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Original Research

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# The impact of probiotics and vitamin C on the prevention of upper respiratory tract symptoms in two preschool children cohorts

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## ABSTRACT

**BACKGROUND/OBJECTIVES:** The efficacy of Lab4 probiotic and vitamin C combination on the prevention of upper respiratory tract infections (URTIs) was investigated in two studies with children. Our objective was to pool dataset of 57 preschool children from the PROCHILD study (ISRCTN28722693) and the dataset of 50 preschool matched cohort from the PROCHILD-2 study (ISRCTN26587549) to evaluate the impact of probiotic/vitamin C combination on the prevention of upper respiratory tract symptoms and provide a more robust assessment of effect using detailed individual level data.
**SUBJECTS/METHODS:** The children were supplemented daily for 6 months with either the multistrain probiotic ($1.25\times10^{10}$ cfu/tablet consisting of two strains of *Lactobacillus acidophilus* CUL21 and CUL60, *Bifidobacterium bifidum* CUL20 and *Bifidobacterium animalis* subsp. *lactis* CUL34) plus 50 mg vitamin C or a placebo.
**RESULTS:** In the pooled analysis of the individual participant data (per protocol population), significant reductions were observed for the incidence (−25%; 95% confidence interval [CI], 0.66, 0.85; $P < 0.0001$) and duration (−14.9 days; 95% CI, −24.8, −5.1; $P = 0.0030$) of typical URTI symptoms in the active group compared with the placebo. The incidence rates of absenteeism from preschool (IR ratio, 0.75; 95% CI, 0.66, 0.86; $P < 0.0001$), paediatric visits (IR ratio, 0.56; 95% CI, 0.47; 0.68; $P < 0.0001$) and antibiotic usage (IR ratio, 0.53; 95% CI, 0.39, 0.71; $P < 0.0001$) were also significantly reduced.
**CONCLUSION:** The pooled analysis findings of comparable preschool cohorts from two studies indicate that the supplementation with probiotic and vitamin C combination is beneficial in the prevention and management of URTI symptoms.

**Keywords:** Lactobacillus; Bifidobacterium; vitamin C; common colds

**Conflict of Interest**

Cultech Limited provided study products. The authors are/have been involved in other collaborative projects with Cultech Limited.

**Author Contributions**

Conceptualisation: Muchová J; Data Curation: Paduchová Z; Formal analysis: Wang D; Investigation: Nagyová Z; Methodology: Wang D; Project administration: Muchová J; Resources: Nagyová Z, Muchová J; Supervision: Paduchová Z; Validation: Muchová J; Visualisation: Paduchová Z; Writing - original draft: Paduchová Z; Writing - review & editing: Nagyová Z, Wang D, Muchová J.

## INTRODUCTION

The incidence of upper respiratory tract infections (URTIs) in young children is elevated by attendance at preschool/day-care facilities [1-3]. On average, preschool children can experience up to 6 to 8 URTI episodes per year [4]. Absenteeism caused by infections has a knock-on effect leading to absence from work for the caring adult and a significant healthcare burden [5,6].

Most URTIs in preschool children are caused by viruses and do not required antibiotic treatment. The unnecessary and inappropriate antibiotic use for URTIs in children is common and can contribute to the development and spread of antibiotic resistance [7-9].

The gut microbiota has an important role in the modulating the immune system and host's defence against viral respiratory infections [10]. The crosstalk between the gut and lung is bidirectional and the role of probiotics in modulating antiviral immune responses is promising [11,12]. Probiotics are defined as 'live microorganisms that, when administered in adequate amounts, confer a health benefit on the host' [13]. A growing evidence indicates that probiotics play a beneficial role in the prevention and management of URTI in children. In the recent Cochrane review, supplementation with probiotics has been shown to reduce the incidence and duration of episodes of acute URTI in children [14]. Furthermore, the use of probiotics to prevent acute respiratory tract and gastrointestinal tract infections in infants and children has been associated with a reduction in antibiotic prescriptions [15].

Vitamin C also has immunomodulatory activity and is recognised for its ability to reduce the incidence of URTI in children [16].

