Pediatr Pract Res. 2026;14(1):1975980
DOI: 10.21765/pprjournal.1975980

ORIGINAL ARTICLE
ORİJİNAL ARAŞTIRMA

The Effect of a Probiotic Containing *Lacticaseibacillus rhamnosus* and *Bifidobacterium longum* subsp. infantis on Clinical Outcomes in Children Aged 2–10 Years with Recurrent Upper Respiratory Tract Infections: A Multicenter Cohort Study

Tekrarlayan Üst Solunum Yolu Enfeksiyonu Olan 2-10 yaş Çocuklarda *Lacticaseibacillus rhamnosus* ve *Bifidobacterium longum* subsp. İnfantis İçeren Probiyotik Kullanımının Klinik Sonuçlar Üzerine Etkisi: Çok Merkezli Kohort Çalışması

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ABSTRACT

Aim: To evaluate the effects of a probiotic combination containing *Lacticaseibacillus rhamnosus* GG and *Bifidobacterium longum* subsp. infantis on the frequency of upper respiratory tract infections (URTI), antibiotic use, school absenteeism, and gastrointestinal symptoms and stool-related indicators in children aged 2–10 years with recurrent URTI.

Material and Method: This retrospective, observational, controlled, multicenter cohort study was conducted across nine pediatric outpatient clinics in Türkiye. The study enrolled 200 children aged 2–10 years with a history of at least three URTI episodes in the preceding six months. Based on documented probiotic exposure in the medical records, children were classified into two groups: Group 1 (n=100) had received a daily six-drop *Lacticaseibacillus rhamnosus* GG+*Bifidobacterium longum* subsp. infantis probiotic combination in addition to standard care, whereas Group 2 (n=100) had received standard care alone. Clinical parameters were assessed at baseline (V0), month 3 (V1), and month 6 (V2). Mann-Whitney U and chi-square tests were used for between-group comparisons; Wilcoxon signed-rank test was applied for within-group longitudinal analysis.

Results: In Group 1, the mean number of URTI episodes decreased from 4.05±0.95 at V0 to 1.39±1.04 at V2 (p<0.001; 65.7% reduction), whereas no significant change was observed in the control group (2.06→2.09; p=0.592). Antibiotic use declined by 58.7%, school absenteeism by 70.9%, and febrile days by 55.5% in Group 1 (all p<0.001). More pronounced clinical improvement was observed in subgroups of children born by cesarean section, those who received no breast milk, and those with a history of frequent antibiotic exposure. Product adherence was 97.5±4.4% and no adverse events were reported.

Conclusion: The *Lacticaseibacillus rhamnosus* GG+*Bifidobacterium longum* subsp. infantis probiotic combination significantly reduces both infection burden and antibiotic use in children with recurrent URTI, with particularly marked clinical efficacy in cesarean-born children, those with minimal breast milk exposure, and those with high prior antibiotic use.

Keywords: Probiotics, upper respiratory tract infection, *Lacticaseibacillus rhamnosus*, *Bifidobacterium longum* subsp. infantis, child

ÖZ

Amaç: Tekrarlayan üst solunum yolu enfeksiyonu (ÜSYE) öyküsü olan 2-10 yaş arası çocuklarda *Lacticaseibacillus rhamnosus* GG ve *Bifidobacterium longum* subsp. infantis içeren probiyotik kombinasyonunun ÜSYE atak sıklığı, antibiyotik kullanımı, okul devamsızlığı ve gastrointestinal mikrobiyota parametreleri üzerine etkisini değerlendirmektir.

Gereç ve Yöntem: Bu retrospektif, gözlemsel, kontrol gruplu, çok merkezli kohort çalışması dokuz farklı çocuk sağlığı ve hastalıkları kliniğinde yürütüldü. Çalışmaya son altı ayda en az üç ÜSYE atağı geçirmiş, 2–10 yaş arasında 200 çocuk dahil edildi. Çocuklar, tıbbi kayıtlarında belgelenen probiyotik kullanım durumuna göre iki grupta sınıflandırıldı: Grup 1 (n=100) standart takibe ek olarak günde altı damla *Lacticaseibacillus rhamnosus* GG+*Bifidobacterium longum* subsp. infantis içeren probiyotik kombinasyonu kullanmış olan çocuklardan, Grup 2 (n=100) ise yalnızca standart takip altında izlenmiş çocuklardan oluşuyordu. Başlangıç (V0), 3. ay (V1) ve 6. ay (V2) vizitlerinde klinik parametreler değerlendirildi. Gruplar arası karşılaştırmada Mann-Whitney U ve ki-kare testleri, grup içi zamana bağlı değişim analizinde Wilcoxon işaret-sırası testi kullanıldı.

Bulgular: Grup 1'de ÜSYE atak sayısı V0'da 4,05±0,95 iken V2'de 1,39±1,04'e geriledi (p<0,001; %65,7 azalma). Kontrol grubunda anlamlı değişim gözlenmedi (2,06→2,09; p=0,592). Grup 1'de antibiyotik kullanım sıklığı %58,7, okul devamsızlığı %70,9 ve ateşli gün sayısı %55,5 oranında azaldı (tüm p<0,001). Sezaryen ile doğan çocuklarda, hiç anne sütü almayanlarda ve yoğun antibiyotik kullananlardan oluşan alt gruplarda daha belirgin klinik iyileşme saptandı. Ürün kullanım uyumluluğu %97,5±4,4 olup hiçbir çocukta yan etki bildirilmedi.

Sonuç: *Lacticaseibacillus rhamnosus* GG+*Bifidobacterium longum* subsp. infantis probiotik içeren probiyotik kombinasyonu, tekrarlayan ÜSYE geçiren çocuklarda hem enfeksiyon yükünü hem de antibiyotik kullanımını anlamlı ölçüde azaltmakta; özellikle sezaryen doğan, az anne sütü alan ve çok antibiyotik maruziyeti yaşayan çocuklarda belirgin klinik etkinlik sergilemektedir.

