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ORIGINAL ARTICLE

Use of *Streptococcus salivarius* K12 to reduce the incidence of pharyngo-tonsillitis and acute otitis media in children: a retrospective analysis in not-recurrent pediatric subjects

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ABSTRACT

BACKGROUND: Previous trials, performed in subjects affected by recurrent streptococcal pharyngo-tonsillar infection, have shown that the use for 90 days of *Streptococcus salivarius* K12 (K12), an oral colonizing probiotic producing lantibiotic bacteriocins, reduces the occurrence of streptococcal and viral pharyngitis and acute otitis media (AOM). The aim was to evaluate the role of K12 in reducing the incidence of streptococcal and viral pharyngo-tonsillitis and AOM when administered in two separate trimesters, from October to December and then from April to June, in pediatric subjects with non-recurrent streptococcal infection.

METHODS: We retrospectively analyzed the incidence of pharyngo-tonsillitis and AOM in 133 children by comparing the number of episodes occurring between September 1st, 2014 and August 31st, 2015, when no treatment with K12 was given, with the period between September 1st, 2015 and August 31st, 2016, when K12 was administered.

RESULTS: Analysis of the findings for the 133 children demonstrated that K12 use decreased the incidence of pharyngo-tonsillitis by about 90% (P<0.001) and the occurrence of AOM by about 70% (P<0.001) and confirms the high safety profile of the strain.

CONCLUSIONS: As already demonstrated in subjects with recurrent streptococcal pharyngo-tonsillar infection, K12, if administered for two trimesters out of 12 months, is associated with a reduced incidence of pharyngitis and AOM in pediatric subjects with non-recurrent streptococcal infection.

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Key words: Probiotics - Streptococcal infections - *Streptococcus salivarius* - Child.

Streptococcus salivarius K12 (K12) is an oral colonizing, persistent, antibiotic-sensitive and safe strain [1-4] which produces two megaplasmid-encoded class I lantibiotics, namely salivaricin A2 and salivaricin B, whose expression limits the growth of *Streptococcus pyogenes*, which is involved in the etiopathogenesis of pharyngo-tonsillar infection, and, to a smaller degree, *Haemophilus influenzae*, *Streptococcus pneumoniae*, and *Moraxella catarrhalis*, which cause acute otitis media (AOM).[5-7] Previous works have

shown that 90-day use of K12 in subjects with a diagnosis of recurrent streptococcal infection (that is with more than two episodes of streptococcal pharyngo-tonsillitis over 6 months or more than three episodes over 1 year) or in children with a diagnosis of secretory otitis media, decreases the number of episodes of streptococcal pharyngo-tonsillitis and AOM.[8-10] In addition, very preliminary results have also suggested that K12 can prevent viral pharyngeal infection.[11] This last action could be due to its ability to elevate salivary γ-interferon concentrations without modifying either IL-1β or TNF-α levels, and by substantially lowering IL-8 release.[7] These biochemical effects on salivary cytokines could possibly explain the positive action shown by the strain when administered in children affected by periodic fever with aphthous stomatitis, pharyngitis, and adenitis (PFAPA).[12] A wider range of clinical action has been recently observed in a trial where 90-day administration of the strain to children with a diagnosis of recurrent streptococcal pharyngeal infection, reduced the number of episodes of some non-streptococcal diseases, including rhinitis, tracheitis, flu, enteritis, and laryngitis.[13] To date, just one trial has examined the benefits of K12 administered for more than 90 days. According to the results of that trial, administration of K12 for 180 days to 3-year-old healthy children during their first year of kindergarten and without a diagnosis of recurrent streptococcal pharyngo-tonsillar infection, resulted in a significant decrease in the number of episodes of streptococcal pharyngo-tonsillitis and AOM recorded in the same year. In the present study, we have retrospectively analyzed the incidence of pharyngo-tonsillitis and AOM in subjects with recurrent non-streptococcal infection by comparing the number of episodes between September 1st, 2014 and August 31st, 2015, when no treatment with K12 was given, with the period between September 1st, 2015 and August 31st, 2016, when K12 was administered daily for a total of 180 days in two separate trimesters, from October to December 2015 and then from April to June 2016.

Materials and methods

BLIS K12 was formulated by SIIT (Trezzano sul Naviglio, Milan, Italy) as slowly-dissolving oral tablets and notified to the Italian Ministry of Health on July 5th, 2011 as Bactoblis® by Omeopiacenza (Pontenure, Italy), according to the provisions of law No. 169 of 2004 (notification no. 53435). The Bactoblis® preparation used in the clinical trial contained 1 billion or more CFU/tablet of *Streptococcus salivarius* K12 (Blis Technologies, Otago, New Zealand) as declared for the expiry date.

