

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

Francesco Di Pierro¹
Guido Donato²
Federico Fomia³
Teresa Adami⁴
Domenico Careddu⁵
Claudia Cassandro⁶
Roberto Albera⁶

¹Scientific Department, Velleja Research, Milano, ²ASL I, Cuneo, ³ASL 3, Brescia, ⁴Infective Diseases, Verona, ⁵ASL I3, Novara, ⁶Surgical Science Department, Università degli Studi, Torino, Italy

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cause of pharyngeal infections in humans, by releasing two bacteriocins named salivaricin A2 and salivaricin B. Unpublished observations indicate that it can also antagonize the growth of other bacteria involved in acute otitis media.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.¹ 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

Correspondence: Francesco Di Pierro
Velleja Research, Viale Lunigiana 23,
20125, Milano, Italy
Tel +39 34 9552 7663
Fax +39 05 2351 1894
Email f.dipierro@vellejaresearch.com

to obtain more specific and durable benefits.² *Streptococcus salivarius* K12, also known as BLIS (bacteriocin-like inhibitory substance) K12, was isolated in New Zealand from the mouth of a healthy child.³ It is known to release two lantibiotic bacteriocins named salivaricin A2 and salivaricin B, with high efficiency.⁴ Via these two lantibiotics, encoded by a 190 kb megaplasmid,⁵ BLIS K12 can effectively counteract the growth of β -hemolytic (group A) *Streptococcus pyogenes*, a common cause of pharyngitis, tonsillitis, and acute otitis media.⁶ 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*.⁷

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 saburreum*, and *Micromonas micros*.⁸ Many of these are potential pathogens in the ear and oral cavity, causing acute otitis media⁹ and halitosis.¹⁰ Preliminary investigations have shown that BLIS K12 colonizes the upper respiratory tract of infants (oral cavity, nasopharyngeal and adenoid tissues)¹¹ and with good persistence, given that after only 3 days of administration, it can still be detected 32 days later.¹² Therefore, because of its good colonization capability and very high safety profile,^{13,14} combined with its reputed ability to counteract oral pathology,¹⁵ 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 not less than three episodes of recurrent pharyngitis and/or tonsillitis in the previous year confirmed by throat swab (positive for group A hemolytic *Streptococcus*). In agreement with their parents, 45 of the 65 children with recurrent oral 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, Bactoblis®, 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, Bactoblis 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 $100(1 - \alpha)\%$ as proposed by Vandenbroucke.¹⁶ 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. These 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 and 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.

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	7
Incidence/month/child	0.054	0.117*
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.

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 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	2
Incidence/month/child	0.020	0.039*
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.

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	n = 42	None
Good	n = 3	n = 1	None
Acceptable		n = 2	None
Unacceptable			

Abbreviations: n, number of children; 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 established previously. Most of this inhibitory activity has been attributed to BLIS-producing *S. salivarius*.¹⁷ Because *S. salivarius* is a member of the normal bacterial flora found in the oral cavity and is considered to be essentially nonpathogenic, it is regarded as an excellent candidate for bacterial interference-mediated prevention of recurrent pharyngitis and tonsillitis.

Our study is based upon the observation that certain strains of *S. salivarius* are capable of preventing the growth of bacteria associated with sore throat due to their production of BLIS. In particular, it has been shown that *S. salivarius*

K12 reduces group A streptococcus acquisition and also the prevalence of sore throat.^{18–21} Therefore, the feasibility of using this harmless strain as a prophylactic agent was investigated in this preliminary study. Ear infections are also common in children, and in severe cases can lead to deafness. Acute otitis media is the most common bacterial infection in young children. It is thought that the bacteria from the nasopharyngeal tissue that infect the middle ear do so via the eustachian tubes. In Italy, over 90% of cases of acute otitis media result in antibiotic treatment. Relapses are common, and repeat treatment may contribute to a reservoir of resistant microorganisms. Published studies have shown that at least 50% of patients acquire a new otitis media infection within 3 months of a previous episode.²² The ability of the normal microflora of the upper airways to inhibit growth of potential pathogens in vitro has been well described.²³ Most of this inhibitory activity has been attributed to alpha-hemolytic streptococci. One as yet unpublished study, (data on file at BLIS Ltd, Dunedin, New Zealand) has 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

Table 9 Episodes of oral streptococcal pathology and acute otitis media in a 6-month follow-up period in children coming from the recurrent-treated and from the recurrent-not-treated groups

Group	Number of episodes	Incidence/ month/child	% versus control
Control (n = 14) (from recurrent-not-treated)	18°	0.214	
Tested (n = 16) (from recurrent-treated)	7°°	0.073*	–65.9

Notes: *P = 0.0278 (Pearson Chi-squared test for difference in proportions, Chi-squared test = 4.84); °oral streptococcal pathology (n = 8) and acute otitis media (n = 10); °°oral streptococcal pathology (n = 4), acute otitis media (n = 2), scarlet fever (n = 1).

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 randomized 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.