Anahtar Kelimeler: Probiyotik, üst solunum yolu enfeksiyonu, *Lacticaseibacillus rhamnosus*, *Bifidobacterium longum* subsp. infantis, çocuk

Cite this article as / Bu makaleye atıf için: Arıca V, Kemalbay HD, Yıldız Özkan H, et al. The Effect of a Probiotic Containing *Lacticaseibacillus rhamnosus* and *Bifidobacterium longum* subsp. infantis on Clinical Outcomes in Children Aged 2–10 Years with Recurrent Upper Respiratory Tract Infections: A Multicenter Cohort Study. Pediatr Pract Res. 2026;14(1):1975980. doi: 10.21765/pprjournal.1975980

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Received/Başvuru Tarihi: 21.06.2026
Accepted/Kabul Tarihi: 18.09.2026

INTRODUCTION

Upper respiratory tract infections (URTIs) represent the most common infectious conditions in childhood and remain a leading cause of primary care visits and antibiotic prescriptions worldwide (1,2). Children of preschool age experience an average of six to eight URTI episodes per year, and recurrent infections impose substantial individual and societal burdens, including school absenteeism, parental productivity loss, and unnecessary antibiotic exposure (3,4). The frequent and often unwarranted use of antibiotics in children with recurrent URTIs contributes to persistent dysbiosis of the gut microbiota and accelerates the emergence of antimicrobial resistance (5).

In recent years, the concept of the gut–lung axis has highlighted the regulatory role of the gastrointestinal microbiota in respiratory immune defense (6). Through this axis, gut dysbiosis compromises mucosal barrier integrity, disrupts T regulatory cell homeostasis, and promotes pro-inflammatory cytokine release, thereby increasing susceptibility to respiratory tract infections (7,8). Cesarean delivery, limited or absent breastfeeding, and early-life antibiotic exposure have been identified as the primary determinants that perpetuate this dysbiotic state (9,10).

Probiotics have attracted growing interest for their potential to restore host microbiota and strengthen both gastrointestinal and respiratory immunity. *Lacticaseibacillus rhamnosus* GG, one of the most extensively studied probiotic strains worldwide, has a robust clinical evidence base demonstrating reductions in diarrhea duration and respiratory infection episodes in children (11,12). *Bifidobacterium longum* subsp. infantis is a dominant early-life microbiota strain that metabolizes human milk oligosaccharides (HMOs), reinforces intestinal epithelial barrier function, and modulates mucosal immune responses (13,14). The synergistic combination of these two strains has been proposed to exert more pronounced effects on both gastrointestinal and immunological parameters compared with single-strain use (15, 16).

Nevertheless, controlled multicenter studies evaluating the *Lacticaseibacillus rhamnosus* GG+*Bifidobacterium longum* subsp. infantis probiotic combination specifically in children with recurrent URTIs remain scarce. This study aimed to assess the effects of this probiotic combination on infection frequency, antibiotic use, school absenteeism, and gastrointestinal symptoms and stool-related indicators using real-world data using real-world data, and to identify differential responses according to mode of delivery, feeding history, and prior antibiotic exposure.

MATERIAL AND METHOD

Study Design and Patients

This was a retrospective, observational, controlled, multicenter cohort study conducted across nine pediatric outpatient clinics. Two hundred children aged 2–10 years with a history of at least three URTI episodes in the preceding six months were enrolled. Exclusion criteria included primary immunodeficiency, chronic pulmonary disease, active malignancy, immunosuppressive therapy, and probiotic use within the preceding two weeks.

Data Sources and Retrospective Data Collection

All data were obtained retrospectively from existing hospital medical records and electronic patient files. V0, V1, and V2 represented documented clinical encounters corresponding approximately to baseline, month 3, and month 6, respectively; these visits were not scheduled prospectively for the purposes of the present study. Clinical outcomes, symptom scores, antibiotic use, emergency visits, school absenteeism, and gastrointestinal variables were extracted from information documented during routine clinical follow-up.

Availability of complete clinical records for the baseline, approximately 3-month, and approximately 6-month follow-up assessments was required for inclusion in the analysis.

Ethical Approval

The study was approved by the İstanbul Prof. Dr. Cemil Taşcıoğlu City Hospital Scientific Research Ethics Committee on June 15, 2026 (Decision No: 274) and was conducted in accordance with the principles of the Declaration of Helsinki. Because the study was retrospective and based on anonymized medical records, no additional intervention was performed. No additional informed consent was obtained for the present retrospective analysis, and all data were anonymized before analysis.

Study Groups and Probiotic Exposure

Group classification was based on documented probiotic exposure in the medical records. Children were included in Group 1 if probiotic supplementation had been recommended by the treating physician during routine clinical care, whereas children who had received standard care without probiotic supplementation were included in Group 2. The treatment decision was made by the treating physician as part of routine clinical practice and was not determined by the investigators for research purposes. Children in Group 1 had received a daily six-drop dose (0.27 mL) of the *Lacticaseibacillus rhamnosus* GG+*Bifidobacterium longum* subsp. infantis probiotic combination in addition to standard care for six months.

The treating clinicians were not blinded to probiotic exposure because treatment had occurred as part of routine clinical care. The present study involved retrospective extraction of data from existing medical records and did not involve prospective blinded outcome assessment.

Clinical Variable Definitions

The respiratory and gastrointestinal symptom scores were retrospectively obtained from routine clinical records. Cough, nasal discharge, and gas/bloating severity were recorded using a 4-point ordinal clinical scale: 0=none, 1=mild, 2=moderate, and 3=severe. Stool consistency was evaluated using the Bristol Stool Form Scale. These symptom ratings represented routine clinical assessments rather than a formally validated disease-specific composite scoring system. The symptom scores were documented by the treating pediatrician during routine clinical visits based on clinical assessment and information provided by the parent or caregiver.

Assessment and Outcomes

Clinical parameters were assessed at baseline (V0), month 3 (V1), and month 6 (V2). The primary outcome was the number of URTI episodes. Secondary outcomes included antibiotic use, emergency visits, school absenteeism, febrile days, respiratory symptom scores, and gastrointestinal symptoms and stool-related indicators, including Bristol stool scores, stool regularity, gas/bloating scores, and post-antibiotic diarrhea.

Statistical Analysis

An a priori sample size calculation was performed using G*Power version 3.1. Assuming a moderate effect size (Cohen's d=0.50), a two-sided alpha level of 0.05, statistical power of 80%, and a 1:1 allocation ratio between the two groups, the required sample size was estimated for comparison of two independent groups. Considering the possibility of incomplete or ineligible records in this retrospective study, the target sample size was increased to 100 children per group, yielding a total planned sample of 200 participants. Data were analyzed using SPSS version 26.0. Mann-Whitney U and chi-square tests were used for between-group comparisons, the Wilcoxon signed-rank test for paired comparisons, and the Kruskal-Wallis test for subgroup analyses. Statistical significance was set at $p<0.05$.

No formal adjustment for multiple comparisons was applied to the exploratory subgroup analyses; therefore, subgroup findings should be interpreted as hypothesis-generating.

