Preliminary pediatric clinical evaluation of the oral probiotic *Streptococcus salivarius* K12 in preventing recurrent pharyngitis and/or tonsillitis caused by *Streptococcus pyogenes* and recurrent acute otitis media

**Background:** The oral probiotic *Streptococcus salivarius* K12 has been shown clearly to antagonize the growth of *Streptococcus pyogenes*, the most important bacterial cause of pharyngeal infections in humans, by releasing two bacteriocins named salivaricin A2 and salivaricin B. Unpublished observations indicate that it can also antagonize the growth of other bacteria involved in acute otitis media. Because of its ability to colonize the oral cavity and its safety profile, we have tested its efficacy in reducing the incidence of streptococcal pharyngitis and/or tonsillitis and episodes of acute otitis media.

**Methods:** We enrolled 82 children, including 65 with and 17 without a recent diagnosis of recurrent oral streptococcal pathology. Of those with recurrent pathology, 45 were treated daily for 90 days with an oral slow-release tablet containing five billion colony-forming units of *S. salivarius* K12 (Bactoblis®), and the remaining 20 served as an untreated control group. The 17 children without a recent diagnosis of recurrent oral pathology were used as an additional control group. After 90 days of treatment, a 6-month follow-up period without treatment was included to evaluate a possible persistent protective role for the previously administered product.

**Results:** The 41 children who completed the 90-day course of Bactoblis showed a reduction in their episodes of streptococcal pharyngeal infection (about 90%) and/or acute otitis media (about 40%), calculated by comparing infection rates in the previous year. The 90-day treatment also reduced the reported incidence of pharyngeal and ear infections by about 65% in the 6-month follow-up period during which the product was not administered. Subjects tolerated the product well, with no side effects or dropouts reported.

**Conclusion:** Prophylactic administration of *S. salivarius* K12 to children with a history of recurrent oral streptococcal pathology reduced episodes of streptococcal pharyngeal infections and/or tonsillitis as well as episodes of acute otitis media.

**Keywords:** BLIS K12, bacteriocin-like inhibitory substance K12, *Streptococcus salivarius* K12, Bactoblis®, pharyngitis, tonsillitis, acute otitis media

**Introduction**

To date, the use of probiotic strains has almost exclusively focused on the gastrointestinal benefits of ingestion of selected bacteria obtained from intestinal sources. However, the potential for probiotic intervention at nonintestinal body sites suggests possible application of effector strains of species selected from alternative target tissues in order
to obtain more specific and durable benefits. Streptococcus salivarius K12, also known as BLIS (bacteriocin-like inhibitory substance) K12, was isolated in New Zealand from the mouth of a healthy child. It is known to release two lantibiotic bacteriocins named salivaricin A2 and salivaricin B, with high efficiency. Via these two lantibiotics, encoded by a 190 kb megaplasmid, BLIS K12 can effectively counteract the growth of β-hemolytic (group A) Streptococcus pyogenes, a common cause of pharyngitis, tonsillitis, and acute otitis media. This inhibitory action is strongly linked to the release of lantibiotics because BLIS K12 P(−), the same strain without the 190 kb plasmid, does not show any antagonism of growth of Streptococcus pyogenes.

In addition to its action against S. pyogenes, BLIS K12 can also inhibit growth of Haemophilus influenzae, Streptococcus pneumoniae, Moraxella catarrhalis, Micrococcus luteus, Streptococcus anginosus, Eubacterium salivarium, and Micromonas micros. Many of these are potential pathogens in the ear and oral cavity, causing acute otitis media and halitosis. Preliminary investigations have shown that BLIS K12 colonizes the upper respiratory tract of infants (oral cavity, nasopharyngeal and adenoid tissues) and with good persistence, given that after only 3 days of administration, it can still be detected 32 days later. Therefore, because of its good colonization capability and very high safety profile, combined with its reputed ability to counteract oral pathology, we decided to evaluate the preventive role of BLIS K12 when administered to children having a history of recurrent streptococcal pharyngitis and/or tonsillitis. Our main endpoint was the number of episodes of streptococcal infections and acute otitis media.

