the combined use of *Clostridium butyricum* enterococci triple viable bacteria can better improve children's lung function. The levels of IL-2, IL-6 and TNF-α in children treated with *Clostridium butyricum* enterococci triple viable bacteria decreased more, indicating that *Clostridium butyricum* enterococci triple viable bacteria can inhibit the level of proinflammatory factors in bronchial asthma. During the recovery process, the intestinal flora stimulates the body to produce biological factors such as interferon and interleukin, and forms a defense system with the help of the circulation of the lymphatic system to make IL-2, IL-4, IL-6. The levels of proinflammatory factors such as TNF-α decreased. After 30 days of treatment, immune function indicators in group B and C were significantly higher than those in group A, suggesting that combined probiotic treatment can better enhance the immune function of children.

CONCLUSION

The combined probiotic treatment has more significant effect than the conventional treatment of BA in children, which can effectively enhance the immune function, lung function and inflammation of children and the effect of *Clostridium butyricum* enterococci triple viable bacteria is better than that of Bifidobacterium triple viable bacteria.

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