

Bifidobacterium animalis subsp. *lactis* BLa80 for preventing allergic, respiratory, and gastrointestinal diseases in young children in China: a randomized double-blind placebo-controlled trial

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Background: Respiratory, gastrointestinal, and allergic diseases can significantly affect children's physical and mental health and quality of life.

Purpose: This study aimed to assess the safety of *Bifidobacterium animalis* subsp. *lactis* BLa80, its preventive effects on morbidities related to respiratory, gastrointestinal, and allergic diseases, and its impact on the gut microbiome of children during the study period.

Methods: Healthy children aged 0–3 years were randomly assigned to an intervention group (IG; n=180) or control group (CG; n=180). Participants received probiotics or placebo for 3 months, followed by a 3-month follow-up period. Children in the IG received one oral probiotic sachet daily for 90 consecutive days starting on the first day of the intervention. Each sachet contained maltodextrin and the BLa80 strain at 5×10^9 colony-forming units (CFUs). Children in the CG received placebo sachets containing maltodextrin only. The primary outcome measure was eczema morbidity during the 6-month study period. Secondary outcomes included acute upper respiratory tract infections (URTIs) and acute tracheitis/bronchitis. Fecal gut microbiota profiles were assessed by 16S rRNA sequencing. Fecal immune biomarkers including calprotectin, human beta-defensin-2 (HBD-2), cathelicidin (LL-37), and secretory immunoglobulin A were also determined. This study was registered with the China Clinical Trial Center (ChiCTR2300074956).

Results: Per-protocol analyses were conducted of 156 and

164 subjects in the IG and CG, respectively. The morbidity rate of eczema during the 6-month period was significantly lower in the IG versus CG (intention-to-treat analysis: 26.1% [47 of 180] vs. 66.7% [120 of 180], $P < 0.01$; per-protocol analysis: 30.1% [47 of 156] vs. 73.2% [120 of 164], $P < 0.01$). Probiotic supplementation was also associated with a lower risk of URTIs (IG vs. CG: 40.3% vs. 20.7%; risk ratio [RR], 0.752; 95% confidence interval [CI], 0.653–0.866) and acute tracheitis/bronchitis (18.8% vs. 9.5%; RR, 0.897; 95% CI, 0.825–0.977). BLa80 intervention increased the relative abundance of *Bifidobacterium bifidum*, *Bifidobacterium kashiwanohense* PV20-2, *Bifidobacterium longum*, and *Enterococcus dispar* ATCC (American Type Culture Collection) 51266 while decreasing the abundance of *Bacteroides thetaiotaomicron*. Postintervention, the IG had significantly lower concentrations of LL-37 (3,509.31±587.89 pg/g vs. 3,720.82±614.90 pg/g, $P = 0.006$) and HBD-2 (202.36±56.35 pg/g vs. 222.65±56.23 pg/g, $P = 0.005$) than the CG. No serious adverse events were reported in either group.

Conclusion: The daily administration of BLa80 at 5×10^9 CFU for 3 months in children aged 0–3 years reduced the risk of eczema, URTIs, and acute tracheitis/bronchitis and beneficially altered the gut microbiome composition, fecal immune biomarkers, and functional gene composition without any adverse effects.

Key words: Probiotics, Child, Gastrointestinal microbiome, Eczema, Respiratory tract infections

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Received: 4 June 2025, Revised: 31 August 2025, Accepted: 1 September 2025

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Probiotic BLa80 for the Prevention of Normal Diseases in Young Children

Summary

To assess the safety of *Bifidobacterium animalis* subsp. *lactis* BLa80 and its preventative effects on the morbidity of respiratory, gastrointestinal, and allergic diseases and the impact on gut microbiome in children.

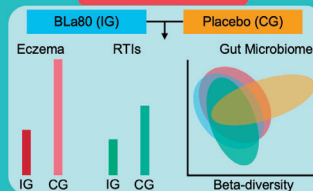
Study Design

Randomized Control Trial
Double-Blinded
Multiple centers

Population

BLa80 (IG, n=180)
Placebo (CG, n=180)
0–3 years old

Results



Conclusion

Probiotic BLa80 at a dosage of 5×10^9 CFU for three months in children can reduce the risks of eczema, URTIs, and acute tracheitis/bronchitis and beneficially altered gut microbiome composition without any adverse effects.

IG, intervention group; CG, control group; RTIs, respiratory tract infections; URTIs, upper respiratory tract infections.

Graphic abstract

Key message

Question: Can probiotic BLa80 bring long-term benefits to the health of young children?

Finding: This trial demonstrated that the daily administration of s BLa80 at 5×10^9 colony-forming units for 3 months in children can reduce the risk of eczema, upper respiratory tract infections, and acute tracheitis/bronchitis as well as beneficially improve the gut microbiome without any adverse effect.

Meaning: BLa80 can bring definite health benefits to young children.

Introduction

The World Health Organization (WHO) estimated that in 2019, approximately 5.3 million children under the age of 5 died globally from various causes. Among these fatalities, infectious diseases accounted for 49.2%, with respiratory and gastrointestinal diseases being the leading causes of mortality, representing 13.9% and 9.1% of deaths, respectively.¹⁾ Meanwhile, the incidence of allergic diseases has greatly increased worldwide over the past 3 decades, posing a significant public health challenge in most developed countries,²⁾ including China.³⁾ According to WHO estimates, approximately 433 million people globally are affected by these conditions.⁴⁾ Preventing respiratory, gastrointestinal, and allergic diseases is therefore essential for the health and development of infants and young children.