**Materials and methods**

This research was carried out in the field of routine clinical practice, following international guidelines and in line with the principles outlined in the Declaration of Helsinki, such that approval from local ethics boards was not required. The study was carried out in five Italian day care centers, located in Cuneo, Brescia, Verona, Novara, and Torino, where it is not mandatory to obtain ethical approval in order to perform experimental protocols on nutraceutical products. Inclusion criteria were: informed signed consent from parents; age 3–12 years; total absence of symptoms of infective disease at the time of enrollment; and diagnosis of recurrent streptococcal (group A hemolytic Streptococcus) pharyngitis and/or tonsillitis in the previous year. Exclusion criteria were: lack of parental signature of informed consent; age below 3 years or above 12 years; diagnosis of obstructive sleep apnea syndrome, respiratory, and/or systemic severe pathologies; asthma; and being a healthy carrier of S. pyogenes. Eighty-two children were enrolled in the study, which was conducted from October 2011 to August 2012. Sixty-five were with and 17 were without a diagnosis of recurrent pathology were selected as the treated group and 20 as the not-treated group. The 17 children enrolled without a diagnosis of recurrent pathology were selected as a not-treated group. The recurrent-treated (n = 45), recurrent-not-treated (n = 20), and not-recurrent-not-treated (n = 17) subjects were followed for 90 days. After this period, 41 of the 45 children in the recurrent-treated group were considered appropriate for the aim of the study, their parents having declared total adherence to the treatment protocol.

In a second part of the study, in accordance with parental consent, 16 of the 41 children in the recurrent-treated group and 14 of the 20 children in the recurrent-not-treated group were enrolled for follow-up lasting a further 6 months, during which the product was not administered. The product, Bactobis®, in agreement with Italian law (169/2004), was notified to the Minister of Health on July 5, 2011 and registered as a food supplement. At the time of manufacturing, Bactobis contains five billion colony-forming units per tablet of S. salivarius K12 ATCC BAA-1 024 (BLIS Technologies Ltd, North Dunedin, New Zealand) and is manufactured by SIIT, (Trezzano S/N, Milan, Italy). In accordance with the treatment protocol, the product is administered as one tablet daily for 90 days. The product, an oral, round-shaped, vanilla-tasting, slow-release tablet (dissolving in about 5 minutes) is administered just before bedtime (ie, after teeth brushing and/or mouthwashing). Correct administration of the product requires that the tablet is not chewed or directly swallowed, but is sucked for about 4–5 minutes. Before administration of the first tablet, a chlorhexidine 0.2% mouthwash is recommended in order to enhance the colonization process of the strain, reducing extreme competition from endogenous S. salivarius inhabiting the mouth.

The primary study endpoints were evaluation by medical visits, results of a throat swab, and otoscopic signs of acute otitis media, and episodes of pharyngitis, tonsillitis, and/or acute otitis media in the recurrent-treated, recurrent-not-treated, and not-recurrent-not-treated groups during 90 days of treatment with the product and during
the 6-month follow-up period in which the product was not administered. Secondary study endpoints were tolerability, compliance, and side effects during the 90 days of treatment. As regards to tolerability and compliance, we defined four terms able to describe the different conditions, ie, very good, good, acceptable, and unacceptable.

**Statistical analysis**

The statistical analysis was performed using the standardized incidence ratio and its confidence interval 100 (1 − α)% as proposed by Vandenbroucke.\(^1\)\(^6\) If the range includes 100%, it is highly likely that the difference between observed and expected values is due to chance (random fluctuations in the data). On the other hand, if the confidence interval does not include 100%, it is very likely that the difference is not due to chance. The statistical comparisons between treatment and past controls are shown in Tables 2–7, where the real number of episodes is reported along with, in table notes, the same value/4 to allow a real statistical comparison, otherwise not possible comparing values obtained in 12 months with values obtained in 3 months.

