Probiotics with vitamin C for the prevention of upper respiratory tract symptoms in children aged 3-10 years: randomised controlled trial

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RESEARCH ARTICLE

Abstract

In a double-blind, randomised, parallel-group, placebo-controlled study, healthy school children aged 3-10 years received a probiotic based supplement daily for 6 months to assess the impact on the incidence and duration of upper respiratory tract infection (URTI) symptoms. The intervention comprised Lab4 probiotic (*Lactobacillus acidophilus* CUL21 and CUL60, *Bifidobacterium bifidum* CUL20 and *Bifidobacterium animalis* subsp. *lactis* CUL34) at 12.5 billion cfu/day plus 50 mg vitamin C or a matching placebo. 171 children were included in the analysis (85 in placebo and 86 in active group). Incidence of coughing was 16% (P=0.0300) significantly lower in the children receiving the active intervention compared to the placebo. No significant differences in the incidence rate of other URTI symptoms were observed. There was significantly lower risk of experiencing five different URTI related symptoms in one day favouring the active group (Risk ratio: 0.31, 95% confidence interval: 0.12, 0.81, P=0.0163). Absenteeism from school and the use of antibiotics was also significantly reduced for those in the active group (-16%, P=0.0060 and -27%, P=0.0203, respectively). Our findings indicate that six months daily supplementation with the Lab4 probiotic and vitamin C combination reduces the incidence of coughing, absenteeism and antibiotic usage in 3 to 10 year old children.

Keywords: cough, school, lactobacilli, bifidobacteria, vitamin C

1. Introduction

The importance of the relationship between the gut microbiome and the development of the immune system is well recognised (Zheng *et al.*, 2020) and there is evidence indicating crosstalk between the gut microbiota and the lungs (Angurana and Bansal, 2020). Probiotics have demonstrated an antiviral activity against common respiratory viruses including influenza, rhinovirus, respiratory syncytial virus and coronavirus and their role in the management of COVID-19 is gaining attention (Baud *et al.*, 2020; d'Ettorre *et al.*, 2020; Tiwari *et al.*, 2020). A predictive study has estimated that probiotic supplementation of the entire US population could prevent up to 54.5 million sick days with respiratory tract infection, 4.2 million missed work days, over a billion dollars expenditure and up to 2.2 million antibiotic prescriptions annually (Lenoir-Wijnkoop *et al.*, 2019).

Upper respiratory tract infections (URTIs) are mostly viral and comprise approximately 90% of total respiratory infections (Marengo *et al.*, 2017). Children are particularly susceptible because of their immune immaturity (Feleszko *et al.*, 2019) and there is growing evidence to suggest that

daily supplementation with probiotics or vitamin C may play role in the management of URTI in children (Emre *et al.*, 2020; Hao *et al.*, 2015; Hemilä and Chalker, 2013; King *et al.*, 2014; Vorilhon *et al.*, 2019). Our own pilot study with young children (3 to 6 year olds) has highlighted potential benefits from 6 months daily supplementation with Lab4 probiotics plus 50 mg vitamin C (Garaiova *et al.*, 2015). The objective of this study was to investigate the impact of the same probiotic based intervention on the prevention of upper respiratory tract symptoms in a broader population of school children aged between 3 and 10 years.

2. Materials and methods

Study design and approval

This was an exploratory multi-centre, double-blind, randomised, parallel-group, placebo-controlled study (PROCHILD-2). The study was conducted in accordance with the principles of the Declaration of Helsinki and the protocol was approved by the Ethics Committee of Bratislava self-governing region, Slovakia (Ref: 07878/2016-HF). The study was registered with the ISRCTN registry (ISRCTN26587549).

Study population, recruitment and randomisation

A total of 234 children (3-10 years old) were recruited from four paediatric health centres/general practices in Slovakia. Recruitment started in December 2016 and continued until March 2017 (n=90), then paused during the late spring and summer seasons, restarted again in October 2017 and continued until March 2018 (n=144). Paediatric physicians recruited the children either during routine preventative visits to a participating study centre or through poster advertisements displayed in study centre waiting rooms. Children were excluded if they were not attending school, were unwell or receiving antibiotics at the time of recruitment, receiving probiotic products regularly or any medication for stimulation of the immune system; if they were sensitive to xylitol/sorbitol. None of the children received the flu vaccine prior to or during the study period. Written informed consent was obtained from parents or legal guardians prior to participation in the study. Eligible children were sequentially assigned to the study by the paediatric physician and allocated in a 1:1 ratio to either of the two arms of the study according to a computer-generated random sequence using block randomisation with a block-size of four and stratified by centre. The randomisation was performed by an independent statistician who had no contact with the participants. The allocation sequence was not available to any member of the research team until the databases had been completed and locked.