Clinical trial

Our retrospective analysis was conducted on 133 (68 males and 65 females) children living in the Genoa area of Italy at the end of 2016. The children were treated with K12 for two trimesters, from October to December 2015 and then from April to June 2016. The number of episodes of streptococcal and viral pharyngo-tonsillitis and AOM from September 1st, 2015 and August 31st, 2016 were compared with the number recorded in the same 133 children the year before when K12 was not administered. The retrospective analysis was conducted in accordance with the Declaration of Helsinki and was approved by the local ethics committee in Genoa (Comitato Etico Regione Liguria Sezione n° 1), Italy. The parents of all study participants were informed of the retrospective analytical methods and signed the appropriate consent and privacy policy documents.

Inclusion and exclusion criteria

All recruited individuals were 3-14 years of age and attending our pediatric outpatient clinics. The 133 children analyzed were administered K12 to prevent possible pharyngo-tonsillar infection or AOM. They had all been diagnosed at least once with pharyngo-tonsillar infection and/or AOM in the previous year. None of the enrolled children had been diagnosed with recurrent streptococcal pharyngo-tonsillitis. Exclusion criteria were as follows: a history of rheumatic disorders, bronchospasm,

severe asthma or allergy requiring corticosteroids; past tonsillectomy or an indication for adeno-tonsillectomy; or severe respiratory or systemic disorders. Individuals were also excluded if they were immunocompromised or had a condition favoring AOM, including severe atopy, acquired or congenital immunodeficiency, cleft palate, craniofacial abnormalities, obstructive adenoids, a chronically ruptured eardrum, sleep apnea, or tympanostomy tubes.

Treatments and diagnosis

The children were told how to administer the oral probiotic. The tablets were to be taken from October to December 2015 for 90 consecutive days and then from April to June 2016 for another 90 days. After teeth were brushed, one tablet was to be dissolved slowly in the mouth just before going to sleep. The tablet was not to be chewed or swallowed whole. Nothing else was to be drunk or swallowed after use of the product. It was recommended that just before the first tablet was administered, the children rinsed their mouth with a 0.2% chlorhexidine mouthwash in order to increase colonization by the strain, by decreasing competition from oral *Streptococcus salivarius*. For the entire 12 months, if the children showed any symptoms of infection they were to be brought immediately to the clinic for examination and a pharyngeal rapid test. A rapid swab positive for group A streptococcus (Test Strep-A, Gima, Gessate, Italy) confirmed streptococcal pharyngo-tonsillar infection. Antibiotic treatment was prescribed if the test was positive. After completion of antibiotic therapy, treatment with K12 resumed and continued until the previously designated 90th day of the trimester. Viral infection was diagnosed according to the following criteria: negative rapid swab for streptococcal disease, absence of petechiae on the palate, absence of submandibular lymphadenopathy, mild dysphagia, and absence of headache, abdominal pain, or hyperpyrexia. Patients presenting with viral pharyngitis had mild dysphagia, low-grade fever, and moderate pharyngeal hyperemia. Acetaminophen or ibuprofen was used to treat viral infections presenting with fever and/or pharyngo-laryngeal pain. Pneumatic otoscopy and clinical signs were used to diagnose AOM, which was treated in accordance with the Italian pediatric guidelines. Any other conditions or diseases were also treated in accordance with Italian pediatric guidelines.

Study aims

The primary endpoint of the study was to evaluate the role of K12 in preventing streptococcal and viral pharyngo-tonsillitis and AOM from September 2015 to August 2016. K12 was administered in two separate trimesters, from October to December 2015 and then from April to June 2016, to subjects with non-recurrent streptococcal infection. The incidence of pharyngo-tonsillar infection and AOM in the same children the previous year before K12 was administered was also retrospectively analyzed. The secondary endpoints were: side effects, tolerability and compliance, number of days absent from kindergarten, school, or work, and doses of antibiotics and anti-inflammatory drugs administered. All secondary endpoints were analyzed by the responsible of the study according to a daily diary filled by parents.

Statistical analysis

The characteristics of enrolled subjects (age, birthweight, type of delivery, breastfed or not, ethnicity, nursery, toy library/kindergarten attendance, use of antihistamines or anti-leukotrienes drugs, and other possible ongoing therapies) were examined using Fisher's exact test. Differences in episodes of infection, days lost and drugs used were determined using Wilcoxon's signed rank sum test. JMP 10 for Mac OsX was used for statistical analysis and statistical significance was set at 95%.