The beneficial effect of the Lab4 probiotic in combination with low dose vitamin C on URTI symptoms was demonstrated in two randomised, double-blind, placebo-controlled studies. In our PROCHILD study, supplementation with the Lab4 probiotic based intervention for 6 months significantly impacted on the incidence and duration of upper respiratory tract symptoms and absenteeism in 3 to 6 years old children attending preschool facilities [17]. Our second URTI focused study (PROCHILD-2) with same intervention also showed beneficial effects in children 3 to 10 years old [18,19]. Daily intake of the probiotic/vitamin C combination resulted in a significant reduction in the incidence rate of coughs and sore throats, absence from preschool/school, paediatrician visits and antibiotic prescriptions over the 6-month study period.

The repetition of a study is an important criterion for establishing the beneficial effects of an intervention and pooling data from multiple trials can provide a robust result about the efficacy of the intervention under test with sufficient power, stronger evidence and more generalisable population. A pooled analysis of two comparable probiotic studies has previously been used to facilitate more robust estimation of probiotic effect size [20]. Positive outcomes from our studies encouraged us to identify a cohort of the preschool children from the PROCHILD-2 study matching the preschool population studied in the PROCHILD study and evaluate the impact of a probiotic based intervention on the prevention of upper respiratory tract symptoms. Pooling of the individual participant data sets is conducted to increase participant numbers and the study power to enable a more robust assessment of effect using detailed individual level data.

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## SUBJECTS AND METHODS

### Study design and participants

Detailed designs, recruitments and intervention of PROCHILD and PROCHILD-2 studies were described in previous work [17,18]. Briefly, both studies were conducted in accordance with the principles of the Declaration of Helsinki and approved by the local Ethical Committees in Slovakia.

In the PROCHILD study (ISRCTN study registration No. ISRCTN28722693), preschool children aged 3–6 years were recruited between October 2010 and March 2011. The exclusion criteria were the use of medication or immunostimulatory products or regular use of probiotics (dairy or supplements) either prior to or at the time of enrolment, or unwillingness to provide blood, urine and saliva samples or a sensitivity to xylitol/sorbitol. The study flow diagram has been described in details elsewhere [17]. Briefly, of 69 children, three children withdrew from the study as they did not provide any records, 3 dropped out due to adverse events (abdominal pain, vomiting) or unknown reason and six children were excluded from the per protocol (PP) analysis due to unauthorized treatment use and failure to complete the follow-up period. 57 participants completed the study (28 in active and 29 in placebo group). The PROCHILD-2 study (ISRCTN26587549) involved 234 children aged between 3 and 10 years recruited between December 2016 and March 2018. Children were excluded if they were not well or taking antibiotics or not attending preschool/school at the time of enrolment, regularly receiving immunostimulatory or probiotic products or if they had a known sensitivity to xylitol or sorbitol. The detailed description of this study flow diagram can be found elsewhere [18]. Eleven children were incorrectly randomised and were excluded from the study, 15 children withdrew shortly after randomisation with no data provided or adverse events (nausea, stomach ache). Thirty-seven children were excluded from the PP analysis due to non-compliance with the protocol or loss of all records. A total of 171 participants completed the study (86 in active and 85 in placebo group). In both studies, none of the children had received the flu vaccine prior to or during the study period. Written informed consent was obtained from parents/legal guardians prior to their children's participation in the studies. The enrolment and sequential assignment of participants to the study interventions were conducted by a paediatrician. The randomisation procedures were carried out by an independent statistician.

The flow diagram of the pooled analysis is shown in the **Fig. 1**. The current analysis relates to a matched cohort of children from the PROCHILD-2 study (the PROCHILD-2 Preschool [P2P] matched cohort) selected with the aim of matching the participants in the PROCHILD study as follows: (i) age range (ii) attendance at preschool (iii) recruited between the months of October and March, and (iv) geographical location. The flow diagrams for the PROCHILD and P2P-matched cohorts are shown in the **Supplementary Fig. 1A and B**, respectively.