Study intervention

Children received daily either one chewable tablet containing *Lactobacillus acidophilus* CUL21 (NCIMB 30156) and CUL60 (NCIMB 30157), *Bifidobacterium bifidum* CUL20 (NCIMB 30153) and *Bifidobacterium animalis* subsp. *lactis* CUL34 (NCIMB 30172) (Lab4) at 1.25×10^{10} cfu in combination with 50 mg vitamin C or an identical looking placebo tablet without the active components for 6 months. Interventions were prepared by Cultech Ltd., Port Talbot, UK. Parents/guardians were instructed to give their children one chewable tablet in the morning after breakfast and to avoid administration within 2 h of any antibiotic intake. In addition, parents were advised to maintain the children's normal diet and lifestyle throughout the study avoiding the consumption of any other probiotic drinks and supplements. Compliance to the intervention was assessed by monitoring the number of unused chewable tablets or from the daily health diaries.

Data collection

At baseline, all children were examined by a paediatric physician and background information, including history of allergy and any antibiotic and/or regular medication use were recorded. Body weight and height were measured using a digital weighing and measuring station with automatic body mass index calculation (kg/m^2, SECA 764, SECA Deutschland, Hamburg, Germany). During the study period, children were examined by a paediatrician at prescheduled 2-, 4- and 6-month appointments when the parents/guardians were instructed how to complete the daily health diaries monitoring the following URTI symptoms (based on guidance from the study paediatrician): sneezing, sore throat, cough, nasal discharge and nasal congestion. In addition, fever, earache, chest wheeze, absenteeism, antibiotic and/or other medication use, gastrointestinal symptoms (stool consistency, stomach-ache and vomiting), physician visits, hospitalisation and intervention compliance were recorded by the child's parents/guardians. Completed daily health diaries and unused tablets were collected and the intervention for next two months were provided at the scheduled visits.

Study endpoints

Primary end points were the incidence and duration of URTI symptoms over the 6-month study period. The symptoms selected to include as representing the Total URTI symptoms were: cough, sore throat, nasal congestion, nasal discharge and sneezing. The secondary end points included the incidence of absenteeism from school, antibiotic usage and gastrointestinal symptoms.

Statistical analysis

The incidence rate represents the number of episodes of each symptom, absenteeism or antibiotic usage divided by the number of days in the study and is expressed per 100 person days. Each distinct episode comprised the number of consecutive days with the symptom and each distinct episode was separated from another episode by a minimum duration of 24 h symptom free. Total URTI symptoms is defined as the incidence of symptom episodes comprising any one or more of the individual symptoms. The duration (mean difference) represents the total number of days with the symptom or absenteeism in the group divided by number of participants within that group. The incidence rates and durations with 95% confidence intervals (CIs) were calculated using a generalised linear model (GLM) that included treatment as a single predictor. For GLM analysis of a continuous endpoint such as duration of URTI symptoms, normal distribution and identity link functions were used; for GLM analysis of recurrent events (such as the number of episodes of URTI symptoms), Poisson distribution and log linkg4 functions were used. Continuous variables were summarised using number of observations, mean (standard deviation), whereas categorical variables were summarised by the number and percentage of events. Post-hoc covariate-adjusted analysis was performed with treatment as study variable and age, gender and history of allergy as covariates. A generalised linear model (GLM) was used for covariate adjusted analysis. Time-to-event for the first symptom in the groups was analysed with the Kaplan-Meier method, and significance was assessed by the Log-rank Mantel-Cox test (GraphPad Prism, version 8.2.2, La Jolla, CA, USA). *P*-values were considered statistically significant when less than 0.05. Data analyses were performed using SAS® version 9.4 (SAS Institute Inc., Cary, NC, USA).