Breastfeeding is considered a cost-effective intervention for preventing these diseases and other causes of death.⁵⁾

Breast milk contains a variety of anti-infective, anti-inflammatory, and immunomodulatory factors that work synergistically to strengthen a child's immune system.^{6,7)} It also serves as a source of commensal bacteria that promote the colonization of beneficial gut microbes.⁶⁾ Probiotics can colonize the gut early in life and help establish the intestinal mucosal barrier, support immune maturation, and protect against pathogenic infections.^{8,9)} Studies have shown that probiotics can prevent and alleviate common childhood diseases by modulating the gut microbiota and immune responses,^{10–13)} including findings from our previous studies.^{14–20)} In recent years, probiotic supplementation has been increasingly recommended as an adjunct for the treatment and prevention of these diseases, with hundreds of products available on the market. These products vary in their excipients, dosages, microbial strains, and activity levels,²¹⁾ and their effects are highly strain-specific.^{22,23)}

Human milk-derived strains are considered promising probiotics, and many studies have focused on isolating such strains for applications in infant health and nutrition.⁶⁾ *Bifidobacterium animalis* subsp. *lactis* BLa80 was isolated from breast milk samples collected in plateau pastoral areas and holds national independent intellectual property rights. The strain is preserved at the China General Microbiology Culture Collection Center (CGMCC No. 22547). BLa80 has been shown to ameliorate intestinal mucosal injury, regulate intestinal flora, inhibit ERK expression, and activate the SCF/c-kit signaling pathway, potentially enhancing gastrointestinal motility in simulated microgravity rat models.²⁴⁾ It can also increase the abundance of bifidobacteria and lactobacilli in the human

intestine.²⁵⁾ In mice, BLa80 supplementation decreased serum concentrations of the proinflammatory cytokines tumor necrosis factor- α , interleukin (IL)-6, and IL-17, while 16S rRNA sequencing revealed that it increased gut microbial diversity and corrected microbiota imbalances associated with ulcerative colitis.²⁶⁾

To our knowledge, no study has thoroughly investigated whether BLa80 can enhance immunity and reduce the morbidity of respiratory, gastrointestinal, and allergic diseases in children. Therefore, we conducted a randomized, placebo-controlled, blinded trial in several communities in Chengdu City to evaluate the prophylactic effect of BLa80 on the morbidity of these common diseases in children. We hypothesized that children supplemented with BLa80 would show reduced morbidity of respiratory, gastrointestinal, and allergic diseases compared with those receiving placebo, accompanied by beneficial changes in gut microbiota and improvements in intestinal immunity.

Methods

1. Study design and participants

This study was a prospective, parallel, multicenter, blind-ed, randomized, placebo-controlled clinical intervention conducted from March 2023 to December 2024. Healthy children of both sexes, aged 0–3 years, were recruited from Chengdu Women's and Children's Central Hospital and its subcenters in Chongzhou, Xindu, Dayi, and Jinniu Maternal and Child Health Hospitals and Baoxing Center for Disease Control and Prevention in Sichuan Province.

The inclusion criteria were: (1) healthy children aged 0–3 years who were artificially fed and born at 37–42 weeks of gestation, with a birth weight between $\geq 2,500$ g and $< 4,000$ g; (2) parents and/or guardians willing to collect fecal samples during the study; (3) no diagnosed allergic diseases (including but not limited to eczema, food allergy, asthma, allergic colitis, allergic rhinitis, and hay fever) at enrollment; (4) no use of other probiotics during the trial; (5) signed written informed consent provided by the parents/guardians.

The exclusion criteria were: (1) history of asphyxia or neonatal intensive care unit hospitalization at birth; (2) congenital defects or anomalies; (3) obstetric risk factors during pregnancy such as gestational hypertension syndrome, eclampsia, gestational diabetes, cholestasis of pregnancy; (4) use of antibiotics within 2 weeks prior to enrollment; (5) diseases affecting growth and development within the month prior to enrollment, including pneumonia, severe diarrhea, severe constipation, malnutrition, gastrointestinal surgery, epilepsy, cerebral palsy, intellec-

tual disability, genetic metabolic diseases, chromosomal disorders, and genetic diseases; (6) use of experimental drugs or participation in other clinical studies prior to screening; (7) use of probiotic products containing BLa80 within 1 month before enrollment; (8) use of glucocorticoids or immunosuppressants; (9) known allergies to any components of the probiotic product used in this study; (10) any other reasons deemed inappropriate for participation by the investigators, such as factors that could affect efficacy evaluation or poor compliance.

Withdrawal criteria: (1) erroneous inclusion or misdiagnosis; (2) lack of clinical records for evaluation; (3) allergic reactions to ingredients in the probiotic product during the trial; (4) inability to ingest the probiotic through the digestive tract; (5) worsening of the participant's condition during treatment requiring admission to the pediatric intensive care unit.

The study protocol was reviewed and approved by the Ethics Committee of Chengdu Women and Children's Central Hospital (approval No.: Scientific Research Ethics Review 2023 [72]). All parents/guardians were informed of the study details and signed the informed consent form. This study was registered in the China Clinical Trial Center (registration number: ChiCTR2300074956) and was supported by the Dipro Medical Research Fund (DiCROH2304400330), which provided probiotic and placebo products, fecal microbiota 16S rRNA analysis and immune biomarkers measurement for the study free of charge.

2. Randomization and blinding

A biostatistician who was not directly involved in conducting the study used the RAND function in Excel to generate random numbers. Participants meeting the inclusion criteria were numbered sequentially and assigned to either the probiotic intervention group (IG) and the placebo control group (CG) according to the randomly generated allocation numbers. Each group included 180 children.

The probiotic and placebo sachets were similar in appearance, taste, and smell, and were packaged in identical sachets with identical labeling, differing only in the subject-specific randomization number. The parents/guardians, clinicians, laboratory personnel, data manager, and statistician remained blinded to group assignments until completion of the data analysis.

3. Sample size

Based on a similar study that used probiotics to prevent eczema in young children,²⁷⁾ the incidence of eczema was 4.2% in the IG and 11.2% in the CG after 6 months of intervention, which was considered clinically significance.

