PROPHYLACTIC EFFECT OF A PROBIOTIC INTERVENTION IN CHILDREN PRONE TO ACUTE UPPER RESPIRATORY TRACT INFECTIONS: A RANDOMIZED CONTROLLED TRIAL

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Relevance. Upper respiratory tract infections (URTIs) are common in children and often progress with secondary complications such as otitis media, bronchitis, or pneumonia, especially in children with recurrent URTIs. Probiotics displayed immunomodulatory effects in children and adults, supporting immune functions to prevent winter diseases or common colds.

Objectives. We assessed the effectiveness of a 6-week prophylaxis with probiotics (*Lactobacillus helveticus* Rosell®-52, *Bifidobacterium infantis* Rosell®-33 and *Bifidobacterium bifidum* Rosell®-71) for preventing primary infections and/or secondary complications in URTI-prone children.

Methods. URTI-prone children were randomly divided into the probiotics (3×10⁹ CFU/day; 6 weeks) or control arm (no preventive intervention). The number of URTIs, duration and related complications were monitored for 6 months. Resistance index, number of medical visits, and antibiotics prescriptions were also recorded.

Results. After 2 months, probiotics reduced the number of URTIs (-2.34±0.13 vs -0.24±0.14; P<0.0001) and duration in (-1.13±0.18 vs -0.18±0.18 days; P=0.0011), and the number of secondary complications (-53% vs -5.8%; RR 0.5313 95% CI [0.3534, 0.7986] P=0.0058). Probiotic lowered resistance index (P<0.0001), number of medical consultations with specialized physicians (P=0.0033) and antibiotics prescriptions (P<0.0001).

Conclusions. Overall, a 6-week prophylaxis with combined probiotic (*Lactobacillus helveticus* Rosell®-52, *Bifidobacterium infantis* Rosell®-33 and *Bifidobacterium bifidum* Rosell®-71) in URTI-prone children exerted a significant and clinically important health benefit, decreasing the number and duration of URTI episodes and secondary complications and reducing the resistance index to a near-normal value. Furthermore, potential societal benefits of reducing health care use and inadequate antibiotic prescriptions in children favours the use of this probiotic product as a preventive strategy against URTIs and their consequences in frequently sick children.

This trial was retrospectively registered on ClinicalTrials.gov (NCT04525040).

Keywords: Prevention, Pediatrics, Ear-Nose-Throat Infections, Resistance Index, Probiotics

Relevance. Upper respiratory tract infections (URTIs), including common cold and other ear-nose-throat infections (ENTIs), are common in children worldwide [1]. The burden of these infections on parents, children and the healthcare system is considered high; they often lead to more severe health complications, especially in cases of recurrent respiratory tract infections [1, 2] which are estimated to occur in approximately 10-15% of children [3]. For example, acute URTIs increase the risk of lower respiratory infections (e. g. bronchitis, pneumonia), of bacterial infections in the middle ear and sinuses, and can act as a trigger of cyclic vomiting (acetonemic) syndrome [4-10]. Consequently, URTIs were associated with an increase in the number of inappropriate antibiotics prescriptions in children [11, 12]. Considering that URTI treatment relies mainly on symptoms alleviation, there is a need to improve preventive strategies against URTIs in order to decrease the occurrence of secondary infections, help limit antibiotics prescription in children, and reduce the collective costs associated with secondary and tertiary health care use for complications (i.e. hospi-

talizations or surgeries).

Current preventive strategies against recurrent URTIs include improving hygiene and social distancing from sick individuals (i.e. sibling, parent, day-care), targeted vaccination and non-specific immunomodulation, the latter comprising, among others, the use of bacterial lysates, immune-promoting vitamins, probiotics, or herbal compounds [13-15]. Probiotics are defined as “live microorganisms that, when administered in adequate amounts, confer a health benefit on the host” [15]. Studies have associated recurrent respiratory infections with a loss of intestinal microbiota diversity [16]. Some probiotic formulations were shown to improve both the innate and adaptive immunity via local and systemic effects. Locally, probiotics can stimulate intestinal mucus secretion, compete with pathogens at the mucosal interface and ameliorate the intestinal barrier function by strengthening tight junctions which reduces intestinal permeability, thereby limiting the translocation of pathogens to the lamina propria and mesenteric lymph nodes. Systemically, probiotics have been shown to alter

the levels of circulating cytokines, regulate the balance between effector and regulatory T-cells and the Th1/Th2 immune responses [17, 18].

Over the past few years, several systematic reviews have concluded to the functional efficacy of probiotics at reducing the incidence and/or duration of acute respiratory infections in children [19-22]. Yet, available evidence from clinical trials was deemed of low to very low quality; more research is required to establish the effectiveness of specific probiotic formulations for the prevention of URTIs in children [19-22]. The high heterogeneity between studies was mainly attributed to the differences in the probiotic strains or formulations tested, their dosage as well as treatment and follow-up durations. Probiotic formula we used containing a combination of three bacterial strains, was shown to modulate immune responses in vitro and in vivo [23-27]. In clinical trials, the combined probiotic we use was previously reported to reduce the incidence of common winter diseases or ENTIs in frequently sick children who received the supplement for 3 to 9 months [23, 27]. Here, we report a functional clinical assessment of its preventive efficacy against URTIs and related complications in frequently sick children following a shorter 6-week preventive intervention course with a 6-month follow-up period.