**Results**

In this study, we attempted to establish the preventive role played by BLIS K12 when administered to children with a history of recurrent pharyngitis and/or tonsillitis of streptococcal origin. The main endpoint was the number of episodes of oral streptococcal pathology and/or acute otitis media. The 82 children enrolled in this study were assigned to one of three groups, ie, recurrent-treated, recurrent-not-treated, or not-recurrent-not-treated. Four children were eliminated because of failure to adhere strictly to therapy. As shown in Table 1, the demographic characteristics of the 78 therapy-adherent enrolled children did not differ statistically.

Statistically significant results were seen during the 90 days of treatment with BLIS K12 (Table 2) in terms of episodes of streptococcal pharyngitis and/or tonsillitis in the 41 children having had more than three episodes of streptococcal pharyngitis and/or tonsillitis in the previous year. 41 children had

### Table 1 Demographic parameters of enrolled children

| Group                        | n   | M  | F  | Age, years* |
|------------------------------|-----|----|----|-------------|
| Recurrent-treated            | 41  | 19 | 22 | 4.5 ± 1.4   |
| Recurrent-not-treated        | 20  | 9  | 11 | 4.2 ± 1.3   |
| Not-recurrent-not-treated    | 17  | 9  | 8  | 5.1 ± 1.5   |

**Note:** *Expressed as the median ± standard deviation.

**Abbreviations:** n, number of children; M, males; F, females.

### Table 2 Episodes of streptococcal oral pathology during 90 days of treatment with BLIS K12 in children (n = 41) with recurrent streptococcal pharyngitis and/or tonsillitis

|                        | Pharyngitis/tonsillitis in the previous year | Pharyngitis/tonsillitis during BLIS K12 |
|------------------------|---------------------------------------------|----------------------------------------|
| Number of episodes     | 152 (1 year)                                | 3 (90 days)                             |
| Incidence/month/child  | 0.309                                       | 0.024*                                  |
| Delta (%)              | −92.2                                       |                                        |

**Notes:** *P < 0.0001 considering 152 episodes and P < 0.01 considering 38 episodes (152/4).

**Abbreviation:** BLIS, bacteriocin-like inhibitory substance.

### Table 3 Episodes of streptococcal oral pathology during 90 days in children (n = 20) with recurrent streptococcal pharyngitis and/or tonsillitis not treated with BLIS K12

|                        | Pharyngitis/tonsillitis in the previous year | Pharyngitis/tonsillitis in 90 days |
|------------------------|---------------------------------------------|------------------------------------|
| Number of episodes     | 78                                          | 27                                 |
| Incidence/month/child  | 0.325                                       | 0.45*                              |
| Delta (%)              | +38.5                                       |                                    |

**Note:** *P < 0.001 considering 78 episodes and not significant considering 19.5 episodes (78/4).

**Abbreviation:** BLIS, bacteriocin-like inhibitory substance.

### Table 4 Episodes of streptococcal oral pathology during 90 days in children (n = 17) without recurrent streptococcal pharyngitis and/or tonsillitis not treated with BLIS K12

|                        | Pharyngitis/tonsillitis in previous year | Pharyngitis/tonsillitis in 90 days |
|------------------------|------------------------------------------|------------------------------------|
| Number of episodes     | 4                                         | 4                                  |
| Incidence/month/child  | 0.020                                     | 0.078*                             |
| Delta (%)              | +290                                      |                                    |

**Note:** *Not significant considering four episodes and P < 0.05 considering one episode (4/4).

**Abbreviation:** BLIS, bacteriocin-like inhibitory substance.

### Table 5 Episodes of acute otitis media during the 90 days of treatment with BLIS K12 in children (n = 41) with recurrent streptococcal pharyngitis and/or tonsillitis

|                        | AOM in previous year | AOM during BLIS K12 |
|------------------------|----------------------|---------------------|
| Number of episodes     | 27                   | 4 (90 days)         |
| Incidence/month/child  | 0.055                | 0.033*              |
| Delta (%)              |                     | −40                 |

**Note:** *P < 0.01 considering 27 episodes and not significant considering 6.75 episodes (27/4).

**Abbreviations:** AOM, acute otitis media; BLIS, bacteriocin-like inhibitory substance.
had 152 episodes in 12 months, and during the 90 days of treatment, only three episodes of oral streptococcal infection were diagnosed, with the calculated incidence per month per child dropping from 0.3109 to 0.024.

