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Original Research

An open-label study to evaluate the effects of *Streptococcus salivarius* K12 given as a powder formula to prevent respiratory infections in young children

Abstract

The purpose of this study was to evaluate the efficacy and safety of Bactoblis® sachets containing *Streptococcus salivarius* K12 (minimum 1 billion CFU/sachet) in preventing acute respiratory infections in young children who had just started attending a kindergarten.

This open-label, single-centre, randomized (1:1), controlled clinical study was conducted between February 2019 and December 2019 at the Poltava Regional Children's Clinical Hospital of the Poltava Regional Council. The randomization of patients in the study was performed by random sampling in a ratio of 1:1.

A total of 58 healthy children aged between two to four years were enrolled; 28 children received a Bactoblis® sachet once daily for 90 days and 30 children who did not receive Bactoblis® served as the control.

The effectiveness of prophylactic administration of the Bactoblis® sachet was assessed as a reduction in the incidence of tonsillopharyngitis, tracheitis, rhinitis, laryngitis and otitis media.

Other outcomes assessed were the requirement to administer antibacterial agents and antipyretics, and the frequency of absence from preschool institutions during the treatment period, and within six months of follow-up.

Children who received Bactoblis® for 90 days had a significant reduction in the incidence of infections during treatment and within six months of follow-up compared to those in the control group (p<0.01).

There was a significant reduction in the use of antipyretics in children treated with the probiotic supplement, and antibiotics were used for a shorter duration compared to the control group (p<0.01).

In addition, the number of days absent from preschool was lower in children treated with Bactoblis® compared to the control group (p<0.01).

Kryuchko TO

Tkachenko OYa

Tetyana Kryuchko,
MD, Professor, Head of the Department of Pediatrics N 2, Ukrainian Medical Stomatological Academy, Shevchenko St., 23, Poltava, 36011, Ukraine

Olha Tkachenko,
MD, PhD, Associate Professor of the Department of Pediatrics N 2, Ukrainian Medical Stomatological Academy, Shevchenko St., 23, Poltava, 36011, Ukraine

**Corresponding author:*
Tetyana Kryuchko

MD, Professor, Head of the Department of Pediatrics N 2, Ukrainian Medical Stomatological Academy, Shevchenko St., 23, Poltava, 36011, Ukraine.
Telephone/fax: (0532) 60-64-91
drkryuchko@gmail.com

No side effects were observed during the study, and 92.9% of patients reported excellent tolerability to the product.
Our results show that treatment with a Bactoblis® sachet for 90 days was effective in preventing acute respiratory infections in children aged between two to four years.
The authors suggest that this could be a useful supplementary probiotic for restoring natural microflora after antibiotic therapy, thus preventing the development of bacterial complications, and increasing resistance to viral infections during seasonal diseases and in organized children's communities.

Keywords: Acute respiratory viral infections, antibiotic use, Bactoblis®, tonsillopharyngitis, *Streptococcus salivarius* K12

Introduction

Probiotics beneficially affect the general health of the oral cavity, ear, nose, throat and respiratory mucosa. Probiotics modify the innate and adaptive immune mechanisms, improve barrier function and positively alter a wide variety of inflammatory diseases [1]. *Streptococcus salivarius* is a member of the bacterial flora usually found in the oral cavity that is recognized to inhibit the growth of group A beta-haemolytic *Streptococcus* (GABHS) [2].
This group of bacteria frequently causes recurrent pharyngotonsillar infections (RPTIs) in young children that necessitate recurrent clinical examinations or specialist consultations, pharmacological treatments with antibiotics and possible surgical intervention [2].
Although antibiotics are the drug-of-choice for treating single acute episodes of GABHS infections, most of these episodes are not complicated. Given the emerging problem of antibiotic resistance, there is a trend towards managing GABHS pharyngitis conservatively, thereby reserving antibiotics for more severe illness. In this context, *S. salivarius* K12 (SsK12) is regarded as a rational candidate for bacterial interference-mediated prevention of pharyngitis and tonsillitis [3].
S. salivarius was isolated from the nasopharynx of children who rarely suffered from throat infections. It was found to produce bacteriocins that inhibit the growth of *S. pyogenes* [4]. Gram-positive bacteria and some genera of Gram-negative bacteria produce bacteriocins or bacteriocin-like inhibitory substances (BLIS), also called lantibiotics, which are heat-stable, ribosomally synthesized small peptide molecules with proven therapeutic potential in treating infectious diseases [5].
The administration of slow-dissolving lozenges containing SsK12 (Bactoblis®) has been demonstrated to reduce the frequency of recurrent pharyngeal infections in children and adult populations by approximately 90 and 80%, re-

spectively, compared to control groups who did not receive probiotic treatment [3, 6].