RESULTS

Demographic Characteristics and Baseline Comparability

A total of 200 children (100 per group) were included in the study. Because the retrospective cohort was defined using complete-record eligibility, all 200 included patients had evaluable baseline and 6-month data; therefore, no additional loss to follow-up occurred within the final analytic cohort. As shown in **Table 1**, the two groups were comparable at baseline in terms of age, sex distribution, weight and height percentiles, mode of delivery, breastfeeding duration, and prior antibiotic use. in terms of age (Group 1: 5.93±2.49 vs Group 2: 5.53±2.44 years; p=0.255), sex distribution (female: 49% vs 52%; p=0.777), weight and height percentiles, mode of delivery, breastfeeding duration, and prior antibiotic use (all p>0.05). Gas/bloating scores (1.28±0.85 vs 0.98±0.93; p=0.003) and stool regularity scores (3.03±1.16 vs 2.38±1.28; p<0.001) were significantly higher in Group 1 at baseline, .indicating a greater burden of gastrointestinal symptoms and stool-related abnormalities at study entry. The distribution of feeding patterns during the first six months of life did not differ significantly between the groups (p=0.115). However, baseline URTI burden was higher in Group 1 than in Group 2, as detailed in **Table 2**.

Primary Outcome: URTI Episode Frequency

As presented in **Table 2** and **Figure 1**, the mean number of URTI episodes in Group 1 decreased significantly from 4.05±0.95 at baseline (V0) to 2.27±0.86 at month 3 (V1) and 1.39±1.04 at month 6 (V2) (p<0.001 for all comparisons; Wilcoxon signed-rank test), corresponding to a 65.7% reduction over six months. In contrast, no statistically significant change was observed in Group 2 (2.06→2.09; p=0.592). At V2, Group 1 had significantly fewer URTI episodes than Group 2 (p<0.001), while groups did not differ at V1 (p=0.607).

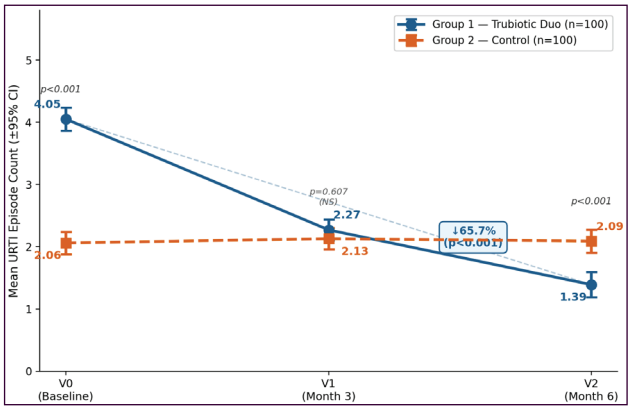

Figure 1. URTI episode frequency over time by group. Error bars represent 95% confidence intervals. The Trubiotic Duo group (Group 1) demonstrated a significant 65.7% reduction from baseline to month 6 (p<0.001), whereas no significant change was observed in the control group (p=0.592). AD: Not significant; GA: Confidence interval.

Table 1. Baseline Demographic and Clinical Characteristics by Group

Characteristic	Group 1 Trubiotic Duo (n=100)	Group 2 Control (n=100)	p value	Statistic	Method
DEMOGRAPHIC FEATURES					
Age, mean±SD (years)	5.93±2.49	5.53±2.44	0.255 (NS)	U=4553	MWU
Sex, Female, n (%)	49 (49%)	52 (52%)	0.777 (NS)	χ^2=0.08	Chi-sq
Weight percentile, mean±SD	3.06±1.51	3.19±1.35	0.294 (NS)	U=4670	MWU
Height percentile, mean±SD	3.82±1.78	3.65±1.70	0.412 (NS)	U=4712	MWU
MODE OF DELIVERY					
Cesarean section (SC), n (%)	76 (76%)	72 (72%)	0.629 (NS)	χ^2=0.23	Chi-sq
Normal vaginal delivery, n (%)	24 (24%)	28 (28%)	0.629 (NS)	χ^2=0.23	Chi-sq
FEEDING (First 6 months)					
Breast milk only, n (%)	16 (16%)	28 (28%)	0.115 (NS)	χ^2=4.32	Chi-sq
Breast milk+formula, n (%)	49 (49%)	44 (44%)	—	—	—
Formula only, n (%)	35 (35%)	28 (28%)	—	—	—
Breast milk duration, mean±SD (months)	5.31±4.54	6.67±5.08	0.052 (NS)	U=4190	MWU
ANTIBIOTIC USE (Last 6 months)					
Antibiotic count, mean±SD	4.41±0.99	4.32±0.68	0.538 (NS)	U=4825	MWU
GASTROINTESTINAL SYMPTOMS AND STOOL-RELATED INDICATORS (Baseline)					
Bristol stool score, mean±SD	2.80±1.18	3.05±0.98	0.190 (NS)	U=4318	MWU
Gas/bloating score (0–3), mean±SD	1.28±0.85	0.98±0.93	0.003**	U=4050	MWU
Stool regularity score (1–5), mean±SD	3.03±1.16	2.38±1.28	<0.001***	U=3704	MWU
Post-antibiotic diarrhea, n (%)	47 (47%)	35 (35%)	0.114 (NS)	χ^2=2.52	Chi-sq

NS: Not significant (p>0.05); MWU: Mann-Whitney U test; Chi-sq: Chi-square test; *** p<0.001, ** p<0.01, * p<0.05. Values are presented as mean±SD or n (%).

Table 2. Clinical Outcomes at V0, V1, and V2 by Group

Clinical Outcome / Visit	Group 1 V0	Group 1 V1	Group 1 V2	Group 2 V0	Group 2 V2	Between-group p (V2)
Urti episode count - primary outcome	4.05±0.95	2.27±0.86	1.39±1.04	2.06±0.92	2.09±0.95	<0.001***
Antibiotic use	4.43±1.16	2.67±1.25	1.83±1.03	2.32±0.94	2.18±0.98	0.017*
Emergency visits	4.49±1.60	3.20±1.12	1.68±0.86	1.62±0.91	1.61±0.86	0.933 (NS)
School absenteeism (days)	19.20±9.16	11.08±5.69	5.58±3.85	3.65±2.36	3.39±2.26	<0.001***
Febrile days	8.90±3.43	6.82±3.19	3.96±2.88	3.30±2.41	3.02±2.27	<0.001***
Nasal discharge score (0–3)	1.87±0.79	1.49±0.73	1.17±0.53	1.45±0.72	1.40±0.65	0.014*
Cough score (0–3)	2.02±0.74	1.66±0.79	1.28±0.59	1.41±0.79	1.64±0.92	0.011*

Wilcoxon signed-rank test (Group 1): V0→V1 p<0.001 and V0→V2 p<0.001 for all primary/secondary outcomes.