Objectives. We assessed the effectiveness of a 6-week prophylaxis with probiotics (*Lactobacillus helveticus* Rosell®-52, *Bifidobacterium infantis* Rosell®-33 and *Bifidobacterium bifidum* Rosell®-71) for preventing primary infections and/or secondary complications in URTI-prone children.

MATERIALS AND METHODS

Study Design. This randomized controlled trial was conducted in Ukraine between September 2016 and June 2017. Study conduct was in accordance with the Declaration of Helsinki and was approved by the ethics board of the Children's Clinical Hospital No. 5 (Kyiv, Ukraine) under the reference number 4/12.09.2016. Before enrolment in the study, a parent or legal tutor signed an informed consent form. This study was registered retrospectively in ClinicalTrials.gov (NCT04525040).

This trial observed a before-after design including a retrospective analysis period (6 months before enrolment) and a prospective observation period of 6 months after probiotic prophylaxis. Enrolled children were randomly assigned, using the sealed envelope method, to the pragmatic control group who received no preventive medication, or the intervention group receiving one probiotic (*Lactobacillus helveticus* Rosell®-52, *Bifidobacterium infantis* Rosell®-33 and *Bifidobacterium bifidum* Rosell®-71) sachet (3×10⁹ CFU) daily for 6 weeks. The randomization list was computer-generated (<http://www.randomization.com/>) with a 3: 2 ratio and block size of 5, with an estimated study population of

60. The clinical observation period started after the probiotic prophylaxis for the intervention arm, and after the baseline visit for the control arm. Children in both groups received standard symptomatic treatment for URTI or secondary complications as needed during the study, except for medications with immunomodulatory properties (e.g., bacterial lysate vaccines, viral vaccines, corticosteroids, immunosuppressants, etc.), which were not permitted.

All participants underwent a physical examination and interview at the beginning and end of the study, and a monthly medical examination with interview during the 6-month follow-up. Data on URTI progression and secondary complications (e.g., otitis media, adenoiditis, bronchitis, pneumonia, or cyclic vomiting syndrome) were collected if a child suffered through an URTI over the specified month. The study was open label, but assessors (i.e., physicians) were blinded.

Participants. Children recruited for this study had frequent URTIs and were under care at the Children's Clinical Hospital №5 and outpatient clinic of family medicine general practice No. 1 in Svyatoshynskiy district, Kyiv. Inclusion criteria were as follows: aged between 3-10 years old, experiencing a high frequency of acute URTIs characterized by a resistance index above 0.33 (i.e., URTI episode occurring more often than once every 3 months) during the past year [28], and the absence of other medications or supplements with immunomodulatory properties in the treatment regimen. Children with chronic pathological conditions (e.g., diabetes, asthma, leukaemia, primary immunodeficiencies, HIV, hepatitis etc.) were excluded. The nomogram method was used for the calculation of sample size. A total of 60 children were anticipated based on a mean clinically significant difference of 0.37 in the resistance index, with a power of 0.80 and alpha of 0.05 [28, 29].

Prophylaxis. Probiotic is composed of *Lactobacillus helveticus* Rosell®-52 (R0052), *Bifidobacterium infantis* Rosell®-33 (R0033), and *Bifidobacterium bifidum* Rosell®-71 (R0071) totalling at least 3×10⁹ CFU/dose, packaged in sachets also containing 750 mg of fructo-oligosaccharides (FOS)/sachet (Lallemand Health Solutions Inc.). Participants in the probiotic arm were instructed to take one sachet daily, with a meal for 6 weeks.

Outcomes. The primary outcome was the number and duration of URTI episodes and the incidence of complications (total cases) in the probiotics versus control groups over the 6-month periods (baseline and follow-up). Secondary outcomes were monthly incidence of URTIs during follow-up, resistance index (calculated as the number of URTI episodes divided by the number of months under medical care), individual complications diagnosed (number of cases), number of medical visits to specialist, and number of antibiotics prescriptions. Adverse events were surveyed at each monthly study visit during follow-up.

Statistical analyses. R version 4.1.0 was used to compare baseline characteristics using Fisher's exact test (proportions) or unpaired Student's t-test (mean±S.E.M.). Between-group comparisons of the change from baseline were analysed with the SAS JMP software v. 15, using the Wilcoxon ranked sum test for paired, non-normally distributed data. R version 4.1.0 was used to calculate the relative risk ratios during follow-up and the score test based 95% confidence intervals.