3. Results

Enrolment, baseline characteristics and compliance

The participant flow diagram is shown in Figure 1. Of the 260 contacted participants, 234 were enrolled between December 2016 and March 2018. Six children in the placebo group and five children in the active group were incorrectly included in the randomisation (did not meet the inclusion criteria) and were excluded. Fifteen children withdrew shortly after randomisation (Figure 1). A total of 37 children were excluded from the analysis; 4 due to lost records, 2 due to non-compliance to the protocol, 21 due to non-authorised treatment usage (vitamin C/ immunostimulants) and 10 due to non-compliance to intervention intake (<80%). The proportions of excluded/ withdrawn children were similar in both arms; 31 children in active group (26.5%) and 32 children in placebo group (27.3%). Thus, the loss of follow-up was assumed to occur at random and no analysis adjustment for the loss of follow-up was made. 171 participants completed the study (85 in placebo and 86 in active group).

Compliance to the interventions by those who were included in the study was 96.1% and comparable between groups. Baseline characteristics of participants are included in Table 1.

Upper respiratory tract infection symptoms

Incidence

The incidence rates of individual and total URTI symptoms are shown in Table 2. Daily supplementation with the Lab4 probiotic plus vitamin C significantly reduced the incidence rate of coughing (-16%, *P*=0.0300) and sore throats (-20%, *P*=0.0373) compared to the placebo. There were no significant between group differences in the incidence rate of any other URTI symptoms. URTI symptoms were not reported for six of the children (3 in each group) over the study period. The incidence rates of fever, wheezing and earache did not differ between groups (Table 2). The results from the *post-hoc* covariate-adjusted analysis are presented in Supplementary Tables S1 and S4. The outcomes remain similar to the unadjusted analysis with the only exception of sore throat.

Time-to-first episode curves for the URTI symptoms are shown in Figure 2. There was no between group difference in the time-to-first URTI symptom episode irrespective of the type of symptom (Figure 2A). However, after approximately 10 days supplementation, the time to first episode of cough for those in the active group was longer than that for the participants in the placebo group and this delay in symptom onset persisted over the duration of the study (Figure 2B). The median time taken for 50% of the children to experience the first episode of coughing was 53.5 days in the active group – double that of the 27 days for the placebo (Figure 2B). For sore throat, the difference in timings was 115 days in the active group compared to 125 days in the placebo (Figure 2C).

The number of total URTI symptoms (cough, sore throat, sneezing, nasal discharge and nasal congestion) recorded per day for each participant in the active and placebo groups has been determined from 1 symptom to 5 symptoms and the incidence rates are presented in Table 3. In the active group, the incidence rate of those episodes including four or five different symptoms on one day was significantly lower than for the placebo (-29%, *P*=0.0278 and -79%, *P*<0.0001, respectively). During the 6-month study period, only 5.8% children (5/86) in the active group had all five symptoms on one day compared to 18.8% children (16/85) in the placebo