MWU: Mann-Whitney U test (between-group); Wilcoxon signed-rank test (within-group paired comparisons). *** p<0.001, ** p<0.01, * p<0.05; NS: not significant. Green values indicate V2 values in Group 1.

Secondary Outcomes

All secondary outcomes are summarized in **Table 2**. As illustrated in **Figure 2**, Trubiotic Duo use was associated with clinically and statistically significant reductions in antibiotic use (−58.7%; p<0.001), school absenteeism (−70.9%; p<0.001), febrile days (−55.5%; p<0.001), nasal discharge scores (−37.4%; p<0.001), and cough scores (−36.6%; p<0.001) over the six-month study period. Emergency visit frequency decreased significantly from V0 to V1 (p<0.001); however, the between-group difference at V2 was not statistically significant (p=0.933).

Subgroup Analyses within Group 1

Detailed subgroup analyses are presented in **Table 3** and **Figures 3–7**.

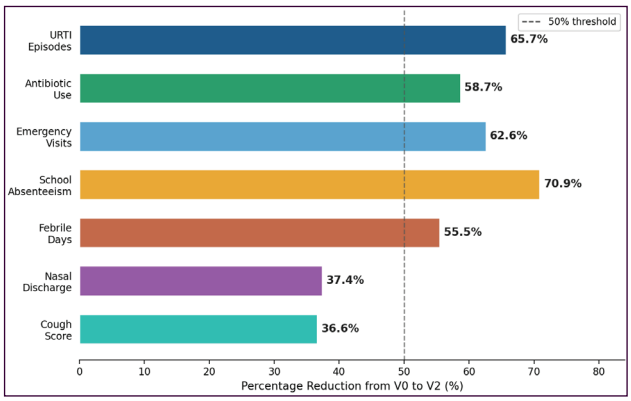

Figure 2. Percentage reduction in all clinical outcomes from baseline (V0) to month 6 (V2) in Group 1 (Trubiotic Duo). School absenteeism showed the greatest reduction (70.9%), followed by antibiotic use (58.7%) and febrile days (55.5%). All reductions were statistically significant (p<0.001).

Table 3. Subgroup Analyses within Group 1 — Clinical Outcomes by Mode of Delivery, Feeding Pattern, Breastfeeding Duration, and Prior Antibiotic Use

Subgroup	n	V0 URTI	V1 URTI	V2 URTI	V0 AB	V2 AB	Gas/Bloating Score
MODE OF DELIVERY							
Cesarean (SC)	76	4.22	2.41	1.51	4.46	1.74	1.50
Normal delivery (NVD)	24	3.50	1.83	1.00	4.33	2.12	0.58
p value (SC vs NVD)	—	0.001**	0.005**	0.037*	0.873	0.168	<0.001***
FEEDING (First 6 months)							
Breast milk only (AS)	16	3.19	1.94	1.12	4.19	1.81	0.25
Breast milk+formula	49	3.69	2.04	1.33	4.41	1.92	1.24
Formula only	35	4.94	2.74	1.60	4.57	1.71	1.80
p value	—	<0.001***	<0.001***	0.263	0.496	0.721	<0.001***
BREASTFEEDING DURATION							
0 months (no BM)	25	5.20	2.88	1.44	4.52	1.44	1.92
1–6 months	52	3.77	2.10	1.38	4.42	2.08	1.25
7+ months	23	3.43	2.00	1.35	4.35	1.70	0.65
p value	—	<0.001***	<0.001***	0.927	0.792	0.061	<0.001***
PRIOR ANTIBIOTIC USE (last 6m)							
3 courses	19	3.32	2.11	1.11	4.11	2.05	0.11
4 courses	38	3.79	1.97	1.37	4.37	1.79	1.26
5 courses	26	4.19	2.31	1.46	4.58	1.88	1.58
6+ courses	17	5.24	3.06	1.65	4.71	1.59	2.18
p value	—	<0.001***	0.001**	0.407	0.329	0.626	<0.001***

MWU: Mann-Whitney U test; Kruskal-Wallis test for ≥3 subgroups. *** p<0.001, ** p<0.01, * p<0.05; NS: not significant. URTI: upper respiratory tract infection; AB: antibiotic; AS: breast milk; SC: cesarean; NVD: normal vaginal delivery.