RESULTS AND DISCUSSION

Flow of participants. Participants' flow throughout the study is presented in Figure 1. Following medical charts pre-screening, the 56 participants assessed for eligibility were enrolled and randomized to the control arm (without preventive intervention, n=23) or the prophylaxis arm (Probiotics, n=33). One participant in the probiotics arm was withdrawn due to an adverse event deemed unrelated to the study supplement, hence 32 participants were analysed for the intervention arm. Among the 23 children enrolled in the comparator control group, 6 were found ineligible after study completion for having had less than one URTI every 3 months despite a similar total number of infections

over the past year and similar nosological profiles at the baseline assessment. These children were excluded from the per-protocol (PP) data analysis (n=17) but were included in the intent-to-treat (ITT) data analysis (n=23), for which results are presented as supplemental figures where indicated.

Demographic characteristics of the study population. The demographic characteristics of the PP population are shown in Table 1. Overall, the study population was composed of 26 females and 23 males, with a similar proportion in each group ($P>0.9999$). The average age of the population was 6.04 ± 0.3 years old, with a similar age group distribution ($P=0.9819$). All children were vaccinated according to the national vaccination schedule (measles, mumps, rubella, diphtheria, tetanus, poliomyelitis before age 6), with no vaccinations occurring in the 6 months before or after the start of the study. However, 3 children in the intervention group and 2 in the control group received vaccinations between 10-6 months before the start of the study.

In the PP population, both groups displayed a similar URTI-related medical history over the past year, typical of children with recurrent URTIs in terms of episodes number (8.7 ± 0.24 vs 8.35 ± 0.42) and duration

Fig. 1. CONSORT flow diagram

Table 1

Demographic characteristics of participants

Population characteristics	Probiotic, n = 32	Control, n = 17	P value
Gender, male (%)	15 (46.9%)	8 (47.1%)	>0.9999 a
Age, mean $\pm$ SEM	Overall: 6.04 $\pm$ 0.3		
	6.0 $\pm$ 0.38	6.12 $\pm$ 0.59	0.9819 b
Age group distribution, n (%)			
3-6 years old	21 (65.6)	11 (64.7)	>0.9999 a
7-10 years old	11 (34.4)	6 (35.3)	>0.9999 a
URTI-related medical history over the past year, mean $\pm$ SEM			
Number of episodes	8.7 $\pm$ 0.24	8.35 $\pm$ 0.42	0.4394 b
Duration of episodes (days)	8.9 $\pm$ 0.21	8.65 $\pm$ 0.31	0.4979 b
Number of related complications	5.6 $\pm$ 0.22	5.53 $\pm$ 0.33	0.8568 b
Number of antibiotic courses	6.0 $\pm$ 0.18	5.76 $\pm$ 0.23	0.4256 b
Resistance index	0.72 $\pm$ 0.02	0.70 $\pm$ 0.04	0.6188 b
Medical assessment of symptoms, n (%)			
Tonsillar hypertrophy	25 (78.1)	13 (76.5)	>0.9999 a
Friable throat	30 (93.8)	15 (88.2)	0.6020 a
Polylymphadenopathy	27 (84.4)	13 (76.5)	0.7001 a
Gastrointestinal symptoms	16 (50.0)	8 (47.1)	>0.9999 a
Paratophy	4 (12.5)	2 (11.8)	>0.9999 a

Note: a – Fisher's exact test; b – unpaired t-test.

(8.9 $\pm$ 0.21 vs 8.65 $\pm$ 0.31 days), number of complications (5.6 $\pm$ 0.22 vs 5.53 $\pm$ 0.33), and antibiotic courses (6.0 $\pm$ 0.18 vs 5.76 $\pm$ 0.23). Both groups harboured a similar 1-year resistance index (0.72 $\pm$ 0.02 vs 0.70 $\pm$ 0.04) well above the 0.33 cut-off value distinguishing frequently from occasionally sick children. In addition, the number of antibiotic courses prescribed to children was slightly higher than the number of complication cases in both groups, which is in accordance with existing data on unjustified antibiotic prescriptions in children with URTI [11, 12]. A similar nosological profile was observed children of both groups at the baseline medical assessment, with characteristics typical of children with recurrent respiratory infections. No child displayed signs of an active URTI at time of enrolment. Tonsillar hypertrophy, friable throat, polylymphadenopathy (with swelling of the palate, anterior cervical and postural groups of lymph nodes observed most often) were diagnosed in a large proportion of the population (76-94%). Nearly half the population reported experiencing a high frequency of gastrointestinal symptoms such as diarrhoea, constipation, dyspepsia, or nausea/vomiting. Conversely, paratophy, defined as superfluous body weight no more than 10-20% of the norm, occurred in approximately 12% of the children although not being considered characteristic of children with recurrent respiratory infections.