The control group, (children enrolled with a diagnosis of recurrent oral streptococcal disease but not-treated) showed an increase in terms of episodes of streptococcal pharyngitis and/or tonsillitis in comparison with the previous year, as shown by the incidence per month per child (Table 3). This increase, from 0.325 to 0.45, is likely due to seasonal reasons being the first value calculated, considering also warm months where the incidence normally decreases, while the second value calculated is only considered during the three winter months.

The other controls, (not-recurrent not-treated children enrolled without a diagnosis of recurrent oral streptococcal disease) also showed an increase in terms of episodes of streptococcal pharyngitis and/or tonsillitis in comparison with the previous year, as shown by the incidence per month per child (Table 4). This value increased from 0.020 to 0.078. This is again likely due to seasonal reasons being the first value calculated considering also warm months where the incidence normally drops down, while the second value is calculated only considering the 3 winter months.

Relevant (~40%) results were seen during 90 days of treatment with BLIS K12 (Table 5) in terms of episodes of acute otitis media in the 41 children enrolled for having had no fewer than three episodes of streptococcal pharyngitis and/or tonsillitis in the previous year. In fact, these 41 children had had 27 episodes in 12 months and four episodes during the 90 days of treatment, with the incidence per month per child dropping from 0.055 to 0.033.

In the control (not-treated) children enrolled with a diagnosis of recurrent oral streptococcal disease, there was an increase in terms of episodes of acute otitis media in comparison with the previous year, as shown by the incidence per month per child (Table 6). This increase, from 0.054 to 0.117, is again likely due the seasonal reasons explained earlier.

In the other control (not-recurrent-not-treated) children enrolled without a diagnosis of recurrent streptococcal oral disease, there was an increase in terms of episodes of acute otitis media in comparison with the previous year, as shown by the incidence per month per child (Table 7). This value increased from 0.020 to 0.039, again likely for seasonal reasons.

The evaluation of tolerability, compliance, and side effects is reported only for the recurrent-treated group as enrolled in terms of number (n = 45). As shown in Table 8, treatment with BLIS K12 seemed to be well tolerated and devoid of side effects. The four children who were excluded from the study were removed because they did not adhere strictly to the study therapy, not because of side effects. According to their parents, they have missed more than 20 days of treatment.

With parental consent, only 16 of the 41 recurrent-treated children and 14 of the 20 recurrent-not-treated children, respectively, continued into the 6-month follow-up period to determine if BLIS K12 had a protective role. As shown in Table 9, the 14 children in the recurrent-not-treated group had eight episodes of oral streptococcal pathology and 10 episodes of acute otitis media over 6 months. The 16 children in the recurrent-treated group were confirmed to be protected, having had four oral streptococcal infections, two episodes of acute otitis media, and one case of scarlet fever, with a reduction by about 65% of incidence.

Discussion

Acute pharyngitis and/or tonsillitis in children are among the most frequent recurrent illnesses presenting to general practitioners and pediatricians. Group A beta-hemolytic streptococci is the most common bacterial cause of acute pharyngitis and tonsillitis. Antibiotic therapy is typically prescribed to treat the acute infection and to prevent development of sequelae, such as rheumatic fever. However, when patients present with sore throat, physicians must also consider a

Table 6 Episodes of AOM during 90 days in children (n = 20) with recurrent streptococcal pharyngitis and/or tonsillitis not treated with BLIS K12

| AOM in previous year | AOM in 90 days |
|----------------------|---------------|
| Number of episodes   | 13            |
| Incidence/month/child| 0.054         |
| Delta (%)            | +116          |

Note: *p < 0.05 considering 13 episodes and not significant considering 3.25 episodes (13/4).
Abbreviations: AOM, acute otitis media; BLIS, bacteriocin-like inhibitory substance.