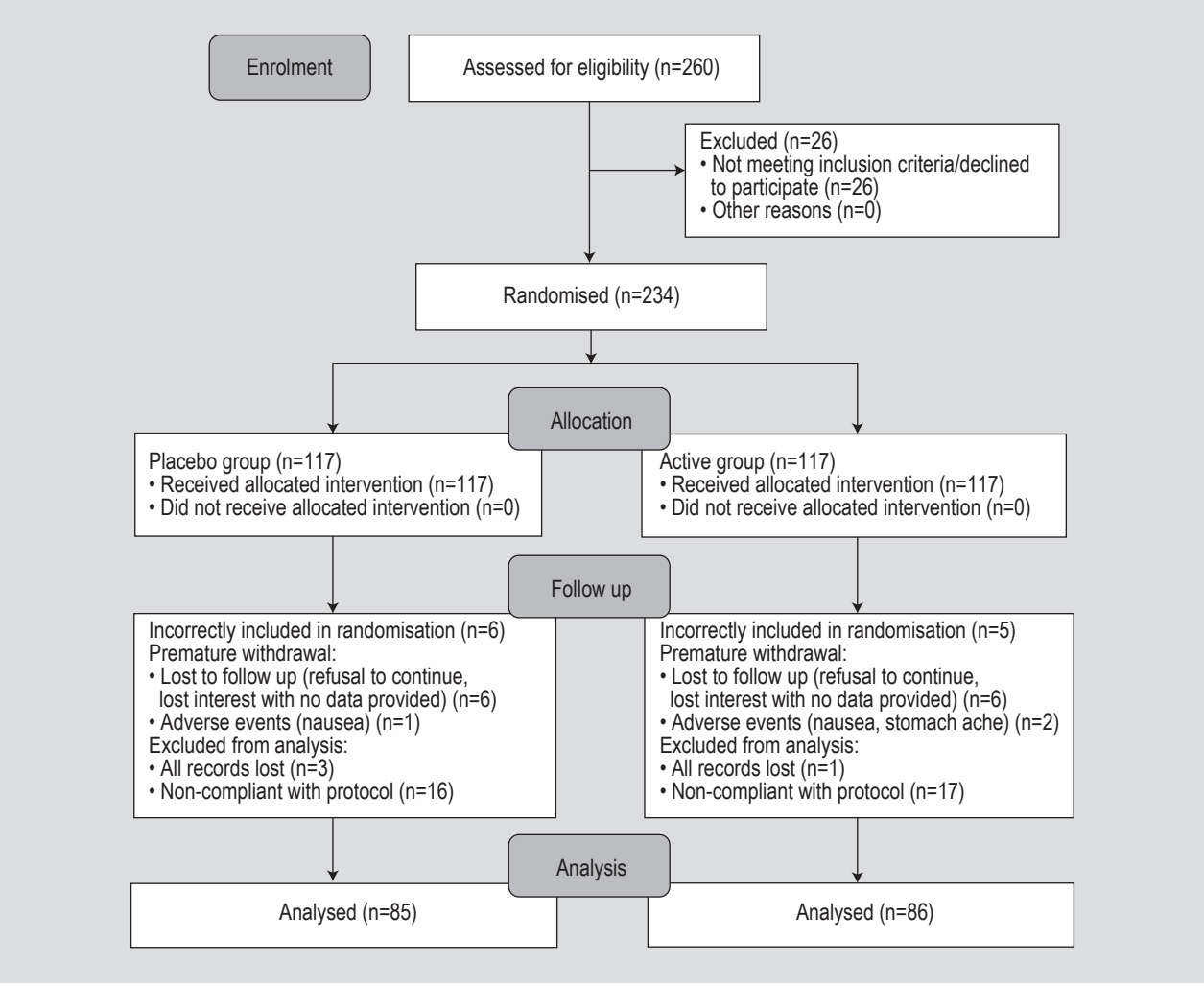

Figure 1. Flow diagram of the study.

Table 1. Baseline characteristics of study participants.

Characteristic[1]		Placebo (n=85)	Active (n=86)	Total (n=171)
Age, years		6.7±2.0	6.5±2.1	6.6±2.0
Gender, n (%)	Girls	48 (56.5%)	35 (40.7%)	83 (48.5%)
	Boys	37 (43.5%)	51 (59.3%)	88 (51.5%)
BMI, kg/m^2		16.1±2.5	16.4±2.5	16.3±2.5
	Girls	15.9±2.1	16.3±2.3	16.1±2.2
	Boys	16.4±2.9	16.4±2.7	16.4±2.8
Centre, n (%)	Centre 1	47 (55.3%)	46 (53.5%)	93 (54.4%)
	Centre 2	19 (22.4%)	13 (15.1%)	32 (18.7%)
	Centre 3	12 (14.1%)	17 (19.8%)	29 (17.0%)
	Centre 4	7 (8.2%)	10 (11.6%)	17 (9.9%)
Allergy, n (%)	Food	0 (0%)	11 (12.8%)	11 (6.4%)
	Asthma	3 (3.5%)	2 (2.3%)	5 (2.9%)
	Atopic eczema	2 (2.4%)	4 (4.7%)	6 (3.5%)
	Hay fever	5 (5.9%)	9 (10.5%)	14 (8.2%)
	Other	5 (5.9%)	4 (4.7%)	9 (5.3%)

[1] Data are presented as mean ± standard deviation. BMI = body mass index.

Table 2. Incidence of upper respiratory tract (URTI) and other symptoms.[1]