Average number and duration of URTI episodes and secondary complications over 6 months. The number and duration of URTI episodes per participant were significantly reduced during the follow-up period vs baseline in both the PP (Figure 2A and B) and ITT populations (Figure 3). As detailed in Table 2, the average number of URTI episodes was markedly decreased in the probiotics group during the follow-up phase compared to baseline (from 4.41 $\pm$ 0.18 to 2.06 $\pm$ 0.15) while remaining stable in the controls (from 4.00 $\pm$ 0.23 to 3.76 $\pm$ 0.24). The change in the number of URTIs was significantly different between groups (-2.34 $\pm$ 0.13 vs -0.29 $\pm$ 0.19; $P < 0.0001$). The average duration of URTI episodes (days) was significantly decreased in the probiotics group compared to controls (-1.13 $\pm$ 0.18 vs -0.18 $\pm$ 0.18; $P = 0.0011$).

The number and duration of complications per participant and resistance index during the follow-up period vs baseline were also significantly reduced between the probiotics and control groups for both the PP (Figure 2C and 2D) and ITT populations (Figure 4).

As detailed in Table 2, the average number of complications per child over 6 months decreased significantly in the probiotics arm from 2.81 $\pm$ 0.15 before enrolment to 0.69 $\pm$ 0.17 during the follow-up period, compared to a marginal decrease from 2.88 $\pm$ 0.22 to 2.59 $\pm$ 0.27 in the control group (-2.13 $\pm$ 0.21 vs -0.29 $\pm$ 0.19; $P < 0.0001$).

Fig. 2. Probiotic prophylaxis significantly reduced the number and duration of URTI episodes, the number of complication cases and resistance index

Note: Violin plots showing the distribution of (A) the number and (B) duration (days) of URTI episodes, (C) the number of complications and (D) the resistance index, averaged over the 6-month follow-up period or 6-month retrospective baseline, among the probiotics ($n=32$) and control group ($n=17$) participants. The red lines denote the median, and the blue lines, quartiles. Stars denote the between-group statistical significance of the absolute change over baseline (Wilcoxon ranked sum test, see Table 2). ** – $P=0.0011$; *** – $P<0.0001$.

Fig. 3. Violin plots showing the distribution of (A) the number and (B) duration (days) of URTI episodes in the ITT population.

Note: Stars denote the between-group statistical significance of the absolute change over baseline (Wilcoxon ranked sum test, ** – $P=0.007$; *** – $P<0.0001$). Before-after plots showing the individual data for the number of URTIs in all participants of the ITT populations in probiotics (C) and control (D) arms.

Fig. 4. Violin plots showing the distribution of (A) the number of complications and (B) the resistance index in the ITT population.

Note: Stars denote the between-group statistical significance of the absolute change over baseline (Wilcoxon ranked sum test, *** – $P < 0.0001$).

Table 2

Summary of URTI-related clinical data over the 6-month baseline and follow-up periods for the PP population

Groups and Outcomes	Baseline (over 6 months)			Follow-up (over 6 months)			Δ (Follow-up - Baseline)		
	Mean	SEM	P Value†	Mean	SEM	P Value†	Mean	SEM	P Value†
Probiotics, n=32									
Number of URTI episodes	4.41	0.18	0.1417	2.06	0.15	< 0.0001	-2.34	0.13	< 0.0001
Duration of URTI episodes (days/episode)	8.72	0.21	0.3277	7.59	0.18	0.0141	-1.13	0.18	0.0011
Number of complications	2.81	0.15	0.4957	0.69	0.17	< 0.0001	-2.13	0.21	< 0.0001
Resistance index	0.73	0.03	0.1417	0.34	0.02	< 0.0001	-0.39	0.02	< 0.0001
Number of antibiotic courses	3.03	0.13	0.0786	0.22	0.07	< 0.0001	-2.81	0.15	< 0.0001
Control, n=17									
Number of URTI episodes	4.00	0.23	-	3.76	0.24	-	-0.24	0.14	-
Duration of URTI episodes (days)	8.59	0.17	-	8.41	0.19	-	-0.18	0.18	-
Number of complications	2.88	0.22	-	2.59	0.27	-	-0.29	0.19	-
Resistance index	0.67	0.04	-	0.63	0.04	-	-0.04	0.02	-
Number of antibiotic courses	2.65	0.15	-	2.41	0.19	-	-0.24	0.20	-
† vs control. Wilcoxon Ranked Sum test.									

Similarly, the average resistance index was significantly reduced in the follow-up period compared to baseline in the probiotics arm (from 0.73 ± 0.03 to 0.34 ± 0.03), while it remained similar in the control group, from 0.67 ± 0.04 during the baseline period to 0.63 ± 0.04 during follow-up (-0.39 ± 0.02 vs -0.04 ± 0.02 ; $P < 0.0001$). Of note, while the 6-month baseline number of URTI episodes and resistance index remained similar in the control group, the average resistance index during follow-up in children who took the probiotic formulation decreased to a value near the cut-off of 0.33 separating occasionally

ill from URTI-prone children. As detailed in Table 3, a reduction in the incidence of URTIs was observed during the 3rd month, peaked during the 4th month of follow-up (RR 0.3585 95% CI [0.1468, 0.8756]; $P = 0.0072$) and remained lower until the end of the follow-up period, except for month 5 where the reduction was important but not significant.