SsK12 was found to be safe for ingestion and had low pathogenic potential [7]. During oral administration, it colonizes the oral and nasopharyngeal cavity [8], and, in competition with pathogenic microflora, it persists for a considerable length of time after the last dose has been received [9]. Results from other studies demonstrate that Bactoblis® prevents not only bacterial infections, but also, viral infections [8, 10, 11], most likely by increasing salivary interferon-gamma secretion [12]. Considering the available evidence, SsK12 can be used to prevent streptococcal pharyngotonsillitis and respiratory infections of viral aetiology in clinical practice. However, previous clinical studies supporting the benefits of SsK12 have been conducted using the lozenge formulation (Bactoblis®), which appeared to be the ideal format to ensure colonization and protection against infections. Nevertheless, lozenges are not suitable for use in children below three years of age.

Therefore, the authors here aimed to evaluate the potential efficacy and safety of using Bactoblis® in a powder format (sachets) containing SsK12 (minimum 1 billion CFU/sachet) in preventing respiratory diseases in young children attending kindergarten.

Materials and methods

This was an open-label, single-centre, 1:1 randomized, controlled clinical study conducted between February 2019 and December 2019 at the Poltava Regional Children's Clinical Hospital of the Poltava Regional Council. The study was conducted in compliance with the Declaration of Helsinki. The local ethics committee approved the protocol (the Commission on Ethics Utility – Poltava Regional Clinical Hospital of the Poltava Regional Council), and parents of participating children provided signed informed consent.

Patient population

A total of 58 children aged between 2 to 4 years who had started attending kindergarten were enrolled in the study. All children were assessed to be clinically healthy on enrolment. Children with autoimmune diseases, congenital diseases of the bronchopulmonary system or abnormalities of the maxillofacial area, immunodeficiencies, concomitant somatic diseases in the stage of decompensation, tuberculosis and those who had episodes of bronchospasm, a rheumatic disease or those who received preventive therapy for recurrent respiratory diseases in the past, were excluded from the study.

Study procedure

The study product (Bactoblis® sachets, also marketed as Streptoblis®) contained SsK12 (minimum 1 billion CFU/sachet) and 5 µg vitamin D3 in a stable powder matrix of maltodextrin and fructooligosaccharides, specifically developed to ensure optimal adhesion of SsK12 to the oral mucosal cells.

The study product has a pleasant strawberry flavour and is packed in an aluminum sachet format to ensure stability. The Bactoblis® sachets were produced according to the standards of GMP, HACCP and ISO 22000, and provided by Bluestone Pharma GmbH.

All children underwent a general clinical examination and, if necessary, were examined by an otolaryngologist to assess local status, and were then assigned to a treatment per a simple 1+1 enrolment key. A total of 28 children received Bactoblis® (sachets) for 90 days once a day, and 30 children who did not receive Bactoblis® formed the control group. The Bactoblis® sachets were administered in the evening before bedtime according to the schedule provided in the instructions.

During the entire study period, including a 6-month follow-up, all parents were requested to bring their child to the clinic as soon as they

displayed oropharyngeal symptoms suggestive of infection. These cases were subjected to a medical examination and a pharyngeal swab test. In the case of a positive result, antibiotic treatment (a combination of amoxicillin and clavulanic acid for 10 days) was prescribed to the child. At the end of the prescribed antibiotic therapy, treatment with Bactoblis® was resumed and continued until the 90th day. Acetaminophen or ibuprofen was prescribed in the case of a viral infection accompanied by pharyngolaryngeal pain and/or a fever.

Any other pathologies were treated according to local paediatric practice guidelines.

Study outcomes

The effectiveness of prophylactic administration of Bactoblis® was evaluated in terms of a reduced incidence of tonsillopharyngitis, tracheitis, rhinitis, laryngitis and otitis media. Other outcomes assessed were the need to administer antibacterial agents and antipyretics, and the frequency of absence from preschool institutions during the entire study period (90 days of treatment and 6 months of follow-up). Diagnosis of these events was carried out by looking at epidemiological history and clinical symptoms according to the current protocols for managing children with respiratory infections [13, 14].

Otoscopy and bacteriological studies were performed as needed. The criteria for prescribing antibacterial drugs were the presence of bacterial complications, such as bacterial rhinosinusitis, otitis media and streptococcal tonsillopharyngitis [15].

The incidence of side effects during the entire study period, and compliance with the study medication protocol, were also assessed.