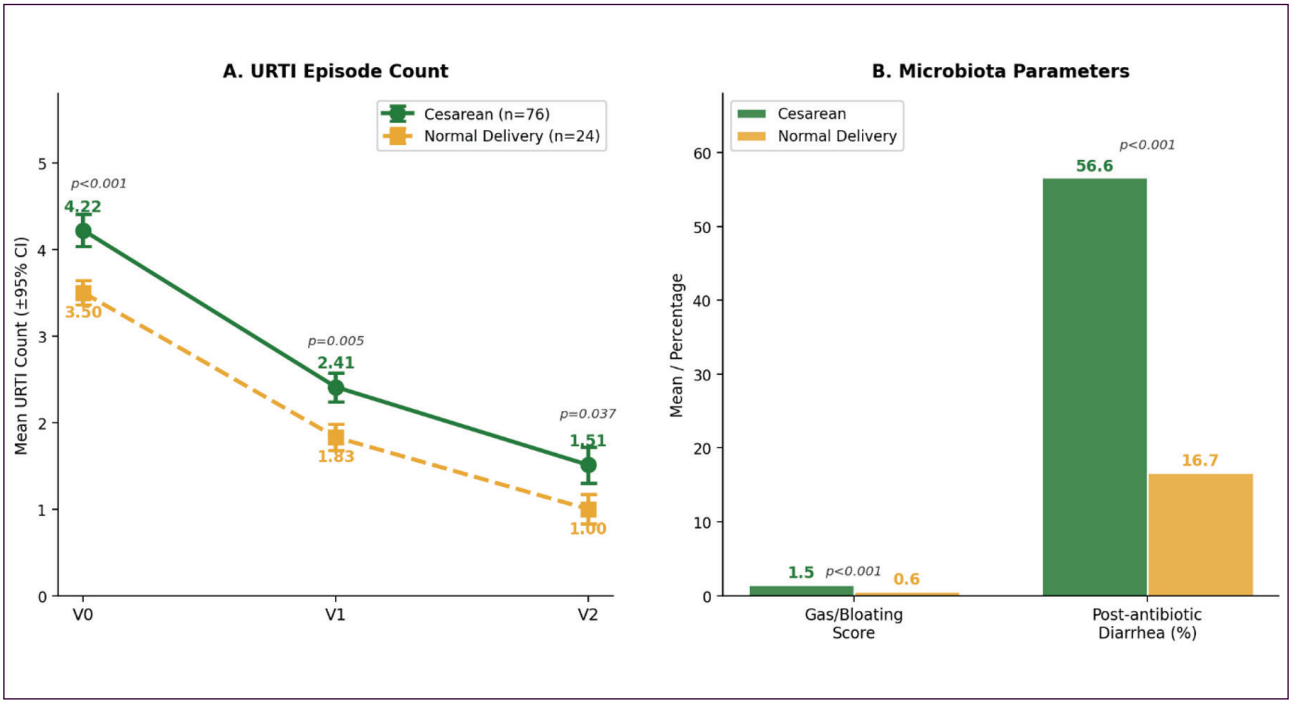

Figure 3. (A) URTI episode trends by mode of delivery in Group 1. (B) Gastrointestinal clinical indicators (gas/bloating score and post-antibiotic diarrhea rate) by mode of delivery. Cesarean-born children exhibited significantly higher gastrointestinal symptom burden (p<0.001). SC: cesarean section; NVD: normal vaginal delivery.

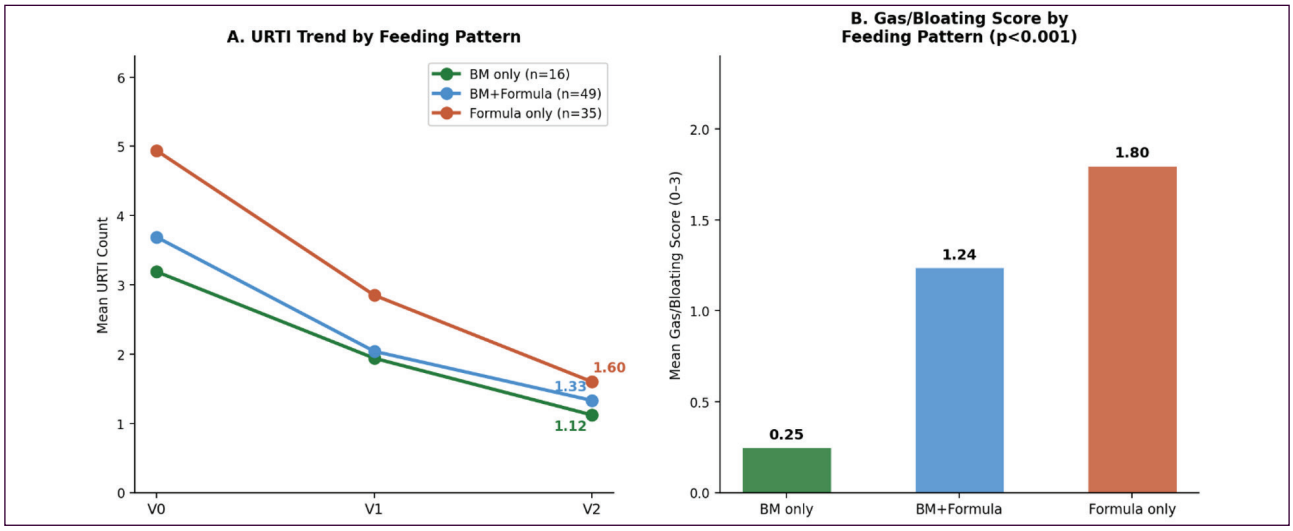

Figure 4. (A) URTI episode trends by early feeding pattern in Group 1. (B) Gas/bloating scores by feeding group. Formula-fed children exhibited the highest gas/bloating symptom burden at baseline (gas score: 1.80) and achieved the greatest absolute reduction with Trubiotic Duo (67.6%). AS: breast milk only; AS+M: mixed feeding; M: formula only.

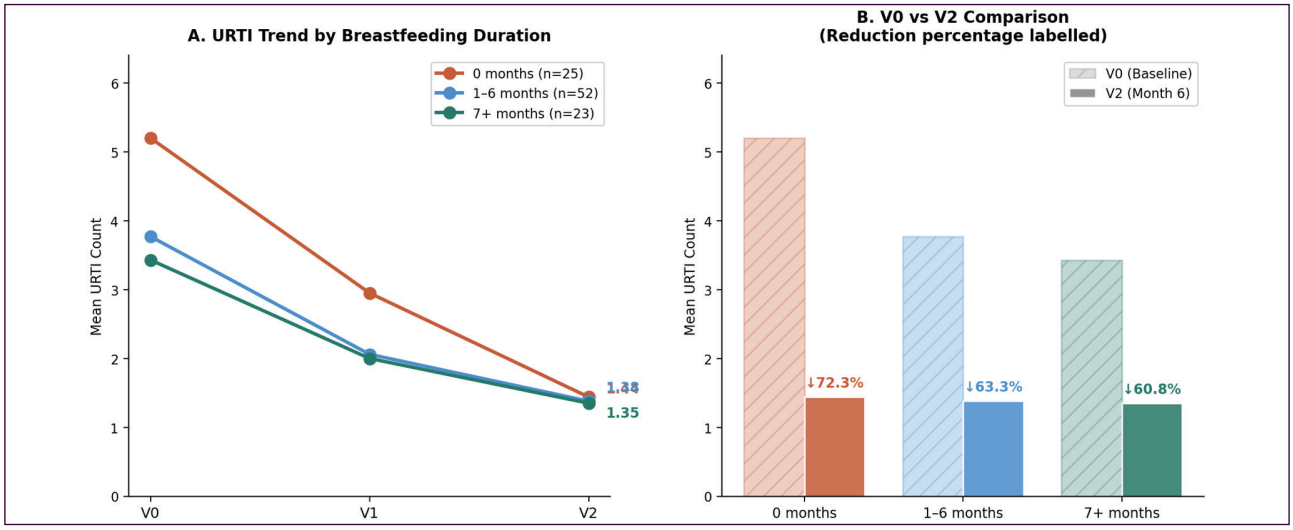

Figure 5. (A) URTI trends by breastfeeding duration in Group 1. (B) Comparison of baseline (V0) and 6-month (V2) URTI counts with percentage reduction. Children who received no breast milk showed the highest baseline burden and the greatest reduction with Trubiotic Duo (72.3%).