At this effect size, the required sample size for each group was calculated to be 150 participants. Allowing for an estimated 20% loss to follow-up, the sample size for both the IG and the CG was set at 180 participants each. Within each group, participants were further stratified into 3 age categories: 0–6 months, 7–12 months, and 13–36 months, with 60 participants in each stratum. The total sample size was therefore 360 participants.

4. Intervention

All eligible children were randomly assigned to the IG or CG. Children in the IG received the oral probiotic in the form of a single sachet (Wecare Probiotics Co, Ltd, Production No: SC10632050900407). The probiotic could be taken directly or mixed with warm water below 45°C, milk, rice paste, or other liquid foods. Each sachet contained maltodextrin and BLA80 strain at 5×10^9 colony-forming units (CFUs). It was taken daily for 90 consecutive days starting on the first day of the intervention. Children in the CG received placebo sachets containing only maltodextrin. The probiotic and placebo were similar in appearance, taste, and smell, and were packaged in identical sachets with identical labeling, differing only by the subject-specific randomization number. If a child vomited within 30 minutes of consuming a sachet, an additional dose was administered (with a maximum of one extra dose allowed within 4 hours). Dosing and redosing were recorded in a case report form (CRF) by the treating physicians. During the intervention and follow-up periods, any illnesses were treated by a pediatrician. If probiotics and oral antibiotics were used simultaneously, a minimum interval of 3 hours was maintained between the two.

5. Data collection

After enrollment, study staff performed assessments, recorded data in the CRF, and collected laboratory samples in accordance with the protocol. The primary outcome was the frequency of allergic eczema episodes, according to the diagnostic criteria for eczema,²⁸⁾ during the 6-month period. The secondary outcomes included episodes of respiratory diseases, defined as the occurrence of symptoms or signs related to the respiratory system within a specific period²⁹⁾; episodes of gastrointestinal diseases, defined as a group of diseases affecting the function of the gastrointestinal tract, typically presenting with symptoms such as vomiting, diarrhea, constipation, or abdominal distension^{30,31)}; biochemical indicators in stool samples, including calprotectin, human beta-defensin-2 (HBD-2), cathelicidin (LL-37), and secretory immunoglobulin A (sIgA); and gut microbiome profiles over the 3-month intervention period. Clinicians used the CRF to record the duration, frequency, therapeutic drugs used,

and related symptoms and signs of respiratory, gastro-intestinal, and allergic diseases within 6 months from the start of the probiotic intervention.

6. Sample collection and biochemical indicator analysis

Fecal samples were collected at week 0 and week 12 in fecal collection tubes containing RNAlater solution and glass beads by the parents/guardians for microbiota analysis. All tubes were sealed tightly and stored at -80°C until further analysis. Biochemical indicators in stool were assessed using human protein-specific enzyme-linked immunosorbent assay kits (Shanghai Enzyme Linked Biotechnology Co., Ltd., China) in accordance to the manufacturer's instructions. Protein concentrations were calculated from standard curves generated with known protein standards and expressed as arbitrary units per milliliter.

7. Microbiota analysis

Total genomic DNA was extracted from the fecal samples using the CTAB/SDS method with a QIAamp Fast DNA Fecal Mini Kit (Qiagen, USA) according to the manufacturer's instructions. The bacterial 16S rRNA gene V3–4 region was amplified using the TransGen AP221-02 Kit (TransGen, China) with the 16S V34 primers 341F-806R. Sequence analysis of the 16S rRNA amplicons was performed using Uparse software (v7.0.1001) and Quantitative Insights Into Microbial Ecology (QIIME, v1.9.1). Sequences with $\geq 97\%$ similarity were clustered into operational taxonomic units (OTUs), and a representative sequence from each OTU was selected for taxonomic annotation using the RDP classifier. Alpha diversity (within sample diversity) was assessed using Shannon, Simpson, Chao1, and ACE indices, while beta diversity (between-sample diversity) was analyzed using principal coordinate analysis (PCoA) based on Bray-Curtis distances. The differential abundance of taxa between groups was analyzed using the Kruskal-Wallis test. To further study the biological function of nutrient synthesis and metabolism, PICRUST2 v2.6.0 (<https://github.com/picrust/picrust2>) was used to predict the functional composition of metagenomes. Based on relevant metabolic pathways, predicted functional capabilities related to nutrient metabolism and synthesis were categorized into short-chain fatty acids (SCFAs), vitamins, and other nutrients.

8. Statistical analysis

The primary outcome analysis was performed using both the intention-to-treat (ITT) and per-protocol (PP) dataset. The Kolmogorov-Smirnov goodness-of-fit test was used to assess the normality of the data. Descriptive statistics were presented as mean±standard deviation or

median (interquartile range) for continuous variable, and as numbers and percentages for categorical variables. For between-group comparisons of normally distributed data with homogeneity of variance, the 2-independent-sample Student *t* test was applied. For skewed data, the Mann-Whitney *U* test was used. Categorical variables were compared using the chi-square test. Negative binomial regression was employed to estimate the risk ratio (RR) and 95% confidence intervals (CIs) for the duration and frequency of symptoms related to respiratory, gastrointestinal, and allergic diseases following probiotic intervention. Binary multivariate logistic regression was conducted to evaluate the effect of the intervention on the incidence rate of these diseases during the study period. All analyses were performed using the IBM SPSS Statistics 29.0.2 for Mac (IBM Co., USA).

Results

1. Baseline characteristics

A total of 360 eligible children completed all intervention procedures, and their clinical and demographic data were collected. All enrolled participants were randomized and included in the ITT analysis. A total of 180 children were assigned to the IG and 180 to the CG. No participants were lost to follow-up, and all completed the CRF. Twenty-three children were excluded from the PP analysis due to major protocol deviations, resulting in 320 participants

in the PP dataset (156 in the IG and 164 in the CG). No adverse events related to study product intake were reported during the study. Fig. 1 presents the flowchart of participant involvement. Before the intervention, there were no significant differences between the 2 groups in sex distribution, age (in months), gestational age at birth, parental education level, monthly household income per capita, registered residence, mode of delivery, or household size ($P > 0.05$ for all, Table 1).