### Study intervention

Children in both studies received daily for 6 months either one chewable tablet of $1.25 \times 10^{10}$ colony forming units (cfu) of Lab4 probiotic consortium in combination with 50 mg vitamin C or an identical looking placebo tablet (Cultech Ltd, Port Talbot, UK). The probiotic consortium comprised *Lactobacillus acidophilus* CUL21 (NCIMB 30156), *Lactobacillus acidophilus* CUL60 (NCIMB 30157), *Bifidobacterium bifidum* CUL20 (NCIMB 30153) and *Bifidobacterium animalis* subsp. *lactis* CUL34 (NCIMB 30172). Parents/guardians were advised to maintain the children's normal diet and lifestyle throughout the study avoiding the consumption of any other probiotic containing


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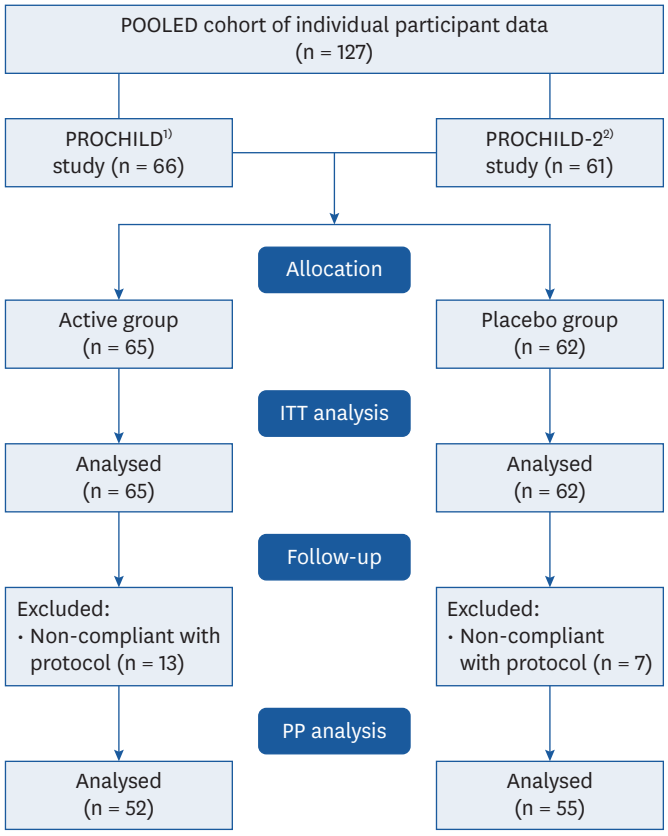


**Fig. 1.** Flow diagram of the pooled analysis.
ITT, intention-to-treat; PP, per protocol.
[1)]Garaiova *et al*., 2015 [17]; [2)]Garaiova *et al*., 2021 [18].

products. The Lab4 probiotic consortium was selected based on previous results from clinical studies in adults supplemented with Lab4 probiotic at a total of $2.5 \times 10^{10}$ cfu/day [21-24] and *ex vivo* work where this probiotic consortium showed beneficial immunomodulatory effect in healthy individuals [25]. In our paediatric studies, we administered a reduced dosage of $1.25 \times 10^{10}$ cfu/day, which is equivalent to half of the dosage used in the adult studies. The decision to include a low dose of vitamin C in the probiotic intervention was made based on the recommendation of study paediatricians.

## Data collection and study endpoints

Daily health diaries monitoring the individual URTI symptoms, such as cough, sore throat, sneezing, nasal discharge and nasal congestion, absence from preschool, antibiotic and other medication usage, physician visits, hospitalisation and intervention compliance were completed by the child's parents/guardians. Compliance to the intervention was assessed by monitoring the number of unused chewable tablets or from completion of the daily health diaries.

The study end points included the incidence and duration of URTI symptoms (cough, sore throat, nasal congestion, nasal discharge and sneezing), absence from preschool and the incidence episodes of paediatric physician visits and antibiotic usage over the 6-month study period.


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### Statistical analysis

For the pooled analysis of the individual participant data, the study variable (1 = PROCHILD, 2 = P2P-matched cohort) was added into the generalized linear models (GLM). The incidence rate (number of episodes divided by number of days in study and expressed as events per 100 person days) of URTI symptoms, absence from preschool, physician visits and antibiotic usage and mean difference (total number of days in the group divided by number of participants within the group) in duration of URTI symptoms or absenteeism with 95% CIs were calculated using GLM that included treatment as a predictor and study as a covariate. Each distinct episode was the number of consecutive days with the symptoms, absenteeism or antibiotic usage, 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 one or more of individual symptoms. For GLM analysis of a continuous endpoint such as duration of URTI symptoms, normal distribution and identity link functions were used. The Poisson distribution and log link functions were used for GLM analysis of recurrence events (such as number of episodes of URTI symptoms). The endpoints for the P2P-matched cohort were calculated using the above GLMs. A $P < 0.05$ was considered as statistically significant. The pooled analysis was based on PP population, supported by an intention-to-treat (ITT) analysis. Data analysis were performed using SAS® version 9.4 (SAS Institute Inc., Cary, NC, USA).