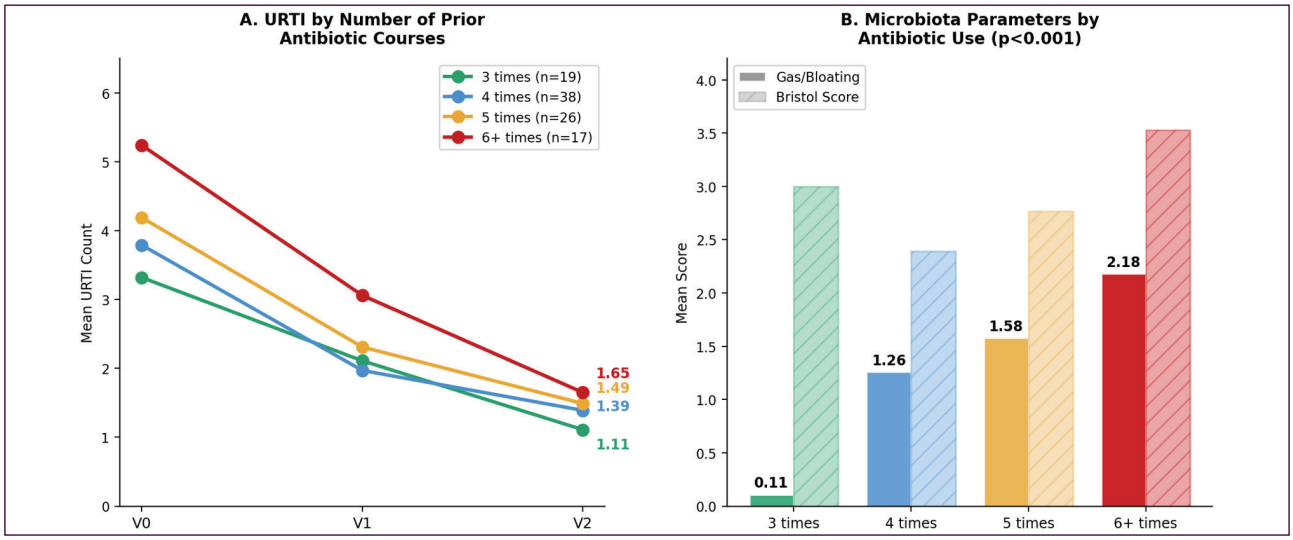

Figure 6. (A) URTI trends by prior antibiotic use in Group 1. (B) Gastrointestinal and stool-related indicators (gas/bloating and Bristol stool scores) by antibiotic use category. A clear dose-response relationship is evident between antibiotic exposure and the severity of gastrointestinal symptoms and stool-related abnormalities (p<0.001 for both parameters).

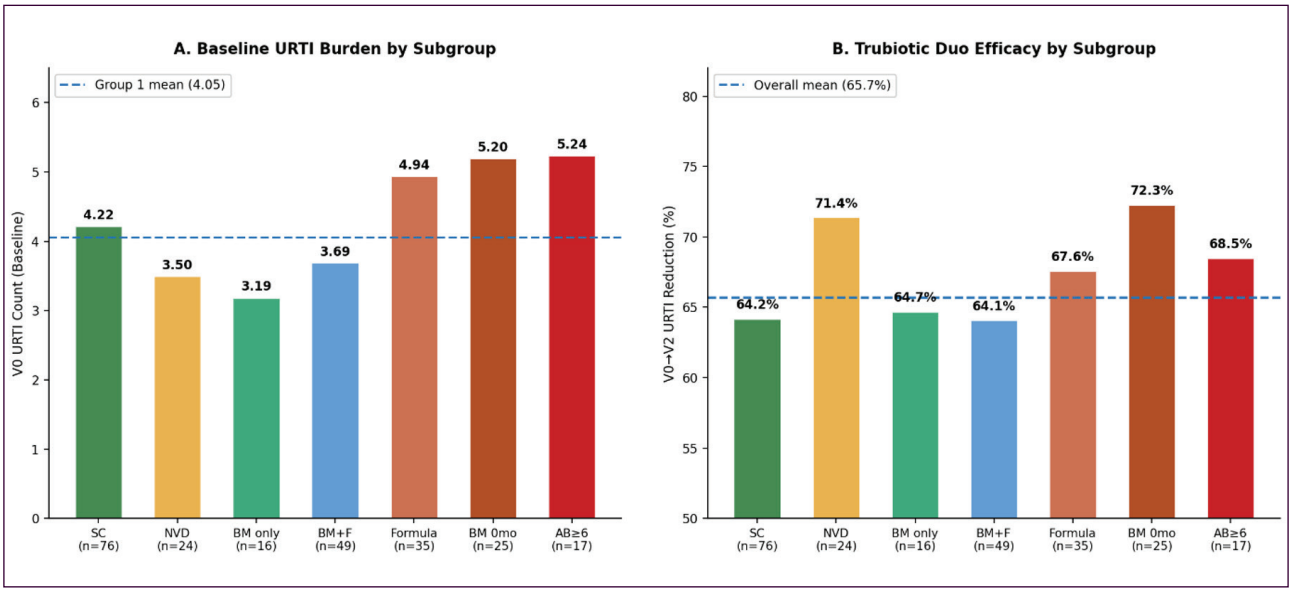

Figure 7. (A) Baseline URTI burden across all subgroups in Group 1. Dashed line indicates the overall Group 1 mean. Children who received no breast milk (0 months) and those with ≥6 prior antibiotic courses had the highest initial burden. (B) Percentage URTI reduction (V0→V2) across subgroups. Formula-fed, no-breast-milk, and frequent-antibiotic-user subgroups achieved the greatest clinical gains with Trubiotic Duo.

Mode of Delivery (Cesarean vs Normal Vaginal Delivery)

Among Group 1 children, 76 (76%) were born by cesarean section (SC) and 24 (24%) by normal vaginal delivery (NVD). As shown in **Figure 3**, SC-born children had significantly higher baseline URTI burden (4.22±0.95 vs 3.50±0.72; p=0.001), gas/bloating scores (1.50±0.77 vs 0.58±0.72; p<0.001), and post-antibiotic diarrhea rate (57% vs 17%; p<0.001). Despite this higher initial burden, both subgroups demonstrated significant URTI reductions following Trubiotic Duo use. The SC group achieved a 64.2% reduction (V0: 4.22→V2: 1.51) and the NVD group a 71.4% reduction (V0: 3.50→V2: 1.00).

Feeding Pattern in the First Six Months of Life

Baseline URTI frequency differed significantly by feeding pattern: exclusively breast-fed (AS) children had a mean of 3.19±0.40 URTI episodes, compared with 3.69±0.58 in the mixed-feeding (AS+M) and 4.94±0.84 in the formula-only (M) group (p<0.001). **Figure 4** demonstrates that gas/bloating scores were also markedly higher in the formula-only group (1.80±0.68 vs 0.25±0.58 in the AS group; p<0.001), reflecting the protective role of breast milk on gut microbiota. Following Trubiotic Duo use, all three subgroups achieved substantial URTI reductions: AS group 64.9%, AS+M group 63.9%, and formula-only group 67.6%, with the formula group showing the highest absolute clinical benefit.