2. Effect of probiotic intervention on the primary outcome

The morbidity of allergic eczema during the 6-month treatment and follow-up period was significantly lower in the IG compared with the CG (26.1% [47 of 180] vs. 66.7% [120 of 180], $\chi^2=59.522$, $P<0.01$) in the ITT analysis. Similarly, the PP analysis showed a significantly lower morbidity in the IG compared with the CG (30.1% [47 of 156] vs. 73.2% [120 of 164], $\chi^2=57.650$, $P<0.01$).

3. Effect of probiotics on the episode days of respiratory, gastrointestinal, and allergic diseases

During the six-month study period, the duration of common respiratory, gastrointestinal, and allergic symptoms, along with their incidence rate per 100 intervention days, are presented in Table 2. Children in the IG experienced significantly shorter duration of common symptoms including cough, runny nose, nasal congestion, fever, dry stools, choking, retching/vomiting, decreased appetite, fussing, and eczema-like skin changes compared

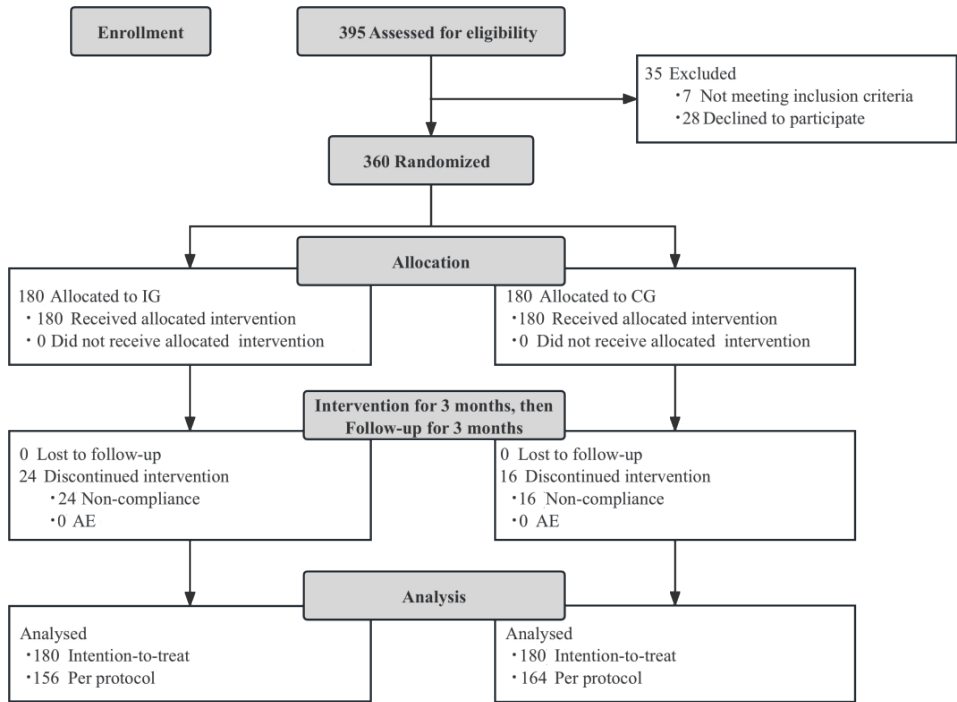


Fig. 1. Flowchart of subject enrollment and study process. AE, adverse events; CG, control group; IG, intervention group.

with those in the CG ($P<0.05$ for all). In contrast, the frequency of defecation was significantly higher in the IG ($P<0.05$).

After adjusting for gender, age in months, gestational age at birth, parental education level, monthly household income per capita, registered residence, mode of delivery, and household size, negative binomial regression analysis showed that probiotic intervention significantly reduced the risk of nasal congestion ($B=-0.635$; 95% Wald CI, -0.887

to -0.384), fever ($B=-0.339$; 95% CI, -0.611 to -0.067), choking ($B=-0.926$; 95% CI, -1.308 to -0.543), vomiting ($B=-0.805$; 95% CI, -1.206 to -0.405), decreased appetite ($B=-0.604$; 95% CI, -0.925 to -0.283), fussing ($B=-0.648$; 95% CI, -0.975 to -0.321), and eczema-like skin changes ($B=-1.577$; 95% CI, -1.815 to -1.34) and increased defecation frequency ($B=1.319$; 95% CI, 1.061-1.577) (Table 2).

Table 1. Baseline clinical and demographic data by study group

Items	CG (n=180)	IG (n=180)	t/χ^2	P value
Sex, boy	102 (56.6)	98 (54.0)	0.18	0.67
Age (mo)	12.25±9.31	13.42±9.90	-1.157	0.248
Parental education level ^{a)}			5.342	0.147
Junior high school and below	27	31		
Senior high school/technical secondary school	37	47		
Junior college/vocational college	68	47		
Bachelor's degree or above	48	55		
Household per capita income (CNY)			13.73	0.001
<5,000	4	33		
5,001–6,000	13	18		
>6,001	163	129		
Registered residence, urban	132 (73.3)	122 (67.0)	0.668	0.414
Delivery mode, vaginal	72 (40.0)	59 (32.7)	0.912	0.339
Household size, n<4	51 (28.3)	27 (15.0)	4.714	0.05

Values are presented as mean±standard deviation or number (%).

CG, control group; IG, intervention group.

^{a)}Selection of the parents' highest education level for the statistical analysis.

Boldface indicates a statistically significant difference with $P<0.05$.