## RESULTS

### Baseline characteristics

Baseline characteristics of participants for the PROCHILD, P2P-matched cohort and pooled population are included in **Table 1** (PP population) and **Supplementary Table 1** (ITT population). The compliance to the intervention was in excess of 90%.

### URTI symptoms

*Incidence*

The forest plots in **Fig. 2** (PP population) and **Supplementary Fig. 2** (ITT population) present the incidence of URTI symptoms for the PROCHILD, P2P-matched cohort and pooled population. In the pooled cohort, significant between group reductions in the incidence rate of symptoms favouring the active group were seen in the incidence of coughing (−38%, $P < 0.0001$, PP; −28%, $P < 0.0001$, ITT, respectively), nasal discharge (−28%, $P < 0.0001$, PP; −24%, $P = 0.0002$, ITT, respectively) and sneezing (−37%, $P < 0.0001$, PP; −34%, $P < 0.0001$, ITT, respectively). In the PP population, a significant reduction in the incidence rate of sore throats was observed in the children receiving the active intervention compared to the placebo (−35%, $P = 0.0031$). No significant between group difference was observed for the ITT population (−20%, $P = 0.0703$). The incidence rate of nasal congestion was 26% significantly lower in the active group compared to the placebo ($P = 0.0034$, ITT), while no

**Table 1.** Baseline characteristics of participants

| Variables | Per protocol population | | | | | |
|---|---|---|---|---|---|---|
| | POOLED cohort | | PROCHILD cohort [17] | | P2P-matched cohort | |
| | Active (n = 52) | Placebo (n = 55) | Active (n = 28) | Placebo (n = 29) | Active (n = 24) | Placebo (n = 26) |
| Age (yrs)[1)] | 4.8 ± 0.9 | 5.0 ± 0.9 | 4.9 ± 0.9 | 5.0 ± 0.7 | 4.7 ± 1.0 | 4.9 ± 1.1 |
| Boys/Girls (%) | 63.5/36.5 | 38.2/61.8 | 57.1/42.9 | 44.8/55.2 | 70.8/29.2 | 30.8/69.2 |
| BMI (kg/m$^2$)[1)] | 15.7 ± 2.5 | 15.5 ± 2.0 | 15.2 ± 1.6 | 15.4 ± 2.1 | 16.2 ± 3.2 | 15.7 ± 1.8 |

P2P, PROCHILD-2 preschool; BMI, body mass index.
[1)]Data are presented as mean ± SD.