	Placebo (n=85)	Active (n=86)		Placebo (n=85)	Active (n=86)
URTI symptoms					
Cough			Total URTI symptoms[3]		
Number of episodes	178	152	Number of episodes	313	330
Incidence rate[2]	1.18	1.0	Incidence rate[2]	2.07	2.16
IRR (95% CI)	0.84 (0.72, 0.98)		IRR (95% CI)	1.04 (0.93, 1.16)	
P-value	0.0300		*P*-value	0.4592	
Sore throat					
Number of episodes	102	83			
Incidence rate[2]	0.68	0.54			
IRR (95% CI)	0.80 (0.66, 0.99)				
P-value	0.0373		Other symptoms		
Nasal congestion			Fever[4]		
Number of episodes	138	144	Number of episodes	70	64
Incidence rate[2]	0.91	0.94	Incidence rate[2]	0.46	0.42
IRR (95% CI)	1.03 (0.87, 1.22)		IRR (95% CI)	0.90 (0.71, 1.15)	
P-value	0.7132		*P*-value	0.4079	
Nasal discharge			Wheezing		
Number of episodes	208	206	Number of episodes	8	5
Incidence rate[2]	1.38	1.35	Incidence rate[2]	0.05	0.03
IRR (95% CI)	0.98 (0.85, 1.12)		IRR (95% CI)	0.62 (0.28, 1.36)	
P-value	0.7597		*P*-value	0.2322	
Sneezing			Earache		
Number of episodes	138	148	Number of episodes	13	15
Incidence rate[2]	0.91	0.97	Incidence rate[2]	0.09	0.10
IRR (95% CI)	1.06 (0.90, 1.25)		IRR (95% CI)	1.14 (0.67, 1.93)	
P-value	0.4855		*P*-value	0.6236	

[1] IRR = incidence rate ratio; CI = confidence interval.

[2] Incidence rate per 100 person-day.

[3] Total URTI symptoms is defined as the incidence of symptom episodes comprising any one or more of the individual symptoms.

[4] Temperature 38 °C or over.

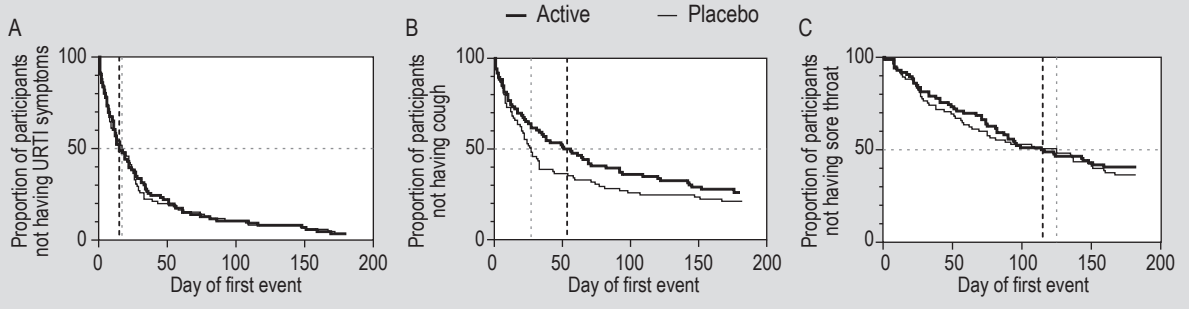

Figure 2. Kaplan-Meier time to event for the first upper respiratory tract infection (URTI) symptom: (A) any URTI symptom, χ^2=0.0094, *P*=0.9227; (B) cough, χ^2=1.915, *P*=0.1664; (C) sore throat, χ^2= 0.3054, *P*=0.5805. The median time to symptom (dotted line) was (A) 15 days for the first URTI symptom in the active group and 17 days in the placebo group; (B) 53.5 days for cough in the active group and 27 days in the placebo group; (C) 115 days for sore throat in the active group and 125 days in the placebo. The statistical significance was calculated by Log-rank Mantel-Cox test.

Table 3. Incidence of number of five common upper respiratory tract infection (URTI) symptoms.[1,2]

Number of symptoms	Placebo (n=85)	Active (n=86)
1 symptom		
Number of episodes	269	311
Incidence rate[3]	1.78	2.04
IRR (95% CI)	1.14 (1.02, 1.28)	
P-value	0.0234	
2 symptoms		
Number of episodes	194	211
Incidence rate[3]	1.29	1.38
IRR (95% CI)	1.08 (0.94, 1.23)	
P-value	0.3033	
3 symptoms		
Number of episodes	102	94
Incidence rate[3]	0.68	0.62
IRR (95% CI)	0.91 (0.75, 1.11)	
P-value	0.3562	
4 symptoms		
Number of episodes	50	36
Incidence rate[3]	0.33	0.24
IRR (95% CI)	0.71 (0.53, 0.96)	
P-value	0.0278	
5 symptoms		
Number of episodes	24	5
Incidence rate[3]	0.16	0.03
IRR (95% CI)	0.21 (0.10, 0.41)	
P-value	<0.0001	