Table 2. Duration of children's common symptoms by study group during the study period

Common symptoms	CG (N=180)		IG (N=180)	
	Total occurrence days	Incident rate per 100 intervention days (%) ^{a)}	Total occurrence days	Incident rate per 100 intervention days (%) ^{a)}
Cough*	1,349	4.16	1,194	3.69
Runny nose*	1,069	3.3	861	2.66
Nasal congestion*	702	2.17	325	1.00
Fever*	359	1.11	240	0.74
Loose stools	328	1.01	390	1.20
Increased defecation frequency*,b)	251	0.77	879	2.71
Colic	78	0.24	66	0.20
Dry stools*,c)	200	0.62	184	0.57
Choking*	149	0.46	60	0.19
Increased belching/bloating/anal gas	82	0.25	53	0.16
Retching/Vomiting*	119	0.37	50	0.15
Stool with milk flaps/food residues/sour odor	119	0.37	143	0.44
Reflux	37	0.11	27	0.08
Decreased appetite*,d)	224	0.69	107	0.33
Fussing*	206	0.64	106	0.33
Eczema-like skin changes*	2,429	7.50	502	1.55

CG, control group; IG, intervention group.

^{a)}A total of 32,400 study days (180 days×180 people). Symptom incidence per 100 intervention days=duration of a symptom/total intervention days×100%. ^{b)}Increased defecation frequency, daily frequency of defecation increased compared with that before the intervention as judged by caregivers/parents. ^{c)}Dry stool defined as Bristol Stool Form Scale types 1–3 stool Bristol score type. ^{d)}Decreased appetite, daily food intake at least 50% lower than before the intervention as judged by the caregivers/parents.

*Intergroup difference was statistically significant ($P<0.05$ for all).

4. Effect of probiotics on the morbidity of respiratory, gastrointestinal, and allergic diseases

Morbidity data were collected over a median of 180 (interquartile range, 166–189) days for both groups. Table 3 presents the incidences and RRs for the episodes of respiratory, gastrointestinal, and allergic diseases during the study period. Probiotic intervention was associated with a lower risk of acute upper respiratory tract infections (URTIs) [CG vs. IG, 40.3% vs. 20.7%; RR, 0.752; 95% CI, 0.653–0.866], acute tracheitis/bronchitis (18.8% vs. 9.5 %; RR, 0.897; 95% CI, 0.825–0.977), and eczema (66.7% vs. 26.1%; RR, 0.450; 95% CI, 0.359–0.563) compared with the CG. For the primary outcome of eczema after the 3-month intervention, probiotic treatment was associated with a reduced risk with marginal statistical significance (18.3% vs. 30.0%; RR, 0.611; 95% CI, 0.372–1.002).

5. Effect of probiotic intervention on growth parameters

There were no significant differences in weight, length, or head circumference between the 2 groups before the intervention ($P>0.05$). By the end of the study, all 3 growth parameters had increased compared with baseline levels, however, no significant differences were observed between the groups ($P>0.05$) (Supplementary Table 1).

6. Effect of probiotic intervention on gut microbiota

1) Effect of intervention on alpha diversity of gut microbiota

Alpha diversity was utilized to assess microbial community diversity within samples (Supplementary Fig. 1). QIIME software was employed to calculate the alpha diversity indices: observed species (Supplementary Fig. 1A) as the richness index, Shannon (Supplementary Fig. 1B) as the diversity index, Pielou_J (Supplementary Fig. 1C) as the evenness index and Pd_faith (Supplementary Fig. 1D) as the evolutionary diversity index. Before the intervention, there were no significant differences in any of the 4 alpha diversity indices between the 2 groups ($P>0.05$). After the

intervention, all 4 indices in the CG were significantly higher than those in the IG ($P<0.05$), while the indices in the IG remained stable ($P<0.05$) before and after the intervention.

2) Effect of intervention on species composition of gut microbiota

The effect of the intervention on gut microbiota composition in children is illustrated in Supplementary Fig. 2. At the phylum level (Supplementary Fig. 2A), the relative abundance of Firmicutes increased and Proteobacteria decreased in the IG. Conversely, the CG showed an increase in Bacteroidota and a decrease in Actinobacteriota. At the class level (Supplementary Fig. 2B), the IG exhibited a decrease in gamma-proteobacteria and an increase in Bacilli. In the CG, the abundances of Actinobacteria, gamma-proteobacteria, and Bacilli decreased, while Bacteroidia and Clostridia increased. At the order level (Supplementary Fig. 2C), the IG showed a decrease in Enterobacterales and an increase in Lactobacillales. In the CG, Lactobacillales, Bifidobacteriales, Enterobacterales, and Lachnospirales decreased, while Bacteroidales and Oscillospirales increased. At the family level (Supplementary Fig. 2D), the IG showed a decrease in Enterobacteriaceae and an increase in Enterococcaceae. In the CG, Bacteroidaceae and Ruminococcaceae increased, while Bifidobacteriaceae and Enterococcaceae decreased. At the genus level (Supplementary Fig. 2E), the IG had reduced abundances of *Klebsiella* and *Streptococcus*, and an increase in *Enterobacterales*. In contrast, the CG showed increased abundances of *Bacteroides* and *Ruminococcus* and decreases in *Bifidobacterium* and *Blautia*. At the species level (Supplementary Fig. 2F), the IG showed increased abundances of *Bifidobacterium kashiwanohense* PV20-2 and *Enterococcus dispar* American Type Culture Collection (ATCC) 51266, while the CG showed a decrease in *Bifidobacterium longum*.