Table 7 Episodes of acute otitis media during 90 days in children (n = 17) without recurrent streptococcal pharyngitis and/or tonsillitis and not treated with BLIS K12

| AOM in previous year | AOM in 90 days |
|----------------------|---------------|
| Number of episodes   | 4             |
| Incidence/month/child| 0.020         |
| Delta (%)            | +95           |

Note: *p < 0.05 considering four episodes and not significant considering one episode (4/4).
Abbreviations: AOM, acute otitis media; BLIS, bacteriocin-like inhibitory substance.
wider range of potential pathogens, including viruses and other bacteria. Apart from a few rare non-group A streptococcal infections, antimicrobial therapy is of no proven benefit to treat causes of pharyngitis other than those provoked by group A beta-hemolytic streptococci. Inappropriate antibiotic therapy imposes unnecessary expense and also contributes to the emergence of antibiotic-resistant bacteria, which are being reported with increasing frequency. Consequently, a conservative approach to managing sore throats is being promoted increasingly, with antibiotic therapy held in reserve until group A beta-hemolytic streptococcal infection is confirmed. Prevention of recurrent infection by nonantibiotic therapy is preferable than having repeated doses of antibiotics. The ability of the normal bacterial microflora in the oral cavity to inhibit the growth of group A streptococci has been well described. Prevention of recurrent infection by nonantibiotic therapy is preferable than having repeated doses of antibiotics. The ability of the normal bacterial microflora in the oral cavity to inhibit the growth of group A streptococci has been well described.

Table 8 Tolerability, compliance and side effects during the 90 days of treatment with BLIS K12 in children (n = 45) with recurrent streptococcal pharyngitis and/or tonsillitis as reported by parents and established by clinician

| Tolerability | Compliance | Side effects |
|--------------|------------|-------------|
| Very good    | n = 42     | None        |
| Good         | n = 3      | None        |
| Acceptable   | n = 2      | None        |
| Unacceptable |           |             |

**Abbreviations:** n, number of children; BLIS, bacteriocin-like inhibitory substance.

Three as yet unpublished studies (data on file at BLIS Ltd, Dunedin, New Zealand) have shown that S. salivarius K12 produces BLIS with activity against S. pneumoniae, S. pyogenes, H. influenzae, and M. catarrhalis, ie, the principal agents known to be causative of acute otitis media. On this basis, we decided to test the role played by S. salivarius K12 in the prevention of streptococcal oral pathology and acute otitis media. Sixty-five children with a history of recurrent oral streptococcal pathology were given Bactoblis, a nutritional supplement containing as its unique active ingredient five billion colony-forming units per tablet of S. salivarius K12 for 90 days.

We also checked the incidence of acute otitis media during treatment. This preliminary investigation was not placebo-controlled, so risks determining incidence values potentially affected by normal fluctuations of mouth and ear pathology. To minimize such a risk, we compared the incidence values in the treated group with those of two untreated groups. The first untreated group had the same characteristics as the treated one, being comprised of children enrolled because of recurrent oral streptococcal pathology. For further control of possible fluctuations in incidence, we monitored disease episodes in a second group of children in whom oral streptococcal pathology had not been recurrent. This methodological approach allowed us to demonstrate that the approximately 90% reduction in incidence observed by administering BLIS K12 for 90 days was not due to random fluctuations during the study period in 2012.

In the second part of the study, we investigated whether administration of BLIS K12 resulted in durable protection in the months following treatment. This evaluation lasted...
from March to August 2012, and demonstrated that prior use of the product provided durable protection against oral streptococcal pathology and acute otitis media, with about 65% reduction compared with controls.

This study has several limitations, in that it is not randomised nor placebo-controlled, and was also not blinded. Furthermore, it was carried out in a relatively small number of children with recurrent oral streptococcal pathology but not specifically with recurrent acute otitis media, with episodes of the latter only being an endpoint. However, in spite of these limitations, the results demonstrate for the first time that use of S. salivarius K12, an oral probiotic, can reduce the incidence of bacterial throat and ear infections in children with a history of recurrent oral streptococcal infection. Our research group is currently organizing a larger, randomized, blinded, placebo-controlled study in children with recurrent oral streptococcal pathology to confirm more precisely what has been observed in this preliminary investigation. This study will be performed with approval and a reference number from our local ethics committee.

**Disclosure**

FDP is the main formulator of the study product. Otherwise, the authors report no conflicts of interest in this work.

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