Profile of URTI-related complications. The nosological profiles of URTI-related complications (Table 4) were similar between groups, with the following diagnostics: otitis media, adenoiditis, bronchitis (simple and obstructive).

Table 3

Monthly incidence of URTIs based on medical examinations of children during the follow-up period for the PP population

Follow-up Month	Probiotics, n = 32		Control, n = 17		Relative Risk [95% CI]	P Value	Average reduction in risk, %
	Without URTI, n (%)	With URTI, n (%)	Without URTI, n (%)	With URTI, n (%)			
1	18 (56.3)	14 (43.8)	7 (41.2)	10 (58.8)	0.7320 [0.3840, 1.3954]	0.3772	26.8
2	20 (62.5)	12 (37.5)	8 (47.1)	9 (52.9)	0.7529 [0.4253, 1.3329]	0.4177	24.7
3	22 (68.8)	10 (31.3)	6 (35.3)	11 (64.7)	0.5134 [0.2589, 1.0181]	0.0351	48.7
4	21 (65.6)	11 (34.4)	4 (23.5)	13 (76.5)	0.3585 [0.1468, 0.8756]	0.0072	64.1
5	23 (71.9)	9 (28.1)	7 (41.2)	10 (58.8)	0.5729 [0.3119, 1.0524]	0.0633	42.7
6	22 (68.8)	10 (31.3)	5 (29.4)	12 (70.6)	0.4278 [0.1976, 0.9264]	0.0149	57.2

tive), pneumonia, and cyclic vomiting syndrome triggered by an URTI episode. The corresponding incidence of these complications at baseline was retrieved from medical charts for the 6 months preceding enrolment. For all secondary complications, a marked reduction in incidence compared to baseline was observed in children who received probiotic prophylaxis versus controls, for whom the number of complications remained comparable to baseline. The number of children with secondary complications was reduced in the probiotics group compared to controls (-53% vs -5.8%), reflecting a significant reduction in the overall risk of complications (RR 0.5313 95% CI [0.3534, 0.7986] $P = 0.0058$). Specifically, there was a significant reduction in the risk of bronchitis (RR 0.2361 95% CI [0.0851, 0.6550] $P = 0.0050$) and adenoiditis (RR 0.2125 95% CI [0.07823318, 0.5772007], $P = 0.0018$) in the probiotics versus control arm. These secondary complications were the most frequently diagnosed in both groups, along with cyclic vomiting syndrome which was reduced in the probiotics group versus baseline although without a significant change in RR. For all complications except pneumonia, the reduced or unchanged incidence between group was mirrored in the ITT population (Figure 5).

For pneumonia, no cases were observed in the probiotics group during follow-up compared to 8 cases at baseline, which resulted in a significant RR reduction in the PP population ($P = 0.0369$) but not in the ITT population.

Number of consultations with a specialized physician and antibiotics prescriptions. Correspondingly with the reduction in the number of URTI episodes and complications (Table 2), there was a significant reduction in the need for medical consultations with specialized physicians during follow-up (Figure 6A) in the probiotics arm compared to controls ($P = 0.0033$). The number of antibiotic courses (Figure 3B) was also significantly

reduced in the probiotics group compared to controls ($P < 0.0001$). Similar results were observed in the ITT analysis (Figure 7).

Adverse events. The combined probiotic used in the study was well tolerated by most children (97%); one child in the probiotics arm was withdrawn from the study for repeated vomiting following the initiation of the intervention. Two attempts were made within a few days' interval. Further analysis of the child's medical data revealed that a similar reaction (vomiting) was sometimes observed following the intake of other medications. Therefore, the adverse event was deemed not specifically associated with the study supplement. No adverse events were observed for other children during the study.

This study shows that a 6-week prophylaxis with a combined probiotic was effective against URTIs and reduced the risk of secondary complications in URTI-prone children over 6 months after intake cessation, with the additional benefits of reducing the need for medical consultations and the number of prescribed antibiotics courses. URTI-prone children who received the probiotic formulation displayed a significantly reduced resistance index approaching the threshold value of occasionally ill children, which is clinically important. Systematic reviews have shown a reduction in URTI incidence and duration in children taking probiotics for periods ranging from 3 to 24 months [19, 20, 22]. However, in addition to the variability in the duration of supplementation, a high level of heterogeneity among studies was also noticed in terms of population studied (i.e. age and health status), probiotic supplement used, dosage, and follow-up time. Notably, few studies have assessed the effect of a shorter preventive probiotic course on infections or included a follow-up period after cessation of the intake. In a study involving healthy 3-12 years old children, the preventive effect of a probiotic formulation containing *L. acidophilus* and *B. lactis* was assessed by initiating supplementa-

Fig. 5. Bar graphs showing the profiles of complications in the (A) PP and (B) ITT populations.