Statistical analysis

Differences in the baseline characteristics of enrolled children between the 2 groups were assessed using Fisher's exact test.

Differences in episodes of infection, days of treatment and medication intake were analyzed using the Wilcoxon rank-sum test.

The JMP Version 10 software for Mac OS X (SAS Institute, Cary, NC, United States) was used for analysis. A statistical significance was set at 95%. All *p* values were 2-sided, and any *p* value less than 0.05 was considered statistically significant.

Results

Of the total of 58 children enrolled in the study, 28 received Bactoblis® sachets containing the strain of SsK12 (minimum 1 billion CFU/sachet). The remaining 30 children who formed the control group did not receive any treatment (**Fig. 1**).

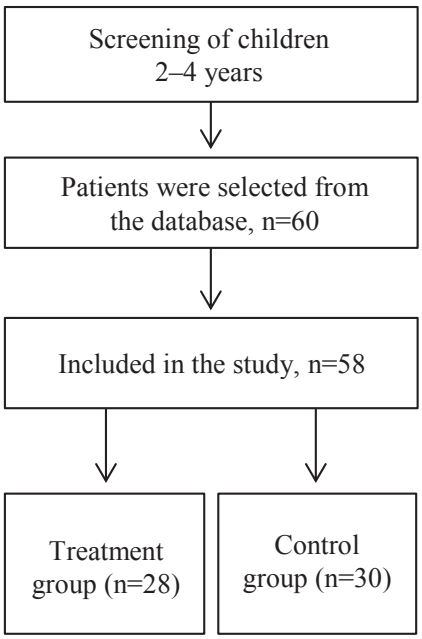

Figure 1 Patient disposition

Children from the study groups did not differ statistically in terms of age, sex and other variables assessed **(Table 1)**.

Table 1 Baseline characteristics of patients

	Treatment group	Control group	*p* value
N	28	30	ns
Males, n	15	16	ns
Females, n	13	14	ns
Age, years	3.4 ± 1.2	3.1 ± 0.9	ns
Birth weight, kg	3.0 ± 0.3	3.2 ± 0.5	ns
Breastfeeding, n	21	24	ns
Formula feeding, n	7	6	ns
Caucasians, n	28	30	ns
Preschool community organized, n	28	30	ns

Data are represented as the mean ± SD unless otherwise indicated; ns = non-significant difference

The results showed that 90 days of treatment with Bactoblis® sachets reduced the incidence of acute respiratory infections at the end of the treatment period **(Table 2)** and during six months of follow-up **(Table 3)** compared to the control group (p<0.01).

Table 2 Incidence of episodes of acute respiratory infections per child during 90 days of Bactoblis® administration

Disease	Treatment group (n=28)	Control group (n=30)	*p* value
Tonsillopharyngitis	0.09 ± 0.18	1.16 ± 1.21	<0.01
Tracheitis	0.23 ± 0.34	0.62 ± 0.43	<0.01
Rhinitis	0.21 ± 0.23	0.54 ± 0.49	<0.01
Laryngitis	0.09 ± 0.11	0.43 ± 0.47	<0.01
Otitis media	0.02 ± 0.01	0.21 ± 0.24	<0.01

Data are represented as the mean ± SD

Table 3 Incidence of episodes of acute respiratory infections per child during the six-month follow-up

Disease	Treatment group (n=28)	Control group (n=30)	*p* value
Tonsillopharyngitis	0.19 ± 0.24	1.08 ± 1.01	<0.01
Tracheitis	0.23 ± 0.28	0.66 ± 0.39	<0.01
Rhinitis	0.21 ± 0.18	0.57 ± 0.44	<0.01
Laryngitis	0.09 ± 0.04	0.31 ± 0.22	<0.01
Otitis media	0.04 ± 0.02	0.43 ± 0.36	<0.01

Data are represented as the mean ± SD

The greatest efficacy was observed in reducing the incidence of tonsillopharyngitis and otitis media.

Treatment with Bactoblis® sachets for 90 days resulted in a significant reduction in the use of antipyretics and these children received antibiotics for a shorter duration compared to the control group (p<0.01) **(Table 4)**.

In addition, the number of days absent from preschool was lower in children treated with Bactoblis® compared to the control group (p<0.01). The data presented in **Table 5** indicate 'excellent' tolerability and compliance during the study.