Results

We retrospectively analyzed the incidence of pharyngo-tonsillitis and AOM by compar-

ing the number of episodes occurring between September 1st, 2014 and August 31st, 2015, when no treatment with K12 was given, with the period between September 1st, 2015 and August 31st, 2016, when K12 was administered daily (Figure 1). The characteristics of the 133 children are reported in Table I. Table II shows that the number of episodes of streptococcal and non-streptococcal pharyngo-tonsillar infections was significantly reduced following prophylaxis with K12 by about 90% in both cases. A significant reduction of about 70% was also observed for AOM. As shown in Table III, compliance and tolerability were both excellent for all but one of the children, a 6-year-old boy who experienced a single episode of mild bronchospasm. Lastly, as shown in Table IV, prophylaxis with K12 significantly reduced the use of both antibiotics and antipyretics/anti-inflammatory drugs by more than 80% and significantly decreased the number of school or work days lost by children or adults by about 85% and 75%, respectively. Our multi-variate analysis (data not shown) showed that the positive results obtained re-

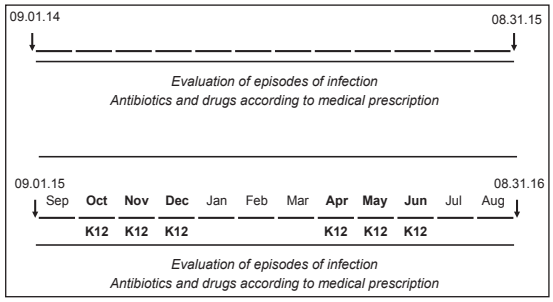

Figure 1.—Study scheme.

TABLE I.—*Characteristics of children enrolled in the study.*

	Males	Females	P value
Total number	68	65	
Age, years)	8.1±3.1	8.2±3.9	NS
Birthweight, kg	3.4±0.3	3.4±0.4	NS
Eutocia	38	38	NS
Dystocia	30	27	NS
Breastfed	48	50	NS
Not breastfed	20	15	NS
Caucasian	67	61	NS
Nursery	14	14	NS
Toy library	4	2	NS
Kindergarten	58	52	NS
PCV13	68	65	NS
Anti-histamine	15	6	NS
Anti-leukotrienes	7	7	NS
Other therapies	22	13	NS

Values are number or mean±SD.
NS: non-significant difference; PCV13: 13-valent pneumococcal vaccine.

TABLE III.—*Tolerability, compliance, and side effects recorded annually in children (N.=133) treated for two trimesters (October-December 2015 and April-June 2016) with K12.*

	Tolerability	Compliance	Side effects
Excellent, N.	132	133	None
Good, N.	0	0	None
Acceptable, N.	1	0	Bronchospasm [a]

[a] This event occurred only once after a few days of treatment with K12 and never happened again.

TABLE IV.—*Days with antibiotics/antipyretics/anti-inflammatory drugs and pre-school/school/working days lost by children and adults in 2014-2015 (no K12) and 2015-2016 (K12 administered for two trimesters).*

Parameter	2014-2015	2015-2016	Δ	P value
Antibiotics, days	3334	395	-88.1%	<0.01
Antipyretics or anti-inflammatory drugs, days	489	75	-84.7%	<0.01
School or pre-school, days	1493	224	-84.9%	<0.01
Work, days	772	193	-75%	<0.01

TABLE II.—*Annual episodes of pharyngo-tonsillitis, caused or not by Streptococcus pyogenes, and occurrence of AOM in 133 children with (2015-2016) or without (2014-2015) K12 administration daily from October to December 2015 and then from April to June 2016.*

Group	2014-2015	2015-2016	P value	Δ
GABHS	1.8±1.2	0.2±0.6	<0.001	-88.9%
Not GABHS	3.1±1.6	0.2±0.5	<0.001	-93.5%
AOM	0.7±1.2	0.2±0.5	<0.001	-71.4%

AOM: acute otitis media; GABHS: pharyngo-tonsillar infection with group A beta-hemolytic streptococcus; not GABHS: pharyngo-tonsillar infection negative to group A beta-hemolytic streptococcus.

garding episodes of infection, drug use, and days lost (pre-school, school, and work) were not associated with sex, age, or any other variable listed in Table I.