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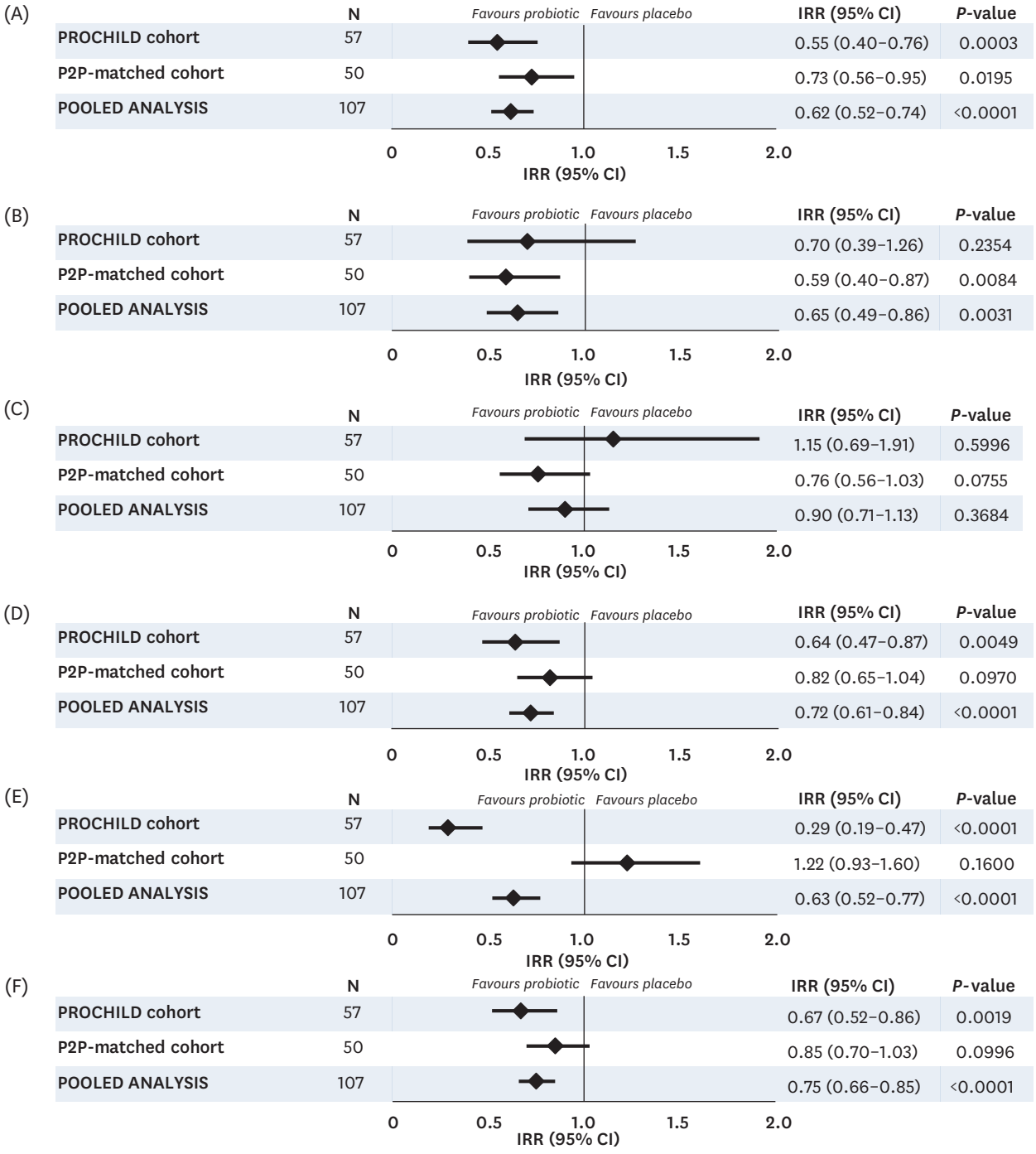


**Fig. 2.** Incidence rate ratio forest plots (per protocol population): (A) cough, (B) sore throat, (C) nasal congestion, (D) nasal discharge, (E) sneezing, (F) Total upper respiratory tract infection symptoms (include at least one symptom of cough, sore throat, sneezing, nasal discharge or nasal congestion).
IRR, incidence rate ratio; CI, confidence interval; P2P, PROCHILD-2 preschool.

significant difference was observed for PP population. The 6-month supplementation with probiotic/vitamin C combination significantly reduced the incidence of total URTI symptoms compared to the placebo (–25%, $P < 0.0001$, PP; –27%, $P < 0.0001$, ITT, respectively).


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*Duration*

The duration of the URTI symptoms for the pooled cohort is shown in **Table 2** (PP population) and **Supplementary Table 2** (ITT population). There was a significant reduction in the duration of total URTI symptoms in the active group compared to the placebo (mean difference: −14.9 days; 95% CI: −24.8, −5.1; $P = 0.0030$, PP; mean difference: −13.0 days; $P = 0.0051$, ITT, respectively). The average number of days with coughing was 11.5 days per child in the active group compared with 20.6 days in the placebo ($P = 0.0031$, PP; 14.9 vs. 20.4 days; $P = 0.0675$, ITT). No significant differences in other symptoms were found. The duration of each episode with at least one URTI symptom was significantly shorter in the active group compared to the placebo (mean difference: −3.2 days; 95% CI: −5.4, −0.9; $P = 0.0058$, PP; mean difference: −2.3 days; 95% CI: −4.2, −0.3; $P = 0.0251$, ITT). The duration of the URTI symptoms for the PROCHILD and P2P-matched cohorts are shown in the **Table 2** (PP) and **Supplementary Table 2** (ITT).