Duration of Breastfeeding

Children who received no breast milk (0 months, n=25) had the highest baseline URTI burden (5.20±0.58) and gas/bloating scores (1.92±0.49), compared with those breastfed for 1–6 months (n=52) and 7+ months (n=23). As shown in **Figure 5**, the 0-month group achieved the greatest URTI reduction following Trubiotic Duo use (72.3% reduction; V0: 5.20→V2: 1.44), compared with 63.4% and 60.6% in the 1–6 month and 7+ month groups, respectively. These findings support the hypothesis that children with the greatest early-life microbiota deficit derive the most benefit from supplementation containing *Bifidobacterium longum* subsp. İnfantis.

Prior Antibiotic Exposure

A clear dose-response relationship was observed between the number of prior antibiotic courses and both baseline URTI frequency and clinical gastrointestinal indicators potentially associated with dysbiosis (**Figure 6**). Children with 6 or more prior antibiotic courses (n=17) had the highest baseline URTI count (5.24±0.56) and gas/bloating score (2.18±0.39), whereas those with only 3 courses (n=19) had the lowest values (3.32±0.48 and 0.11±0.32, respectively; p<0.001). Despite having the greatest gastrointestinal symptom burden the 6+ group achieved the highest URTI reduction percentage (68.5%; V0: 5.24→V2: 1.65), demonstrating that heavily antibiotic-exposed children benefit most from Trubiotic Duo.

Treatment Adherence and Safety

As summarized in **Table 4**, the mean adherence rate in Group 1 was 97.5±4.4%, and all 100 children (100%) achieved ≥80% adherence. No adverse events were recorded in any child throughout the study period (0/100). These findings confirm that the six-drop daily formulation of Trubiotic Duo is well tolerated and practically applicable in pediatric outpatient settings.

Table 4. Treatment Adherence, Safety, and Overall Clinical Efficacy Summary

Parameter	Group 1 Trubiotic Duo (n=100)	Group 2 Control (n=100)
Adherence rate, mean±SD (%)	97.5±4.4	—
Adherence ≥80%, n (%)	100 (100%)	—
Adverse events, n (%)	0 (0%)	—
URTI reduction V0→V2 (%)	65.7%	3.4% (NS)
Antibiotic reduction V0→V2 (%)	58.7%	6.0% (p=0.009)
School absenteeism reduction V0→V2 (%)	70.9%	7.1% (p=0.031)
Febrile day reduction V0→V2 (%)	55.5%	8.5% (p=0.021)

V0: baseline; V2: month 6; NS: not significant; —: not applicable (Group 2 did not receive study product).

DISCUSSION

General Overview

This retrospective multicenter cohort study demonstrates that a six-month course of the probiotic combination containing *Lacticaseibacillus rhamnosus* GG+*Bifidobacterium longum* subsp. infantis probiotic significantly reduces infection frequency, antibiotic use, and school absenteeism in children aged 2–10 years with recurrent upper respiratory tract infections (URTIs). The 65.7% reduction in URTI episode count is consistent with findings from existing studies evaluating the effects of probiotics on respiratory tract infections in children (16).

Hojsak et al. (11) reported that Lactobacillus GG use significantly reduced URTI frequency in children attending day-care centers. Similarly, Leyer et al. (12) demonstrated that probiotic supplementation markedly reduced the incidence and duration of cold and influenza-like symptoms in children. In our study, the use of a synergistic dual-strain combination rather than a single strain may have enabled simultaneous modulation of the gut-lung axis at both gastrointestinal and immunological levels, thereby contributing to the observed clinical improvement.

In a systematic review and meta-analysis conducted by King et al., probiotic use was found to significantly shorten the duration of acute respiratory tract infections in children. Our findings are in alignment with this evidence base and are further supported by real-world multicenter data (17).

Complementary symptom-directed approaches have also been investigated in pediatric respiratory infections. For example, Arıca and Adıgüzel reported clinical improvement in influenza-related symptoms with intranasal olopatadine hydrochloride; however, this approach concerns symptomatic management rather than infection prevention and is therefore mechanistically distinct from the probiotic strategy evaluated in the present study (18).

Impact on Antibiotic Use

In our study, a significant 58.7% reduction in antibiotic use frequency was achieved over the six-month period with Trubiotic Duo. This finding is of particular importance given the growing challenge of antibiotic resistance in the pediatric population (2, 5). Hicks et al. (5) emphasized that unnecessary antibiotic prescribing in outpatient settings continues to be a major problem in the United States. It is well established that probiotic interventions can indirectly reduce antibiotic demand by decreasing URTI frequency, thereby contributing to the prevention of antimicrobial resistance (17).

Furthermore, antibiotic use disrupts the diversity of the gut microbiota, predisposing to dysbiosis and impairing immune responses. Korpela et al. (15) demonstrated that antibiotic exposure leaves lasting effects on microbiota composition in early life and increases susceptibility to recurrent infections (15). The reduction in antibiotic use achieved with Trubiotic Duo not only lowers pharmacological burden but also creates a protective cycle that preserves microbiota health.

Gut-Lung Axis and Gastrointestinal Clinical Indicators

The improvement observed in gastrointestinal clinical indicators, including gas/bloating scores and stool regularity, is consistent with the growing body of evidence supporting a potential relationship between gut health and respiratory immunity (6, 7). Marsland et al. (6) demonstrated that respiratory tract health is substantially dependent on the composition of the gastrointestinal microbiota. Trompette et al. (8) showed that microbial metabolic products—in particular short-chain fatty acids (SCFAs)—directly regulate pulmonary mucosal immune responses.

The unique capacity of *Bifidobacterium longum* subsp. infantis to metabolize human milk oligosaccharides (HMOs) to metabolize human milk oligosaccharides (HMOs) supports its potential to reinforce the intestinal epithelial barrier and modulate mucosal immune responses. Henrick et al. (14) demonstrated that colonization with *Bifidobacterium longum* subsp. infantis significantly reduced intestinal inflammation in neonates. The improvement observed in gastrointestinal symptoms and stool-related indicators among cesarean-born children and those who received no breast milk is clinically consistent with this theoretical framework; however, microbiota composition itself was not directly assessed in the present study.