Discussion

The concept of oral probiotics is relatively new.[7] Probiotics are known to help the host combat gastrointestinal disorders including diarrhea, constipation, and irritable bowel syndrome.[14] The development of the strain K12, selected from the oral cavity commensal species *Streptococcus salivarius*, suggests oral health benefits can be achieved with probiotic therapy. Isolated originally from the oral cavity of a young child without recent experience of infection with *Streptococcus pyogenes*, the strain K12 has subsequently been shown inhibit *Streptococcus pyogenes* and oral cavity bacterial pathogens associated with AOM. Its previous clinical use has shown that, used as a prophylactic agent in subjects diagnosed with recurrent streptococcal pharyngeal and/or tonsillar infection, it can reduce the incidence of streptococcal pharyngo-tonsillar episodes by more than 80% during the time of K12 administration and by about 60% during a 6-month [8, 9] or 9-month follow-up.[15] In these studies, where subjects did not have recurrent AOM, the protective effect of K12 on episodes of AOM only showed a tendency and never reached statistical significance. However, it was observed that prophylactic use of K12 significantly decreased the incidence of AOM in subjects with more severe ear involvement, such as secretory otitis media.[10] In all of these studies, K12 was administered daily for a maximum of 90 days. Recently, a prospective study examined the role of K12 prophylaxis in healthy 3-year-olds without recurrent infections. The study demonstrated that daily administration of K12 for 180 consecutive days significantly reduced the incidence of streptococcal infections and AOM.[16] In our retrospective study we wanted to verify if administration of K12 for 180 days divided into two different trimesters, October-December and April-June, periods when more streptococcal infection often occurs, could protect subjects with no features of recurrent streptococcal infection from both pharyngo-tonsillar infection and AOM. Our results confirm our hypothesis. In subjects with non-recurrent streptococcal infections, the prophylactic administration of K12 during trimesters with increased incidence of infection significantly reduced the number of streptococcal and viral pharyngo-tonsillar infections and AOM episodes. Consequently, there was also a significant reduction in antibiotic and drug use and a decrease in the number of pre-school, school, and work days lost.

The strain K12 is endowed with a "bacteriocins action" exerted *versus Streptococcus pyogenes* and partially *versus Moraxella catarrhalis*, *Haemophilus influenzae* and *Streptococcus pneumoniae*.[7] This, at least in part, could explain the effects observed against streptococcal oral disease and AOM. Moreover, some recent investigations [11, 12] have clearly shown that strain K12 could antagonize also not streptococcal diseases like viral sore throat and PFAPA may be due to its ability to elevate salivary γ-interferon concentrations without modifying either IL-1β or TNF-α levels, and by substantially lowering IL-8 release.[7] This could a possible explanation for the effect observed in the not streptococcal infections.

Limitations of the study

Our work has some limitations, particularly the lack of a control group. We know the number of illnesses such as streptococcal pharyngotonsillar infection and AOM in children tends to reduce spontaneously year on year, as seen in one of our previous studies. In that study, a 90% reduction in incidence was observed in children treated with K12 compared with the number of episodes seen in the same children the previous year, but a 50% reduction was also observed in the control group where children were untreated but one year older. In our current study, the reduction percentage was calculated by comparing the number of episodes of infection seen in 2014-2015 with those evaluated in 2015-2016. This means that the same possible bias could be present in the study. However, even if 50% of

the reduction we found is due to an age effect, the other 50% should be due to K12 administration. Last, cannot be excluded that during the year of the study a reduced rate of infection could be occurring. On the basis of our experience anyway the rates of infection of the two years considered were not different. Finally, as shown, we have demonstrated that K12 is a safe probiotic, with very high tolerability.

Conclusions

In conclusion, as previously demonstrated in subjects with recurrent streptococcal pharyngo-tonsillar infection, the use of K12, if prophylactically administered for two trimesters out of 12 months, is associated with reduced incidence of pharyngitis and AOM in subjects with non-recurrent streptococcal infection, leading to a significant reduction in the use of antipyretics, antibiotics, and anti-inflammatory drugs, together with a decrease in the number of pre-school, school, and work days lost.

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Authors' contributions.—Francesco Di Pierro wrote the paper and prepared the figure and tables. Paolo Risso performed the statistical analysis. All the other authors were involved in the diagnosis and treatment.

Conflicts of interest.—Francesco Di Pierro is the main formulator of the tested product and is on the scientific council of the company (Omeopiacenza) which markets the tested product in Italy. The other authors do not report any conflict of interest.

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