Table 4 Frequency of administration of antibiotics/antipyretics and absence from preschool institutions during treatment and the six-month follow-up of children

Parameter	Treatment group (n=28)	Control group (n=30)	Δ	*p* value
Antibiotics, days	68	207	-67.1%	<0.01
Antipyretics (anti-inflammatory agents), days	39	152	-74.3%	<0.01
Absence from preschool institutions, days	156	434	-64.1%	<0.01

Table 5 Tolerability, side effects and compliance reported for children treated with Bactoblis® (n=28)

Result	Tolerability	Side effects	Compliance
Excellent, n	26	None	25
Good, n	2	None	3
Satisfactory, n	0	None	0

Discussion

Children admitted to child care centres or kindergartens tend to get more upper respiratory tract infections and acute ear infections than children who are cared for in their own homes [16]. In addition to having an immature immune system [17], children in

group settings come in close contact with many children, share toys and touch each other during play, so they have a much greater chance of getting an infection from another child or toys. The present results demonstrate the efficacy of Bactoblis® in reducing acute respiratory infections during the treatment period and within six months of follow-up in children who had just started attending a kindergarten, reiterating the efficacy of this probiotic strain administered in powdered form in reducing acute respiratory infections shown in previous studies [18].

The long-term use of Bactoblis® restores the mucosal immunity of the upper respiratory tract and protects the mucous membranes from pathogenic bacterial colonization.

This was demonstrated in our previous study, where the microbiological examination of oropharyngeal smears in children treated with Bactoblis® showed restoration of mucosal immunity with a significant decrease in bacterial colonization by *Staphylococcus aureus*, *Haemophilus influenzae* and pneumococcus [19].

In numerous previously conducted clinical trials involving paediatric patients (3 to 10/14 years old) with recurrent upper respiratory tract infections, Bactoblis® lozenges (SsK12) prevented relapse of infections associated with GABHS and tonsillopharyngitis of another aetiology, acute otitis media, rhinitis and laryngitis [19, 20].

However, those clinical studies were conducted in children with a history of recurrent infections. To our knowledge, there is only one published study reporting benefits in healthy children without a history of recurrent infections, and in this study SsK12 was administered as a lozenge [18].

The results of our present study support the findings of Di Pierro *et al*., where young children attending kindergarden (daycare) with presumed higher exposure to pathogens had significantly fewer episodes of tonsillopharyngitis and otitis media when using Bactoblis® [18]. In our study, the reduction in the number of episodes of tonsillopharyngitis and otitis media was 80 and 90%, respectively, compared to the control group during the six-month period after using Bactoblis® sachets for 90 days. Hence, the powder formula tested in the present study, Bactoblis® sachets, seems to provide a similar level of clinical benefit as reported for SsK12 lozenges. Our results contradict preliminary findings by Cohen *et al*., where SsK12 used to prepare milk formula did not confer any advantage in terms of preventing the incidence of otitis media [21]. Another study where SsK12 was administered in powder form to young otitis media-prone children reported substantially lower colonization efficacy than typically achieved with lozenges [22].

Hence, the composition of the powder formula seems to have a significant impact on the ability of SsK12 to colonize the oral mucosa, and thereby, confer protection from infections. Furthermore, the duration of application may play a role, and repeated courses of administration may also be required. It should be noted that no side effects were recorded, even with prolonged administration of Bactoblis® sachets, and this is of particular significance in paediatric practice.

The 90-day treatment course allowed the authors to record an entirely predictable prophylactic effect over six months. Moreover, the data presented in **Table 5** show 'excellent' tolerability of the study product, and 89.3% of children were fully compliant with the treatment.

Preventing infection by non-antibiotic therapy is preferred to repeated treatment with antibiotics. Notably, during the entire observation period (90 days of application of Bactoblis® and six months of follow-up), a significant decrease in the frequency of administration of antibiotics and antipyretics in children was seen **(Table 4)**. Moreover, using probiotics not only allows regeneration of the natural microbiocenosis of the oral cavity and

nasopharynx, but it also helps reduce antibiotic resistance and avoid unwanted side effects to antibiotic therapy in children.

The study product Bactoblis® sachet contained vitamin D3 that may have an impact on non-specific and adaptive immune mechanisms [23]. Hence, we cannot rule out that vitamin D3 may have augmented the protective effects of SsK12 against respiratory infections observed in the present study. However, the rather low dose of vitamin D3 (5 µg) is unlikely to have exerted the clinical benefits observed here, which are most probably attributable to SsK12.

Conclusion

The results of this study support the preventive potential of SsK12 administered in a powder format to young healthy children who do not have a history of recurrent streptococcal infection or acute otitis media. Moreover, the SsK12 powder formula tested was found to be safe and well accepted. Altogether, Bactoblis® can be considered an effective prophylactic treatment for reducing the most common upper respiratory tract infections in young children attending kindergarden.

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