Cesarean Section Subgroup

In cesarean-born children, early-life gut microbiota development is disrupted due to the absence of exposure to the maternal vaginal microbiota. Shao et al. demonstrated that infants born by cesarean section

exhibit a marked reduction in beneficial anaerobic bacteria, particularly Bifidobacterium, along with increased colonization by opportunistic pathogens. In our study, cesarean-born children had a greater gastrointestinal symptom burden, characterized by higher gas/bloating scores and a higher post-antibiotic diarrhea rate (57% vs 17%), together with a greater baseline URTI burden. These findings are in full agreement with the existing literature (9, 19).

Trubiotic Duo produced a significant URTI reduction in the cesarean-born subgroup as well (V0: 4.22→V2: 1.51; 64.2% reduction). The capacity of the *Bifidobacterium longum* subsp. infantis probiotic strain to restore the Bifidobacterium colonization that is deficient in early life may represent the primary mechanism underlying the clinical improvement in this subgroup. Supporting evidence for this biological plausibility has been reported by Arrieta et al., whose study demonstrated that early-life microbiota disruption predisposes to long-term immune dysregulation (19).

Breast Milk and Feeding Pattern

The immunological and microbiota-supporting effects of breast milk are well established, and breastfeeding has been identified as a protective factor against recurrent URTI in numerous studies (10). Victora et al. showed that breastfeeding provides strong, duration-dependent protection against respiratory tract infections in childhood. The significantly lower baseline URTI burden (3.19±0.40) and gas/bloating scores (0.25±0.58) observed in exclusively breastfed children in our study corroborate this protective effect.

Breast milk contains HMOs, the preferred carbon source of B. longum subsp. infantis (13). Stiemsma and Michels (20) demonstrated that insufficient HMO exposure in early life impairs gut barrier integrity and adversely affects immune development. The capacity of the *Bifidobacterium longum* subsp. infantis probiotic strain to metabolize HMOs and thereby compensate precisely for this deficit emerges as the primary biological mechanism explaining the pronounced clinical improvement observed in formula-fed children.

Antibiotic Exposure and Microbiota Disruption

A clear dose-response relationship was identified between the number of prior antibiotic courses and both baseline URTI frequency and microbiota disruption markers. This finding is in concordance with the study by Korpela et al. (15), which documented lasting adverse effects of early-life antibiotic use on gut microbiota composition in Finnish preschool-aged children. Furthermore, Doré and Blottière (20) demonstrated that dysbiosis of the gut microbiota amplifies both local and systemic inflammation, thereby reinforcing susceptibility to infections.

Notably, children with six or more prior antibiotic courses had the highest gas/bloating scores (2.18±0.39) yet achieved the greatest URTI reduction with Trubiotic Duo (68.5%). These findings suggest that probiotic supplementation may be particularly relevant in children with frequent prior antibiotic exposure and greater gastrointestinal symptom burden.

Adherence and Safety

A mean adherence rate of 97.5±4.4% and a zero adverse event profile provide strong support for the safe use of Trubiotic Duo in pediatric outpatient settings. Vandenplas et al. (16) emphasized that probiotics are generally safe and well tolerated in the pediatric population, but underscored the necessity of product-specific clinical validation. The adherence and safety data from our study confirm the practical applicability of the six-drop daily formulation under real-world conditions.

Study Limitations

This study has several limitations. First, its retrospective observational design does not allow causal inference and may be subject to residual confounding. Second, no direct microbiome sequencing or microbiota composition analysis was performed; therefore, gas/bloating scores, stool regularity, Bristol stool scores, and post-antibiotic diarrhea should be interpreted as gastrointestinal clinical and stool-related indicators potentially associated with dysbiosis rather than direct measures of the gut microbiota. Third, the probiotic-exposed group had a substantially higher baseline URTI frequency than the control group. Consequently, baseline imbalance and regression to the mean may have contributed to the magnitude of the observed within-group reduction, and the effect estimates should therefore be interpreted cautiously. Prospective randomized controlled studies incorporating direct microbiome analyses and immunological biomarkers are warranted to confirm these findings.

Requiring complete follow-up records may have introduced selection bias by excluding patients with incomplete documentation or irregular follow-up.

The absence of blinding may have introduced observer or documentation bias, particularly for subjective symptom outcomes.

CONCLUSION

This multicenter retrospective cohort study found that documented use of the *Lacticaseibacillus rhamnosus* GG+*Bifidobacterium longum* subsp. infantis probiotic combination was associated with substantial reductions in URTI episode frequency, antibiotic use, and school absenteeism over six months in children with recurrent URTIs. The most critical findings of the study may be summarized as follows:

- Microbiota disruption (gas/bloating, post-antibiotic diarrhea) is significantly more severe at baseline in cesarean-born children; Trubiotic Duo produces meaningful improvements in both URTI burden and gastrointestinal parameters in this group.
- Children who received no breast milk and those fed exclusively with formula have the highest baseline URTI burden and the most pronounced microbiota disruption. The unique HMO-metabolizing capacity of the *Bifidobacterium longum* subsp. infantis probiotic strain emerges as the primary mechanism explaining the greatest clinical benefit observed in this subgroup.
- The strong dose-response relationship between the number of prior antibiotic courses and the degree of microbiota disruption indicates that the greatest observed clinical improvement was seen in children with frequent antibiotic exposure.
- An adherence rate of 97.5% and an adverse event rate of 0% confirm that Trubiotic Duo can be used safely in pediatric clinical practice and that families readily accept this product.

In the context of growing evidence supporting the role of the gut-lung axis in recurrent URTIs, the *Lacticaseibacillus rhamnosus* GG+*Bifidobacterium longum* subsp. infantis probiotic combination offers clinicians a valuable tool for reducing both the infectious burden and the antibiotic pressure of recurrent URTIs through mechanisms encompassing early-life microbiota support, gut barrier reinforcement, and mucosal immune modulation. Our findings support the consideration of Trubiotic Duo as an adjunct to standard care in children with recurrent URTIs—particularly in those born by cesarean section, those with limited breastfeeding exposure, and those with a history of frequent antibiotic use.

The real-world data and multicenter design of our study strengthen the generalizability of the findings. Nevertheless, prospective randomized controlled studies incorporating immunological biomarkers and microbiome analyses will be needed to further validate these results at a higher level of evidence..

ETHICAL DECLARATIONS

Ethics Committee Approval: he study was approved by the İstanbul Prof. Dr. Cemil Taşcıoğlu City Hospital Scientific Research Ethics Committee (Date: 15.06.2026, Decision No: 274)

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

Financial Disclosure: The authors declared that this study has received no financial support.

Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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