Note: Stars denote the statistical significance of the RR during follow-up (* – $P < 0.05$; ** – $P < 0.01$).

Table 4

Nosological forms profile and incidence of complications for the PP population

Variable	Probiotics, n = 32						Control, n = 17						Relative Risk [95% CI]	P Value
	Baseline		Follow-up		Δ		Baseline		Follow-up		Δ			
	#	n (%)	#	N (%)	#	n	#	n (%)	#	n (%)	#	n		
Pneumonia	8	8/32 (25.0)	0	0/32 (0)	-8	-8	4	4/17 (23.5)	3	3/17 (17.6)	-1	-1	0.0909 § [0.0000, 0.6447]	0.0369
Bronchitis	22	18/32 (56.2)	4	4/32 (12.5)	-18	-14	14	10/17 (58.8)	12	9/17 (52.9)	-2	-1	0.2361 [0.0851, 0.6550]	0.0050
Cyclic vomiting syndrome	23	14/32 (43.7)	10	7/32 (21.9)	-13	-7	13	9/17 (52.9)	12	7/17 (41.2)	-1	-2	0.5313 [0.2233, 1.2641]	0.1929
Adenoiditis	24	20/32 (62.5)	5	4/32 (12.5)	-19	-16	12	9/17 (52.9)	12	10/17 (58.8)	0	+1	0.2125 [0.0782, 0.5772]	0.0018
Otitis Media	13	12/32 (37.5)	3	3/32 (9.4)	-10	-9	6	5/17 (29.4)	5	5/17 (29.4)	-1	0	0.3984 [0.1006, 1.5783]	0.2172
Total	90	32/32 (100)	22	15/32 (46.8)	-68	-17	49	16/17 (94.1)	44	15/17 (88.2)	-5	-1	0.5313 [0.3534, 0.7986]	0.0058

Note: Δ – change from baseline (6-month Follow-up – 6-month retrospective Baseline); # – number of cases diagnosed; n – number of participants with at least one episode diagnosed. § – 0.5 was added to all numbers to correct for 0 count.

Fig. 6. Significant reduction in the number of medical visits and antibiotics prescriptions in the probiotics arm.

Note: Bar graphs showing: A) the number of medical visits in each group over the 6 months of follow-up. (2-way ANOVA; overall, $P=0.0033$; Tukey's multiple comparisons test, adjusted $P=0.0466$), and B) the average number of antibiotic prescriptions over 6-months in each group at baseline and follow-up. (2-way ANOVA with Bonferroni's multiple comparison test, adjusted $P < 0.0001$).

Fig. 7. Bar graphs showing (A) the number of medical visits in the ITT population in each group over the 6 months of follow-up.

Note: (2-way ANOVA; overall, $P=0.0010$; Tukey's multiple comparisons test, adjusted $P=0.0147$), and (B) the average number of antibiotic prescriptions in the ITT population over 6-months in each group at baseline and follow-up. (2-way ANOVA with Bonferroni's multiple comparison test, adjusted $P < 0.0001$)

tion (5 billion CFU/d; 2 weeks) at the first appearance of respiratory infections in a member of the household. The duration of URTI episodes was reduced, but not the incidence of URTIs or secondary complications (otitis media, sinusitis, bronchitis, or pneumonia) between treated and control children [30]. Aside from the fact that the study population was composed of otherwise healthy children, this could suggest that administration after the assumed infection time upon exposure to the sick family member or the short supplementation period did not allow for a full preventive action.

In our study, the sustained effect in time observed after intake cessation could be explained by the timing of administration; childhood is a critical window for the immune system maturation. Indeed, this probiotic formulation strengthens the immune system and promotes host defence development on three fronts: counteracting opportunistic pathogens, maintaining the integrity of the intestinal barrier, and modulating cellular and systemic

immunity [23-27, 31-33]. In rodents, probiotic formula we used in the study induced tight junction proteins expression and mucin production, which contributes to the maintenance of intestinal permeability [34-37]. Specifically, *L. helveticus* R0052 was shown to increase the amount of circulating IgM, which are produced in responses to infections, and IgG which constitute a secondary response [38]. In vitro, the mechanisms were assessed using intestinal epithelial cells challenged with Poly(I:C) which elicits a response mimicking an infection by RNA viruses. Synergy was observed between the strains with a marked attenuation of the Poly(I:C) immune response and reduction in pro-inflammatory Th1 cytokines [25, 26].