Table 3. Morbidities related to respiratory, gastrointestinal, and allergic diseases in children by study group

Episodes	Morbidity (%)		RR	95% CI	Adjusted RR ^{a)}	95% CI
	IG (N=180)	CG (N=180)				
URTIs *	20.7	40.3	0.752	0.653–0.866	0.701	0.621–0.839
Acute tracheitis/bronchitis*	9.5	18.8	0.897	0.825–0.977	0.863	0.754–0.918
Bronchopneumonia	8.4	13.8	0.941	0.874–1.012	0.877	0.791–1.139
Diarrheal disease	21.8	17.1	1.060	0.957–1.173	0.962	0.889–1.143
Functional constipation	2.2	5.5	0.966	0.927–1.007	1.121	0.872–1.108
Functional dyspepsia syndrome	6.1	8.8	0.971	0.916–1.030	1.059	0.883–1.172
Infantile intestinal spasms	4.5	7.7	0.966	0.916–1.018	0.934	0.812–1.129
Eczema *	26.1	66.7	0.450	0.359–0.563	0.501	0.368–0.632

IG, intervention group; CG, control group; RR, risk ratio; CI, confidence interval; URTI, upper respiratory tract infection.

^{a)}Adjusted for sex, age, parental education level, household per capita income, registered residence, delivery mode, and household size.

*Significant intergroup difference ($P<0.05$).

7. Effect of intervention on beta diversity of gut microbiota

1) PCA analysis of beta diversity

Principal component analysis (PCA) of beta diversity between groups before and after the intervention (Supplementary Fig. 3) showed that axis 1 (PC1) accounted for 11.9% of the variability, while axis 2 (PC2) accounted for 10.85%. Before the intervention, samples from the IG and CG were spatially close to each other. However, after the intervention, the 2 groups were clearly separated in the PCA plot.

2) PCoA analysis of beta diversity

The PCoA plots based on Bray-Curtis, weighted Unifrac, and unweighted Unifrac distances are shown in Supplementary Fig. 4A-C). In Supplementary Fig. 4A, the PCoA based on Bray-Curtis distance showed that axis 1 (PC1) explained 13% of the variability, while axis 2 (PC2) explained 9%. There were no significant differences in PC1 component between the IG and CG prior to the intervention ($P>0.05$); however, significant differences emerged between the groups following the intervention ($P<0.05$). Specifically, PC1 increased significantly in the CG and PC2 increased significantly in both the IG and CG.

In Supplementary Fig. 4B, the PCoA based on weighted Unifrac distance revealed that PC1 accounted for 8% of the variability, while PC2 accounted for 4%. No significant differences in PC1 were observed between the IG and CG prior to the intervention ($P>0.05$). After the intervention, significant differences were observed in both PC1 and PC2 ($P<0.05$). Specifically, PC1 increased significantly in both groups, with notable intergroup differences, while PC2 increased without significant intergroup differences.

In Supplementary Fig. 4C, the PCoA based on unweighted Unifrac distance indicated that PC1 accounted for 26% of the variability, while PC2 accounted for 20%. Before the intervention, there were no significant differences in the PC1 or PC2 between the groups ($P>0.05$). After the intervention, significant differences were observed between the IG and CG ($P<0.05$), with PC1 increasing significantly in the CG.

8. Comparison of the TOP10 species-level relative abundance differences between groups

The top10 differences in species-level relative abundance between the 2 groups before and after the intervention are shown in Supplementary Fig. 5. After the intervention, the abundances of *Bifidobacterium bifidum*, *Bifidobacterium kashiwanohense* PV20-2, *Bifidobacterium longum*, and *Enterococcus dispar* ATCC 51266 were higher in the IG than in the CG, while the abundance of *Bacteroides*

thetaiotaomicron was lower in the IG compared with the CG ($P<0.05$ for all).

9. Changes of functional gene composition about nutrient metabolism in gut microbiota

To investigate the effects BLa80 intervention on physiological functions, PICRUSt was utilized to analyze and predict the composition of functional genes involved in nutrient metabolic pathways. The results showed significant differences in nutrient metabolism-related functional genes within the gut microbiota before and after BLa80 treatment, suggesting a potential impact on nutrient metabolic pathways of the gut microbiota.

As shown in Supplementary Fig. 6, after BLa80 treatment, gene expression related to carbohydrate metabolism in the gut microbiome increased significantly, whereas expressions related to vitamin B2, vitamin B7, hydrogen, butanoate, and propanoate metabolism decreased markedly ($P<0.05$). Following the intervention, children in the IG exhibited significantly higher gene expressions for vitamin C, acetate, and carbohydrate metabolism compared with those in the CG, while showing significantly lower expression for vitamin B2, vitamin B7, hydrogen sulfide, hydrogen, butanoate, and propanoate metabolism ($P<0.05$). These findings provide preliminary evidence that BLa80 supplementation can significantly influence the composition of functional genes involved in the metabolism of specific nutrients within the gut microbiota.

10. Effect of probiotic intervention on fecal immunity and inflammatory biomarkers

The present study found no significant differences in fecal levels of sIgA, LL-37, calprotectin, and HBD-2 between the 2 groups before the intervention ($P>0.05$ for all). However, after the intervention, the concentrations of LL-37 (IG vs. CG: $3,720.82\pm614.90$ pg/g vs. $3,509.31\pm587.89$ pg/g, $P=0.006$), and HBD-2 (IG vs. CG: 222.65 ± 56.22 pg/g vs. 202.35 ± 56.35 pg/g, $P=0.005$) were significantly higher in the IG compared with the CG (Supplementary Fig. 7, detailed data are provided in Supplementary Table 2).

11. Occurrence of probiotic-related adverse reactions during the study period

During the study period, no adverse reactions such as abdominal cramps, nausea, vomiting, fever, diarrhea, constipation, changes in appetite, and allergies were observed in infants and young children that could be directly attributed to the probiotic intervention.