## Absenteeism from preschool

From the pooled analysis (**Table 3** and **Supplementary Table 3**) it can be seen that there was significantly less absence from preschool for the probiotic/vitamin C supplemented children compared to the placebo (−25%, $P < 0.0001$, PP; −22%, $P < 0.0001$, ITT, respectively). The duration of absenteeism from preschool in the active group was found to be a school week less than that for the placebo group (11.2 vs. 16.9 days per child, mean difference: −5.7 days per child; 95% CI: −11.2, −0.2; $P = 0.0426$, PP; 12.1 vs. 16.9 days per child, mean difference: −4.8 days per child; 95% CI: −9.8, 0.3; $P = 0.0653$, ITT). The incidence rates of absence from preschool for the PROCHILD and P2P-matched cohorts are shown in **Table 3** (PP) and **Supplementary Table 3** (ITT).

**Table 2.** Duration of URTI symptoms

| Variables | Per protocol population | | | | | |
|---|---|---|---|---|---|---|
| | POOLED cohort | | PROCHILD cohort [17] | | P2P-matched cohort | |
| | Active (n = 52) | Placebo (n = 55) | Active (n = 28) | Placebo (n = 29) | Active (n = 24) | Placebo (n = 26) |
| Cough | | | | | | |
| Mean ± SD, days | 11.5 ± 11.8 | 20.6 ± 19.5 | 11.9 ± 10.1 | 23.5 ± 20.3 | 11.0 ± 13.7 | 17.3 ± 18.3 |
| MD (95% CI) | −9.1 (−15.2, −3.1) | | −11.6 (−19.9, −3.4) | | −6.3 (−15.1, 2.6) | |
| *P*-value | 0.0031 | | 0.0056 | | 0.1654 | |
| Sore throat | | | | | | |
| Mean ± SD, days | 2.4 ± 4.5 | 3.7 ± 5.1 | 1.9 ± 2.7 | 2.8 ± 4.2 | 2.9 ± 6.0 | 4.7 ± 5.8 |
| MD (95% CI) | −1.3 (−3.1, 0.5) | | −0.9 (−2.7, 0.9) | | −1.7 (−4.9, 1.5) | |
| *P*-value | 0.1552 | | 0.3315 | | 0.2862 | |
| Nasal congestion | | | | | | |
| Mean ± SD, days | 5.4 ± 7.5 | 10.3 ± 19.8 | 4.9 ± 7.8 | 9.8 ± 23.8 | 6.0 ± 7.3 | 10.8 ± 14.4 |
| MD (95% CI) | −4.9 (−10.6, 0.8) | | −5.0 (−14.1, 4.1) | | −4.8 (−11.1, 1.5) | |
| *P*-value | 0.0907 | | 0.2845 | | 0.1342 | |
| Nasal discharge | | | | | | |
| Mean ± SD, days | 13.0 ± 15.8 | 20.0 ± 22.3 | 11.5 ± 15.6 | 21.4 ± 25.5 | 14.8 ± 16.2 | 18.5 ± 18.5 |
| MD (95% CI) | −7.0 (−14.3, 0.3) | | −10.0 (−20.8, 0.9) | | −3.7 (−13.1, 5.8) | |
| *P*-value | 0.0595 | | 0.0720 | | 0.4474 | |
| Sneezing | | | | | | |
| Mean ± SD, days | 4.4 ± 8.2 | 8.3 ± 13.1 | 2.3 ± 4.0 | 9.6 ± 14.8 | 6.9 ± 10.9 | 6.8 ± 11.1 |
| MD (95% CI) | −3.9 (−8.0, 0.2) | | −7.4 (−12.9, −1.8) | | 0.1 (−5.9, 6.1) | |
| *P*-value | 0.0649 | | 0.0093 | | 0.9816 | |
| Total URTI symptoms[1)] | | | | | | |
| Mean ± SD, days | 22.4 ± 20.7 | 37.3 ± 30.9 | 22.1 ± 21.0 | 43.1 ± 35.4 | 22.8 ± 20.8 | 30.9 ± 24.1 |
| MD (95% CI) | −14.9 (−24.8, −5.1) | | −21.0 (−35.9, −6.0) | | −8.1 (−20.3, 4.2) | |
| *P*-value | 0.0030 | | 0.0059 | | 0.1980 | |

URTI, upper respiratory tract infection; P2P, PROCHILD-2 preschool; MD, mean difference; CI, confidence interval.
[1)]Total URTI symptoms include at least one symptom of cough, sore throat, sneezing, nasal discharge or nasal congestion.