Importantly, the immunomodulatory effects of this formulation observed *in vitro* and *in vivo* are in accordance with those seen in clinical trials, supporting the efficacy of combined probiotic for the prevention of common winter diseases and respiratory infections in

children [23, 27]. A 3-month course in 2-7 year-old children prone to winter diseases significantly reduced the relative risk of most common winter diseases by 25%. Furthermore, only 25.8% of children from the probiotic group missed ≥ 1 schooldays, compared to 42.5% of children in the placebo group ($p < 0.05$) [23]. A 9-month combined probiotic course reduced the incidence of ENTIs in atopic children younger than 5 suffering from respiratory infections or wheezing. The preventive effect was observed after 3 months and maintained until the end of the 9-month study, which was correlated with an increase in serum IgA, IgG and IgM levels after 3 months, and a decrease in serum IgE levels after 9 months of continuous intake. This study suggests that measurable effects on the immune system in allergic children (as per skin prick sensitization testing) appears to require a longer supplementation period before IgE level normalization [27]. Combined probiotic was shown to increase or maintain the levels of secretory IgA (sIgA) in children [33, 39-41], which suggests a role for the formulation at enhancing mucosal immunity. Specifically, salivary sIgA levels were increased and pro-inflammatory cytokines IL-6 and IL-17 were significantly reduced in children with non-infectious diarrhoea who received probiotic [40]. In healthy 3-12 month-old children, the anti-inflammatory IL-10/IL-12 ratio was increased significantly after an 8-week supplementation period with *B. infantis* R0033, while this ratio was increased in the *L. helveticus* R0052 and placebo groups after the intervention period [31]. In addition, formula-fed new-borns receiving probiotic maintained higher faecal sIgA levels than formula-fed children not taking probiotics, which suggests an effect of the formulation on the maturation of the immune system [41].

While harbouring a few limitations, it is improbable that these have affected the conclusions of our study. Six ineligible participants were enrolled in the control group who were found after study completion to deviate from the frequently sick enrolment criterion based on the number of URTIs, despite a similar total number of infections over the past year and displaying a similar set of symptoms at the baseline examination. Hence, the PP analysis paradigm was used to ensure these 6 participants with no URTIs for the 6-month baseline (Figure 3D), who could only display an increase over the follow-up period, were not inflating the between-group differences observed in the ITT analyses. However, results were similar despite the lower study power of the PP population, which demonstrates that these 6 participants were not driving the differences seen in the ITT population. Strain persistence was not assessed in the stools and we did not assess the levels of immunoglobulins in the population; this information could have allowed to confirm the mechanism behind the lasting effect of probiotic prophylaxis. However, long-term colonization of the intestinal tract is unlikely; generally, probiotics are no longer detected in stools within a month after intake cessation [32]. Additionally, the cause of the primary URTI infec-

tions was not determined. However, it is well recognized that URTIs usually result from a viral infection [42]; it may be relevant to validate the specific URTI viral aetiology in future studies. The control group was observational and did not receive placebo. Nevertheless, a placebo effect is unlikely in this context considering the non-subjective nature of the outcomes and assessors blinding. A longer follow-up period would have been informative about the duration of the protection conferred by probiotic; improvement in RRs reached a plateau between 3 and 6 months, which does not allow to exclude that the benefit could extend for longer. Further studies are required to establish whether the duration of the preventive probiotics course harbours a linear relationship with the duration of the benefit in time, and to identify the duration of prophylaxis appropriate for specific subgroup of patients, such as URTI-prone, atopic, or otherwise healthy children.

CONCLUSIONS

This study confirms the safety and preventive effect of combined probiotic (*Lactobacillus helveticus* Rosell®-52, *Bifidobacterium infantis* Rosell®-33 and *Bifidobacterium bifidum* Rosell®-71) against URTIs in URTI-prone children. Considering the significant reduction in the average number and duration of URTI episodes, as well as reduction of average number of secondary complications and decreased the resistance index in URTI-prone children, the use of this probiotic formulation as a preventive strategy could be considered in paediatric medical practice. Furthermore, although no formal cost-benefit analyses were conducted, the non-negligible potential benefit of reducing health care use and inappropriate antibiotics prescriptions should also be confirmed in follow-up studies.

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ПРОФІЛАКТИЧНИЙ ЕФЕКТ ПРОБІОТИЧНОГО ВТРУЧАННЯ У ДІТЕЙ, СХИЛЬНИХ ДО ГОСТРИХ ІНФЕКЦІЙ ВЕРХНІХ ДИХАЛЬНИХ ШЛЯХІВ: РАНДОМІЗОВАНЕ КОНТРОЛЬОВАНЕ ДОСЛІДЖЕННЯ

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Вступ. Інфекції верхніх дихальних шляхів (ІВДШ) поширені у дітей і часто прогресують із вторинними ускладненнями, такими як отит, бронхіт або пневмонія, особливо у дітей з рецидивуючими інфекціями. Пробиотики виявляли імуномодулюючу дію у дітей та дорослих, підтримуючи імунні функції для профілактики зимових захворювань або застуди.

Ціль: оцінити ефективність 6-тижневої профілактики пробіотиками (*Lactobacillus helveticus* Rosell®-52, *Bifidobacterium infantis* Rosell®-33 та *Bifidobacterium bifidum* Rosell®-71) для запобігання первинним інфекціям та/або вторинним ускладненням у дітей, схильних до ІВДШ.