Discussion

1. Effect of probiotic intervention on clinical outcomes

Recently, there has been growing support for the use of probiotics, which exert beneficial effects on the immune system and significantly boost host defense mechanisms.³²⁾ Probiotics can help restore healthy gut and respiratory microbiota, thereby reducing the clinical manifestations of various diseases, including respiratory, gastrointestinal, and allergic diseases.³³⁻³⁷⁾

The present study demonstrated that supplementation with BLa80 significantly reduced the duration of cough, runny nose, nasal congestion, fever, dry stools, choking, retching/vomiting, decreased appetite, fussing, and eczema-like skin changes. It also decreased the morbidity of eczema, URTIs, and acute tracheitis/bronchitis during a 3-month intervention and a 6-month observation period. These findings are generally consistent or partially consistent with the results reported in previous studies.

Reports from both caregivers and clinicians supported the positive outcomes associated with BLa80, highlighting its beneficial effects on respiratory, gastrointestinal, and immune system health. Notably, BLa80 improved health outcomes without adverse effects or hindrance on children's physical growth. No participants experienced any adverse reactions, such as abdominal cramps, nausea, vomiting, fever, diarrhea, or allergic reactions that could be directly linked to the use of BLa80. Although our findings align with the clinical outcomes of many other studies, it is important to note that strain-specific effects are closely tied to the characteristics of individual strains.^{38,39)} Several similar intervention studies in children using different strains have found no significant effects on the incidence of respiratory, gastrointestinal, or allergic diseases.⁴⁰⁻⁴²⁾ This highlights substantial heterogeneity among studies with respect to probiotic strains, dosages, and treatment durations. As a result, it remains unclear which specific probiotics, along with their optimal dosages and regimens, are most effective in preventing these diseases.

While BLa80 supplementation did not significantly affect the incidence of gastrointestinal diseases in this study, it did shorten the duration of related symptoms. Since the sample size calculation was based on eczema incidence as the primary outcome, and the incidence of gastrointestinal diseases was much lower than that of eczema, the study may have been underpowered to detect a significant effect for this outcome. Future studies with larger sample sizes may yield significant results.

2. Effect of probiotic intervention on gut microbiota

In the present study, we also investigated alterations in gut microbiota composition within the intestinal micro-

ecology and examined how BLa80 influenced this ecosystem. These changes are closely associated with both the preventive effects and clinical progression of respiratory, gastrointestinal, and allergic diseases.

Intervention with *Bifidobacterium animalis* subsp. *lactis* has been shown to have extensive beneficial impacts on the intestinal microecology of children, as demonstrated in multiple intervention studies. One of our previous studies revealed that administering BLa80 at a dose of 5×10^9 CFU/day to children with diarrhea increased the abundances of *Bifidobacterium breve* and *Collinsella aerofaciens*, improved alpha diversity, and was associated with better clinical outcomes.²⁵⁾ Another of our studies demonstrated that supplementation with XLTG11 at a dose of 1×10^{10} CFU/day in children with diarrhea resulted in a beneficial shift toward dominance of *Bifidobacterium longum*, *Bifidobacterium breve*, and *Escherichia coli*.¹⁸⁾ Similarly, a study performed in Uganda found that supplementation with *Lactobacillus rhamnosus* GG and *Bifidobacterium animalis* subsp. *lactis* BB-12 in children with severe acute malnutrition did not influence β -diversity at discharge or follow-up, but did increase the number of observed species.⁴³⁾ Van Rossum et al.⁴⁴⁾ also reported that a multi-strain probiotics containing 2 *Bifidobacterium animalis* subsp. *Lactis* strains did not reduce the incidence of multidrug-resistant organisms at day 30 of life in preterm infants, but modulated the microbiome toward eubiosis. Moreover, other studies on *Bifidobacterium animalis* subsp. *Lactis* strains BB-12⁴⁵⁾ and CP-9⁴⁶⁾ demonstrated increased relative abundance of *Bifidobacterium animalis*, the order Lactobacillales, and the genus *Bacteroides* in children after supplementation.

In our current study, BLa80 supplementation increased the abundances of *Bifidobacterium bifidum*, *Bifidobacterium kashiwanohense* PV20-2, *Bifidobacterium longum*, and *Enterococcus dispar* ATCC 51266, which coincided with improvements in common symptoms and reduced morbidity of eczema, URTIs, and acute tracheitis/bronchitis.

Although both groups experienced various respiratory, gastrointestinal, and allergic conditions during follow-up, the disease profiles and symptom durations differed notably between them. This discrepancy may be partially attributed to distinct alterations in intestinal microbiome composition. *Bifidobacterium* plays a pivotal role in the development of gut immunity and overall health in children, particularly during early growth.⁴⁷⁾ It is among the first microbes to colonize the human gastrointestinal tract and is widely recognized for its beneficial effects, including pathogen defense and immune regulation. Interestingly, we observed that the relative abundance of *Bacteroides thetaiotaomicron* increased in the CG

after placebo intervention but decreased in the IG. *Bacteroides thetaiotaomicron* is a major component of the human intestinal microflora and can influence host physiology by modulating gene expression related to mucosal barrier reinforcement, immune regulation, and nutrient metabolism.^{48,49} Some studies have found that interventions such as probiotics, nutritional supplements, dietary changes, and specific foods can increase its abundance,⁵⁰⁻⁵² and that this increase is often positively associated with disease recovery or health maintenance. However, we do not yet have sufficient evidence to fully explain the contrasting changes observed in our study. Possible explanations included: (1) *Bacteroides thetaiotaomicron* has a strong ability to regulate intestinal microecological homeostasis. Differences in intestinal immune and inflammatory status between the CG and IG may have triggered self-regulation of intestinal flora. (2) The relative decrease in other beneficial bacterial species in the CG may have contributed to a proportional increase in *Bacteroides thetaiotaomicron*. More research is needed to confirm these findings and clarify the underlying mechanisms.

