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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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/m², 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.
The participant flow diagram is shown in Figure 1. Of the
Enrolment, baseline characteristics and compliance
3 Results

3.1 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.

<table>
<thead>
<tr>
<th>Characteristic¹</th>
<th>Placebo (n=85)</th>
<th>Active (n=86)</th>
<th>Total (n=171)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>6.7±2.0</td>
<td>6.5±2.1</td>
<td>6.6±2.0</td>
</tr>
<tr>
<td>Gender, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Girls</td>
<td>48 (56.5%)</td>
<td>35 (40.7%)</td>
<td>83 (48.5%)</td>
</tr>
<tr>
<td>Boys</td>
<td>37 (43.5%)</td>
<td>51 (59.3%)</td>
<td>88 (51.5%)</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>16.1±2.5</td>
<td>16.4±2.5</td>
<td>16.3±2.5</td>
</tr>
<tr>
<td>Girls</td>
<td>15.9±2.1</td>
<td>16.3±2.3</td>
<td>16.1±2.2</td>
</tr>
<tr>
<td>Boys</td>
<td>16.4±2.9</td>
<td>16.4±2.7</td>
<td>16.4±2.8</td>
</tr>
<tr>
<td>Centre, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Centre 1</td>
<td>47 (55.3%)</td>
<td>46 (53.5%)</td>
<td>93 (54.4%)</td>
</tr>
<tr>
<td>Centre 2</td>
<td>19 (22.4%)</td>
<td>13 (15.1%)</td>
<td>32 (18.7%)</td>
</tr>
<tr>
<td>Centre 3</td>
<td>12 (14.1%)</td>
<td>17 (19.8%)</td>
<td>29 (17.0%)</td>
</tr>
<tr>
<td>Centre 4</td>
<td>7 (8.2%)</td>
<td>10 (11.6%)</td>
<td>17 (9.9%)</td>
</tr>
<tr>
<td>Allergy, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Food</td>
<td>0 (0%)</td>
<td>11 (12.8%)</td>
<td>11 (6.4%)</td>
</tr>
<tr>
<td>Asthma</td>
<td>3 (3.5%)</td>
<td>2 (2.3%)</td>
<td>5 (2.9%)</td>
</tr>
<tr>
<td>Atopic eczema</td>
<td>2 (2.4%)</td>
<td>4 (4.7%)</td>
<td>6 (3.5%)</td>
</tr>
<tr>
<td>Hay fever</td>
<td>5 (5.9%)</td>
<td>9 (10.5%)</td>
<td>14 (8.2%)</td>
</tr>
<tr>
<td>Other</td>
<td>5 (5.9%)</td>
<td>4 (4.7%)</td>
<td>9 (5.3%)</td>
</tr>
</tbody>
</table>

¹ Data are presented as mean ± standard deviation. BMI = body mass index.
Table 2. Incidence of upper respiratory tract (URTI) and other symptoms.¹

<table>
<thead>
<tr>
<th>URTI symptoms</th>
<th>Placebo (n=85)</th>
<th>Active (n=86)</th>
<th>Placebo (n=85)</th>
<th>Active (n=86)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cough</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of episodes</td>
<td>178</td>
<td>152</td>
<td>313</td>
<td>330</td>
</tr>
<tr>
<td>Incidence rate²</td>
<td>1.18</td>
<td>1.0</td>
<td>2.07</td>
<td>2.16</td>
</tr>
<tr>
<td>IRR (95% CI)</td>
<td>0.84 (0.72, 0.98)</td>
<td>1.04 (0.93, 1.16)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P-value</td>
<td>0.0300</td>
<td>0.4592</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Sore throat</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of episodes</td>
<td>102</td>
<td>83</td>
<td>70</td>
<td>64</td>
</tr>
<tr>
<td>Incidence rate²</td>
<td>0.68</td>
<td>0.54</td>
<td>0.46</td>
<td>0.42</td>
</tr>
<tr>
<td>IRR (95% CI)</td>
<td>0.80 (0.66, 0.99)</td>
<td>0.90 (0.71, 1.15)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P-value</td>
<td>0.0373</td>
<td>0.4079</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Nasal congestion</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of episodes</td>
<td>138</td>
<td>144</td>
<td>138</td>
<td>148</td>
</tr>
<tr>
<td>Incidence rate²</td>
<td>0.91</td>
<td>0.94</td>
<td>0.91</td>
<td>0.97</td>
</tr>
<tr>
<td>IRR (95% CI)</td>
<td>1.03 (0.87, 1.22)</td>
<td>1.06 (0.90, 1.25)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P-value</td>
<td>0.7132</td>
<td>0.4855</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Nasal discharge</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of episodes</td>
<td>208</td>
<td>206</td>
<td>8</td>
<td>5</td>
</tr>
<tr>
<td>Incidence rate²</td>
<td>1.38</td>
<td>1.35</td>
<td>0.05</td>
<td>0.03</td>
</tr>
<tr>
<td>IRR (95% CI)</td>
<td>0.98 (0.85, 1.12)</td>
<td>0.62 (0.28, 1.36)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P-value</td>
<td>0.7597</td>
<td>0.2322</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Sneezing</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of episodes</td>
<td>138</td>
<td>148</td>
<td>13</td>
<td>15</td>
</tr>
<tr>
<td>Incidence rate²</td>
<td>0.91</td>
<td>0.97</td>
<td>0.09</td>
<td>0.10</td>
</tr>
<tr>
<td>IRR (95% CI)</td>
<td>1.06 (0.90, 1.25)</td>
<td>1.14 (0.67, 1.93)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P-value</td>
<td>0.4855</td>
<td>0.6236</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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, \( \chi^2=0.0094, P=0.9227; \) (B) cough, \( \chi^2=1.915, P=0.1664; \) (C) sore throat, \( \chi^2=0.3054, P=0.5805. \) 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.
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).

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**Table 3. Incidence of number of five common upper respiratory tract infection (URTI) symptoms.**

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

\(^1\) 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.

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**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 (1×10^9 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 (2×10^9 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.

Acknowledgements


Conflicts of interest

Cultech Limited provided study products and funding. I.G., D.R.M. and S.F.P. are employees of Cultech Ltd and had no role in the recruitment, data collection and analysis. Z.P., Z.N., D.W., J.R.M., Z.Ď. and J.M. are have been involved in other collaborative projects with Cultech Limited.
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