[1] IRR = incidence rate ratio; CI = confidence interval.
[2] URTI symptoms included (cough, sore throat, nasal congestion, nasal discharge and sneezing). [3] Incidence rate per 100 person-day.

group (Risk ratio: 0.31, 95% CI: 0.12, 0.81, *P*=0.0163). There was a significantly higher incidence rate of episodes with one symptom (predominantly nasal discharge) in the active group compared to the placebo (*P*=0.0234). The results from the post-hoc covariate-adjusted analysis are similar (Supplementary Tables S2 and S5).

Duration

The findings for sore throats indicate a 33% reduction in the average number of days with sore throats for children in the active group compared to the placebo (3.0 vs 4.5 days per child, mean difference: -1.4 days, 95% CI: -3.0, 0.1, *P*=0.0717). No significant changes in the duration of the total URTI and other individual symptoms were observed between groups.

The average number of days per episode (episode length) of the total URTI symptoms was 5.5 days for the active group; significantly shorter than the 6.1 days observed for the placebo (mean difference: -0.66 days, 95% CI: -1.29, -0.04; *P*=0.0371). The average number of days per episode of cough was also significantly shorter in the active group compared to the placebo (4.1 vs 5.3 days per episode, mean difference: -1.2 days, 95% CI: -2.0, -0.3; *P*=0.0083). The episode length for the other individual symptoms showed no between group differences.

Absenteeism

Overall, there was a 16% significant reduction in the incidence rate of absenteeism from school in the Lab4 probiotic/vitamin C supplemented children compared to the placebo (0.0145 vs 0.0174, respectively, *P*=0.0060, Figure 3). The result from the post-hoc covariate-adjusted analysis were similar (Supplementary Figure S1). The average number of days absent from school per child was 8.3 days in the active group compared to 9.4 days in the placebo (mean difference: -1.1 days, 95% CI: -4.2, 1.9; *P*=0.4570).

Antibiotic usage

The forest plot in Figure 3 presents the incidence rate ratio of antibiotic use (irrespective of the number or type of antibiotic). The incidence rate of total antibiotic use in the active group was 27% lower than in the control group (*P* = 0.0203). The result from the *post-hoc* covariate-adjusted analysis is similar to the unadjusted analysis (-29%, *P*=0.0121, Supplementary Figure S1).

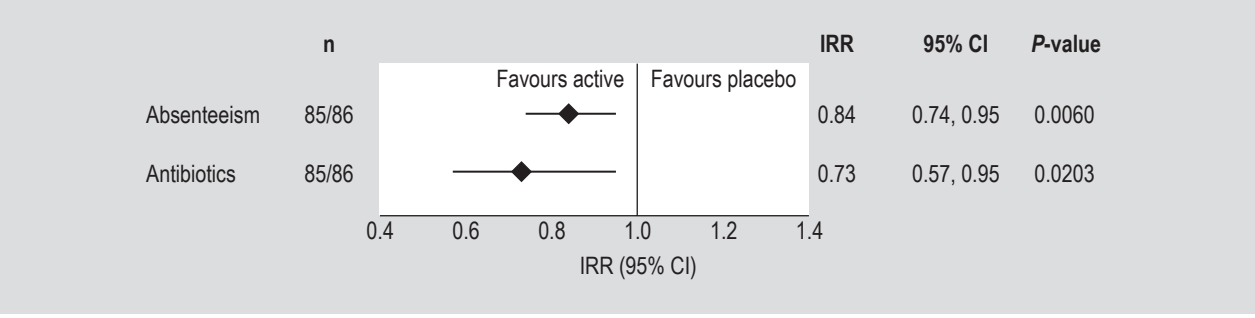

Figure 3. Incidence rate ratio (IRR) forest plot of absenteeism and antibiotic usage. CI = confidence interval.

Additional parameters

In the active group the incidence rate of total paediatric physician visits (scheduled/unscheduled) was reduced compared to the placebo (IRR: 0.81, 95% CI: 0.69, 0.95, P=0.0077). There were significantly less changes in normal stool consistency in the active group compared to the placebo (IRR: 1.12, 95% CI: 1.07, 1.18, P<0.0001) together with a significant reduction in the incidence rate of watery stools episodes (IRR: 0.56, 95% CI: 0.45, 0.71, P<0.0001). The results from the post-hoc covariate-adjusted analysis are similar (Supplementary Table S3 and S6). There were no between group differences in incidence of stomach-ache or vomiting. Four children were hospitalised during the study period (2 placebo/2 active) for illness/reasons unrelated to upper respiratory tract infections.