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**Table 3.** Incidence of absenteeism, physician visits and antibiotic usage

| Variables | Per protocol population | | | | | |
|---|---|---|---|---|---|---|
| | POOLED cohort | | PROCHILD cohort [17] | | P2P-matched cohort | |
| | Active (n = 52) | Placebo (n = 55) | Active (n = 28) | Placebo (n = 29) | Active (n = 24) | Placebo (n = 26) |
| Absence from preschool | | | | | | |
| Incidence rate[1)] | 2.02 | 2.70 | 1.98 | 2.82 | 2.08 | 2.60 |
| IRR (95% CI) | 0.75 (0.66, 0.86) | | 0.70 (0.55, 0.91) | | 0.80 (0.66, 0.97) | |
| *P*-value | < 0.0001 | | 0.0069 | | 0.0255 | |
| Physician visits | | | | | | |
| Incidence rate | 0.94 | 1.66 | 1.09 | 1.79 | 0.76 | 1.50 |
| IRR (95% CI) | 0.56 (0.47, 0.68) | | 0.60 (0.48, 0.77) | | 0.50 (0.37, 0.68) | |
| *P*-value | < 0.0001 | | < 0.0001 | | < 0.0001 | |
| Antibiotic usage | | | | | | |
| Incidence rate | 0.34 | 0.65 | 0.37 | 0.69 | 0.31 | 0.61 |
| IRR (95% CI) | 0.53 (0.39, 0.71) | | 0.55 (0.37, 0.81) | | 0.50 (0.32, 0.80) | |
| *P*-value | < 0.0001 | | 0.0025 | | 0.0038 | |

P2P, PROCHILD-2 preschool; IRR, incidence rate ratio; CI, confidence interval.
[1)]Incidence rate per 100 person-day.

### Physician visits and antibiotic usage

The incidence rates of total paediatric physician visits (scheduled/unscheduled) and antibiotic usage (irrespective of the number or type of antibiotic prescriptions) for the pooled cohort are shown in **Table 3** (PP population) and **Supplementary Table 3** (ITT population). Significant between group reductions in the incidence rate of physician visits (−44%, $P < 0.0001$, PP; −23%, $P = 0.0007$, ITT, respectively) and antibiotic usage (−47%, $P < 0.0001$, PP; −38%, $P = 0.0002$, ITT, respectively) favouring the active group were observed. The incidence rates of physician visits and antibiotic usage for the PROCHILD and P2P-matched cohorts are shown in **Table 3** (PP) and **Supplementary Table 3** (ITT).

## DISCUSSION

Pooled analysis of the individual participant data showed that supplementation with the multistrain probiotic consortium in combination with low dose vitamin C resulted in a significant reduction in the incidence rate and duration of URTI symptoms. In addition, the significant reduction in the incidence rate of absence from preschool, paediatric visits and antibiotic usage were observed. The beneficial impact of supplementation with the multistrain probiotic and low dose vitamin C combination on the incidence and duration of URTI in preschool children was first demonstrated in the PROCHILD study [17]. Positive outcomes from this study encouraged us to identify a cohort of the preschool children from the PROCHILD-2 study [18] matching the preschool population studied in the PROCHILD study and combining them into a pooled analysis to increase the sample size and provide a more robust intervention assessment.

The impact of probiotics at different doses and study durations on URTIs has been investigated in many randomised intervention studies and evaluated in meta-analysis and systematic reviews [14,26,27]. In children attending day care/preschool facilities, the results are variable with some showing a benefit on URTI symptoms while others have found no effect. In line with our findings, the beneficial effects on URTI incidence and/or duration were observed with the *Lacticaseibacillus rhamnosus* GG (LGG) administered in milk product to children aged 1 to 7 years at doses between $10^8$ to $10^9$ cfu/day for periods of 3 or 7 months [28-30]. Similarly, the supplementation with a fermented dairy drink containing *Lacticaseibacillus paracasei subsp. paracasei* DN-114 001/CNCM I-1518 at a dose of $2 \times 10^{10}$ cfu/day for 3 months resulted in the