3. Effect of probiotic intervention on fecal biochemical parameters

Although the effects of BLA80 on intestinal inflammation indicators observed in this study are not entirely consistent with findings from studies on other strains of the same species,^{17,53,54} they remain largely in agreement with the majority of existing research results.^{15,55-57}

After the intervention, fecal levels of HBD-2 and LL-37 were significantly higher in the IG compared with the CG, which may be attributed to several factors. HBD-2 pertains to the beta-defensin family and is a small cationic peptide with broad-spectrum antibacterial activity, capable of effectively targeting bacteria, fungi, and viruses.⁵⁸ LL-37 is also an antimicrobial peptide produced by neutrophils and belonging to the cathelicidin family. It possesses multiple biological functions, including antibacterial, anti-inflammatory, and immunomodulatory effects.⁵⁹ In this study, children in the IG experienced significantly shorter durations of common disease symptoms and lower incidences of URTIs, acute tracheitis/bronchitis, and eczema compared with those in the CG. These findings suggest that the intestinal anti-inflammatory status in the IG may have been more effective than in the CG, leading to higher levels of protective anti-inflammatory indicators such as HBD-2 and LL-37. In other words, elevated levels of these protective markers may, to some extent, reflect a lower inflammatory state in the intestine. It should also be noted that the initial sample size was calculated based on clinical outcomes. Therefore, the sample size for fecal biomarker analysis may have been insufficient to detect

significant postintervention differences between the groups for certain markers, such as sIgA and calprotectin, which might be more sensitive than others.

4. Effect of probiotic intervention on functional gene composition related to nutrient metabolism in the gut microbiota

According to functional gene prediction analysis, BLA80 treatment enriched/induced the expression of functional genes involved in metabolic pathways of the gut microbiome, including, but not limited to, those related to vitamins (vitamin C, vitamin B2, vitamin B7), carbohydrates, hydrogen sulfide, hydrogen, and SCFAs (acetate, butanoate, and propanoate). Hydrogen and hydrogen sulfide,^{60,61} produced through intestinal microbiota metabolism, can influence children's health in multiple ways. These effects span functional gastrointestinal diseases, growth and development, microbiota composition, inflammation, nutrient absorption, and mental health, encompassing both protective roles and potential risks. SCFAs, as key mediators of host-microbiota interactions, play multidimensional roles in immunity, metabolism, and neurodevelopment in children. Dietary fiber intake and probiotic supplementation have been shown to optimize SCFA levels, offering a promising strategy to support children's health.^{62,63} One of our previous trials²⁵ also demonstrated that BLA80 supplementation can enrich the expression of other functional genes, including those involved in DNA repair and recombination proteins, purine metabolism, ribosome, peptidases, pyrimidine metabolism, chromosome, and amino acid related enzymes. Collectively, these findings suggest that BLA80 may exert protective effects on children's health and reduce the risk of respiratory, gastrointestinal, and allergic diseases, by regulating the metabolic pathways of various microbiota-derived products. However, it is imperative to emphasize that these hypotheses, including the observed reductions in certain gene expressions, require further validation through well-designed animal and *in vitro* studies to confirm their accuracy and elucidate the precise mechanisms of BLA80's action.

5. Limitation analysis

Firstly, as outlined in the initial study design, the sample size was calculated based on the relatively high clinical incidence of eczema. Consequently, the statistical power may have been insufficient to detect significant effects for diseases with lower incidence rates or for certain fecal biochemical indicators. Secondly, administering a single dose of BLA80 at 5×10^9 CFU/day limited our ability to investigate the optimal dose-response relationship of this strain in preventing respiratory, gastrointestinal, and allergic diseases. Lastly, the effects of exogenous

probiotic supplementation on intestinal function can be influenced by various potential confounding factors, such as mode of delivery, feeding method, dietary pattern, and living environment. Although some of these factors were accounted for, it is possible that the intervention effects of BLA80 may still have been partially affected. Future studies should consider increasing the sample size and extending the observation period to enhance the reliability and generalizability of the findings. Additionally, further investigation into the long-term effects of BLA80 on children's health, particularly its role in regulating functional gene metabolic pathways, would hold significant value.

6. Conclusion

To conclude, our study found no adverse effects associated with BLA80 supplementation during the observation period, supporting its safety for young children aged 0–3 years. Administering BLA80 at a dosage of 5×10^9 CFU per day for 3 months reduced the risk of eczema, URTIs, and acute tracheitis/bronchitis, while also beneficially modulating gut microbiome composition, fecal immune function, and functional gene composition without any adverse effects.

Footnotes

Supplementary materials: Supplementary Tables 1, 2 and Supplementary Figs. 1–7 are available at <https://doi.org/10.3345/cep.2025.01256>.

Conflicts of interest: No potential conflict of interest relevant to this article was reported.

Funding: This was supported by the Dipro Medical Research Fund (DiCROH2304400330).

Acknowledgments: We express our gratitude to all the parents or primary caregivers and their children for participating in this study. We also thank the healthcare workers involved in the field trial, whose names are not individually mentioned, for their diligent assistance. Raw 16S rRNA gene sequences for all fecal samples used in this study have been deposited in the National Center for Biotechnology Information BioProject database with the BioProject ID PRJNA1233578. (<http://www.ncbi.nlm.nih.gov/bioproject/1233578>).

Author contribution: Conceptualization: KC; Data curation: KC; Formal analysis: KC; Funding acquisition: KC; Methodology: KC; Writing - original draft: KC, XZ, KZ, JZ,

SJ, YN, PY, NH, HC, YC, YF, ZF, WJ, CL; Writing - review & editing: KC, XZ, KZ, JZ, SJ, YN, PY, NH, HC, YC, YF, ZF, WJ, CL

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How to cite this article: Chen K, Zhang X, Zeng K, Zhong J, Jin S, Nie Y, et al. *Bifidobacterium animalis* subsp. *lactis* BLA80 for preventing allergic, respiratory, and gastrointestinal diseases in young children in China: a randomized double-blind placebo-controlled trial. *Clin Exp Pediatr* 2026;69:150-62.