4. Discussion

Daily intake of the probiotic-based supplement resulted in a significant reduction in the incidence rate of coughs, absenteeism and antibiotic usage over the 6-month study period. Children taking the intervention had a 69% lower risk of experiencing all five URTI symptoms (cough, sore throat, sneezing, nasal discharge and nasal congestion) on one day compared to children taking placebo.

Both probiotics and vitamin C have been shown to possess immunomodulatory capability (Baud *et al.*, 2020; Carr and Maggini, 2017; Maldonado Galdeano *et al.*, 2019). The innate immune system provides the host's first line of defence against a viral challenge and involves, in part, the production of interleukin (IL)-12 and IL-1β by tissue residing immune cells such as macrophages (Arango Duque and Descoteaux, 2014). These pro-inflammatory cytokines play key roles in the resolution of infection by regulating the secretion of chemokines and other cytokines and promoting the differentiation, recruitment and activation of immune cells (Guo *et al.*, 2019; Rathinam and Fitzgerald, 2010). *In vitro* work with the Lab4 probiotic consortium has demonstrated enhanced production of IL-12 and IL-1β by macrophages undergoing simulated viral challenge thus indicating the potential to heighten the immune response to infection (Davies *et al.*, 2018). Similar *in vitro* findings in macrophages have been observed in response to stimulation with *Lactobacillus rhamnosus* GG (Miettinen *et al.*, 2012). Vitamin C is thought to possess virucidal properties and has been shown to induce the expression of anti-viral interferons during the early stages of viral infection (Colunga Biancatelli *et al.*, 2020). The benefits of the Lab4 probiotic and low dose vitamin C combination on the incidence and duration of URTI symptoms were first demonstrated in our PROCHILD study with children aged 3 to 6 years, however, no between group differences in plasma levels of cytokines were detected although blood samples were not taken when the children were symptomatic (Garaiova *et al.*, 2015).

In other probiotic multi-strain combination studies, beneficial effects on URTIs in children have been observed with *L. acidophilus* NCFM/*B. animalis* subsp. *lactis* Bi-07 (1×10^{10} cfu/day) (Leyer *et al.*, 2009) and also *L. acidophilus/B. bifidum* (4×10^{9} cfu/day) (Rerksuppaphol and Rerksuppaphol, 2012). Little or no effects were reported with *B. animalis* subsp. *lactis* BB12 in combination with *L. rhamnosus* GG ($2x10^{9}$ cfu/day) (Laursen *et al.*, 2017) or *Lactobacillus plantarum* HEAL9/*Lactobacillus paracasei* 8700:2 (1×10^{9} cfu/day) (Lazou Ahren *et al.*, 2020).

Meta-analysis of vitamin C intervention studies has shown that in children supplemented with 200 mg/day or more of vitamin C, the duration of URTI was reduced by 14% and with higher doses of vitamin C (1 to 2 g/day) achieving 18% reduction. Limited evidence exists to support any beneficial effects of low vitamin C dose (<200 mg per day) on URTI (Hemilä and Chalker, 2013). To the best of our knowledge, there is no published evidence by other research groups showing beneficial effects for probiotics with vitamin C (under 100 mg/day) in the prevention or management of upper respiratory tract infection in children. One study demonstrated a favourable effect with a herbal preparation containing echinacea, propolis and vitamin C (100 mg to 150 mg/day) in the management of respiratory tract infections in children aged 1-5 years (Cohen *et al.*, 2004). Low doses of vitamin C (10-50 mg) were included as a component of the placebo in some early vitamin C studies (Vorilhon *et al.*, 2019).

Coughing is common in children and is most often caused by acute respiratory infection. In a large population-based prospective cohort study, 69% of children aged 1 to 18 years were reported to have a cough with colds irrespective of age (Jurca *et al.*, 2017). In Chinese children (aged 3 to 5 years) attending day-care, supplementation with *L. acidophilus* NCFM combined with *B. animalis* subsp. *lactis* Bi-07 at a dose of 10^{10} cfu/day for 6 months resulted in the significant reduction of the incidence of cough (Leyer *et al.*, 2009). Coughing was also reduced in Thai children aged 8-13 years supplemented with an *L. acidophilus/B. bifidum* combination at a dose of 4×10^{9} cfu/day for 3 months (Rerksuppaphol and Rerksuppaphol, 2012). In our study with children aged 3 to 10 years, we observed significant reductions in the incidence and episode length of coughing together with an indication of a delay in the onset of the first coughing event suggesting that the active intervention might reduce the susceptibility of these children to cough.