Матеріали та методи. Дітей, схильних до ІВДШ, випадковим чином поділили на групу, що приймала пробіотики (3×109 КУО/день; 6 тижнів), і контрольну групу (без профілактичного втручання). Кількість ІВДШ, тривалість та пов'язані з ними ускладнення відстежувались протягом 6 місяців. Також реєстрували індекс резистентності, кількість медичних відвідувань та призначення антибіотиків.

Результати. Через 2 місяці пробіотики зменшили кількість ІВДШ (-2,34±0,13 проти -0,24±0,14; P<0,0001) та їх тривалість у (-1,13±0,18 проти -0,18±0,18 днів; P=0,0011), а також кількість вторинних ускладнень (-53% проти -5,8%; RR 0,5313 95% ДІ [0,3534, 0,7986] P=0,0058). Пробиотики зменшили індекс резистентності (P<0,0001), кількість медичних консультацій у спеціалістів (P=0,0033) та призначення антибіотиків (P<0,0001).

Висновки. Загалом 6-тижнева профілактика з використанням комбінованих пробіотиків (*Lactobacillus helveticus* Rosell®-52, *Bifidobacterium infantis* Rosell®-33 та *Bifidobacterium bifidum* Rosell®-71) у дітей, схильних до ІВДШ, зменшила кількість та

тривалість епізодів ІВДП та вторинних ускладнень та знизила показник резистентності до майже нормального значення. Крім того, потенційна суспільна користь від зменшення використання медичної допомоги та неадекватного призначення антибіотиків у дітей сприяє використанню цього пробіотичного продукту як профілактичної стратегії проти ІВДП та їх наслідків у часто хворих дітей.

Це дослідження було зареєстровано на ClinicalTrials.gov (NCT04525040).

Ключові слова: профілактика, педіатрія, інфекції вуха-горла, індекс резистентності, пробіотики

ПРОФИЛАКТИЧЕСКИЙ ЭФФЕКТ ПРОБИОТИЧЕСКОГО ВМЕШАТЕЛЬСТВА У ДЕТЕЙ, СКЛОННЫХ К ОСТРЫМ ИНФЕКЦИЯМ ВЕРХНИХ ДЫХАТЕЛЬНЫХ ПУТЕЙ: РАНДОМИЗИРОВАННОЕ КОНТРОЛИРУЕМОЕ ИССЛЕДОВАНИЕ

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Актуальность. Инфекции верхних дыхательных путей (ИВДП) распространены у детей и часто прогрессируют со вторичными осложнениями, такими как отит, бронхит или пневмония, особенно у детей с рецидивирующими инфекциями. Пробиотики проявляли иммуномодулирующее действие у детей и взрослых, поддерживая иммунные функции для профилактики зимних заболеваний или простуды.

Цель: оценить эффективность 6-недельной профилактики пробиотиками (*Lactobacillus helveticus* Rosell®-52, *Bifidobacterium infantis* Rosell®-33 и *Bifidobacterium bifidum* Rosell®-71) для предотвращения первичных инфекций и / или вторичных осложнений у детей, склонных к ИВДП.

Материалы и методы. Детей, склонных к ИВДП, случайным образом разделили на группу, принимающую пробиотики (3×10⁹ КОЕ/день, 6 недель), и контрольную группу (без профилактического вмешательства). Количество ИВДП, продолжительность и связанные с ними осложнения отслеживались в течение 6 месяцев. Также регистрировали индекс резистентности, количество медицинских посещений и назначения антибиотиков.

Результаты. Через 2 месяца пробиотики уменьшили количество ИВДП (-2,34±0,13 против -0,24±0,14; p<0,0001) и их продолжительность в (-1,13±0,18 против -0,18±0,18 дня; P = 0,0011), а также количество вторичных осложнений (-53% против -5,8%; RR 0,5313 95% ДИ [0,3534, 0,7986] P=0,0058). Пробиотики уменьшили индекс резистентности (P<0,0001), количество медицинских консультаций у специалистов (P=0,0033) и назначения антибиотиков (P<0,0001).

Выводы. В общем 6-недельная профилактика с использованием комбинированных пробиотиков (*Lactobacillus helveticus* Rosell®-52, *Bifidobacterium infantis* Rosell®-33 и *Bifidobacterium bifidum* Rosell®-71) у детей, склонных к ИВДП, уменьшила количество и продолжительность эпизодов ИВДП и вторичных осложнений, а также снизила показатель резистентности до почти нормальных значений. Кроме того, потенциальная общественная польза от уменьшения использования медицинской помощи и неадекватного назначения антибиотиков у детей способствует использованию этого пробиотического продукта как профилактической стратегии против ИВДП и их последствий у часто болеющих детей.

Это исследование было зарегистрировано на ClinicalTrials.gov (NCT04525040).

Ключевые слова: профилактика, педиатрия, инфекции уха-горла, индекс резистентности, пробиотики.