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significantly lower incidence rate of URTI in children aged 3 to 6 years [31,32]. In 3 to 5 years old Chinese children attending day care centres and supplemented for 6 months with *L. acidophilus* NCFM at $10^{10}$ cfu/day, the reductions in the incidence and duration of fever, coughing and rhinorrhoea with improved outcomes when combined with *Bifidobacterium animalis* subsp. *lactis* Bi-07 were observed [33]. In our cohort, we observed significant reductions in the incidence of sore throats, coughing, sneezing and nasal discharge in preschool children supplemented with multistrain probiotic based intervention. Similar findings were reported in Malaysian children (aged 2 to 6 years) supplemented with *Bifidobacterium longum* BB536 at a dose of $5 \times 10^9$ cfu/day for 10 months [34]. Other studies have shown little or no effect [35-38]. Hay *et al.* [39] reported the duration of URTI on average of 8 days in children in the community. In our preschool placebo cohort, each episode with URTI symptoms lasted an average of 8.7 days, however the supplementation with the active intervention shorter the duration of episode by 36% (mean difference: −3.17 days; 95% CI: −5.42, −0.92; $P = 0.0058$, PP; −27%, mean difference: −2.26 days; 95% CI: −4.24, −0.28; $P = 0.0251$, ITT). Evidence for the be beneficial effects of vitamin C alone on URTI at dose under 200 mg per day is limited [16]. To the best of our knowledge, there is no published evidence from other research groups that demonstrates beneficial effects of probiotics with low dose vitamin C in the prevention of URTI in children.

The overprescribing of antibiotics for acute respiratory tract infections in the paediatric population is still common, raising the public health concern of antibiotic resistance [40]. A meta-analysis on the prevention of acute URTIs has shown that infants and children supplemented with probiotics had a 41% lower risk of being prescribed antibiotics compared to placebo [14]. In our pooled analysis, the significant reduction in the incidence and duration of URTI symptoms in preschool children receiving the probiotic and vitamin C intervention may be associated with fewer paediatric physician visits observed alongside a 47% reduction in the overall antibiotic usage.

The mechanism of action of probiotics in the management of acute respiratory infections involves their ability to modulate both local and systemic immunity. *In vitro* and *in vivo* studies indicate that probiotics are able to induce cytokine and chemokine production, engage Toll-like receptors/interferon pathways, improve mucosal barrier by increasing mucin expression and secretion, inhibit the replication of respiratory viruses and decrease viral load, modulate antiviral and pro-inflammatory gene expression, alleviate infection symptoms and boost host immunity [41,42]. The antiviral potential of Lab4 probiotic consortium has been demonstrated by ability to enhance the acute inflammatory response to viral challenge *in vitro* [43] and the systemic anti-inflammatory capability by reduction of pro-inflammatory cytokines interleukin-6 and tumor necrosis factor-α, and pro-inflammatory keratinocyte chemoattractant/growth regulated oncogene *in vivo* [44].

The strengths and limitations of the PROCHILD and PROCHILD-2 studies have been described in details elsewhere [17,18]. Briefly, both studies were carried out at multiple paediatric centres and used the same probiotic based intervention for the same duration with no formal power calculation performed, nor probiotic alone or vitamin C alone study arms included. Pooling of the individual participant data sets from two comparable studies is introduced in the current work to enable more robust estimation of effect size in repeated studies focused on URTI in children. However, we are aware that the result of the pooled analysis may have been influenced by the inclusion of a sub-population extracted from a larger study, which may have impacted on the randomisation. Every effort was made to ensure that all participants were matched for age range, recruitment period and geographical location.


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The findings of this pooled analysis with preschool children aged 3 to 6 years indicate the beneficial impact of supplementation with the multistrain probiotic in combination with a low dose vitamin C on the incidence and duration of URTI symptoms and absence from preschool with a concurrent reduction in paediatric physician visits and antibiotic usage.

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## SUPPLEMENTARY MATERIALS

### Supplementary Table 1
Baseline characteristics of participants

### Supplementary Table 2
Duration of URTI symptoms

### Supplementary Table 3
Incidence of absenteeism, physician visits and antibiotic usage

### Supplementary Fig. 1
Flow diagram of the PROCHILD (A) and P2P-matched cohorts (B) analysis.

### Supplementary Fig. 2
Incidence rate ratio forest plots (intention-to-treat population): (A) cough, (B) sore throat, (C) nasal congestion, (D) nasal discharge, (E) sneezing, (F) Total upper respiratory tract infection symptoms (include at least one symptom of cough, sore throat, sneezing, nasal discharge or nasal congestion).

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