In addition, we have observed the improvement in sore throats with active intervention, but not in fever, nasal congestion/discharge, sneezing, wheezing or earache. Other studies with multistrain probiotic interventions

have reported reductions in fever and rhinorrhoea alongside coughing (Leyer *et al.*, 2009; Rerksuppaphol and Rerksuppaphol, 2012). The authors did not evaluate the probiotic effect on the incidence of sore throat.

Reduced incidence of URTI can contribute to less absence from school for the child and less demand on parents/guardians and healthcare providers. Our study showed a significant reduction in the incidence rate of absenteeism with the active intervention in line with other probiotic studies (Hatakka *et al.*, 2001; Hojsak *et al.*, 2010; Leyer *et al.*, 2009; Rerksuppaphol and Rerksuppaphol, 2012).

Inappropriate antibiotic use for URTIs in children is a global issue (Holstiege *et al.*, 2014) and strategies are required to minimise the increased spread of antibiotic resistance associated with inappropriate usage and any potential detrimental impacts on long-term health. It has been found that infants and children supplemented with probiotics to prevent acute respiratory and gastrointestinal tract infections had a 29% lower relative risk of being prescribed antibiotics (King *et al.*, 2019). We also observed a significant 27% reduction in the incidence of antibiotic usage alongside a significantly reduced incidence rate of paediatric physician visits.

One of the main strengths of our study is that this is our second long-term study with the same probiotic based intervention focusing on the prevention of URTI symptoms and absenteeism in children but in this case the age range was broadened. Moreover, the recruitment of participants was performed in four discrete paediatric centres in two different cities of south-west Slovakia to minimise any single centre limitation and allow better generalisation of our findings. On the other hand, we are aware of the lack of a formal power calculation due to exploratory nature of the study with broader children's age range. We did not include probiotic alone or vitamin C alone study arms as our aim was to follow the protocol from our first study where the low dose of vitamin C was included to the probiotic intervention in response to suggestions made by the paediatricians who would be recruiting children for the study (They anticipated better parental acceptance to participate in a study with vitamin C as a component of the intervention). For future studies it would be useful to include an assessment of the severity of the symptoms as an additional outcome. It would be also interesting to look at the relationship between URTI symptoms and the incidence and severity of fever.

Our findings suggest a beneficial impact of supplementation with the Lab4 probiotic consortium in combination with a low dose vitamin C on the incidence of coughing, absenteeism and antibiotic usage in children aged 3 to 10 years.

Supplementary material

Supplementary material can be found online at https://doi.org/10.3920/BM2020.0185.

Table S1. *Post-hoc* covariate adjusted analysis for incidence of upper respiratory tract infection and other symptoms with treatment as study variable and age, gender and history of allergy as covariates.

Table S2. *Post-hoc* covariate adjusted analysis for incidence of number of five common upper respiratory tract infection symptoms with treatment as study variable and age, gender and history of allergy as covariates.

Table S3. *Post-hoc* covariate adjusted analysis for incidence of paediatric physician visits and stool consistency with treatment as study variable and age, gender and history of allergy as covariates.

Table S4. *Post-hoc* covariate adjusted analysis for incidence of upper respiratory tract infection and other symptoms with treatment as study variable and age, gender and history of allergy as covariates (b values and 95% CI).

Table S5. *Post-hoc* covariate adjusted analysis for incidence of number of five common upper respiratory tract infection symptoms with treatment as study variable and age, gender and history of allergy as covariates (b values and 95% CI).

Table S6. *Post-hoc* covariate adjusted analysis for incidence of paediatric physician visits, stool consistency, absenteeism and antibiotic usage with treatment as study variable and age, gender and history of allergy as covariates (b values and 95% CI).

Figure S1. *Post-hoc* covariate adjusted analysis for incidence rate ratio forest plot of absenteeism and antibiotic usage with age, gender and history of allergy as covariates.

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Conflicts of interest

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