References

- Sathyabama S, Vijayabharathi R, Bruntha Devi P, Ranjith Kumar M, Priyadarisini VB. Screening for probiotic properties of strains isolated from feces of various human groups. *J Microbiol*. 2012;50:603–612.
- Tagg JR, Dierksen KP. Bacterial replacement therapy: adapting 'germ warfare' to infection prevention. *Trends Biotechnol*. 2003;21:217–223.
- Tagg JR. Prevention of streptococcal pharyngitis by anti-Streptococcus pyogenes bacteriocin-like inhibitory substances (BLIS) produced by Streptococcus salivarius. *Indian J Med Res*. 2004;119 Suppl:13–16.
- Jack RW, Tagg JR, Ray B. Bacteriocins of Gram-positive bacteria. *Microbiol Rev*. 1995;59:171–200.
- Hyink O, Wescombe PA, Upton M, Ragland N, Burton JP, Tagg JR. Salivaricin A2 and the novel lantibiotic salivaricin B are encoded at adjacent loci on a 190-kilobase transmissible megaplasmid in the oral probiotic strain Streptococcus salivarius K12. *Appl Environ Microbiol*. 2007;73:1107–1113.
- Sharma S, Verma KK. Skin and soft tissue infection. *Indian J Pediatr*. 2001;68 Suppl 3:S46–S50.
- Wescombe PA, Burton JP, Cadieux PA, et al. Megaplasms encode differing combinations of lantibiotics in Streptococcus salivarius. *Antonie Van Leeuwenhoek*. 2006;90:269–280.
- Burton JP, Chilcott CN, Tagg JR. The rationale and potential for the reduction of oral malodour using Streptococcus salivarius probiotics. *Oral Dis*. 2005;11 Suppl 1:29–31.
- van Zon A, van der Heijden GJ, van Dongen TM, Burton MJ, Schilder AG. Antibiotics for otitis media with effusion in children. *Cochrane Database Syst Rev*. 2012;9:CD009163.
- Burton JP, Chilcott CN, Moore CJ, Speiser G, Tagg JR. A preliminary study of the effect of probiotic Streptococcus salivarius K12 on oral malodour parameters. *J Appl Microbiol*. 2006;100:754–764.
- Power DA, Burton JP, Chilcott CN, Dawes PJ, Tagg JR. Preliminary investigations of the colonisation of upper respiratory tract tissues of infants using a paediatric formulation of the oral probiotic Streptococcus salivarius K12. *Eur J Clin Microbiol Infect Dis*. 2008;27:1261–1263.
- Horz HP, Meinelt A, Houben B, Conrads G. Distribution and persistence of probiotic Streptococcus salivarius K12 in the human oral cavity as determined by real-time quantitative polymerase chain reaction. *Oral Microbiol Immunol*. 2007;22:126–130.
- Burton JP, Wescombe PA, Moore CJ, Chilcott CN, Tagg JR. Safety assessment of the oral cavity probiotic Streptococcus salivarius K12. *Appl Environ Microbiol*. 2006;72:3050–3053.
- Burton JP, Cowley S, Simon RR, McKinney J, Wescombe PA, Tagg JR. Evaluation of safety and human tolerance of the oral probiotic Streptococcus salivarius K12: a randomized, placebo-controlled, double-blind study. *Food Chem Toxicol*. 2011;49:2356–2364.
- Tagg J, Wescombe P, Burton J. Oral streptococcal BLIS: heterogeneity of the effector molecules and potential role in the prevention of streptococcal infections. *International Congress Series 1289*. 2006:347–350.
- Vandenbroucke JP. A shortcut method for calculating the 95 per cent confidence interval of the standardised mortality ratio. *Am J Epidemiol*. 1982;115:303–304.
- Tagg JR. A longitudinal study of Lancefield group A streptococcus acquisitions by a group of young Dunedin school children. *N Z Med J*. 1990;103:429–431.
- Fantinato VC, Shimizu MT. Production of bacteriocin-like inhibitory substances (BLIS) by Streptococcus salivarius strains isolated from the tongue and throat of children with and without sore throat. *Revista de Microbiologia*. 1999;30:332–334.
- Ragland N, Tagg JR. Applications of bacteriocin-like inhibitory substance (BLIS) typing in a longitudinal study of the oral carriage of beta-haemolytic streptococci by a group of Dunedin schoolchildren. *Zentralbl Bakteriell*. 1990;274:100–108.
- Tagg JR. Significance of bacteriocin production by oral streptococci. In: R Lutticken R, editor. *Proceedings of the Xth Lancefield Symposium*. New York, NY: Gustav Fischer Verlag; 1990.
- Dierksen KP, Tagg JR. The influence of indigenous bacteriocin-producing Streptococcus salivarius on the acquisition of Streptococcus pyogenes by primary school children in Dunedin, New Zealand. In: Martin DR, Tagg JR, editors. *Streptococci and Streptococcal Diseases Entering the New Millennium*. Auckland, New Zealand: Securacopy; 2000.
- Roos K, Hakansson EG, Holm S. Effect of recolonisation with "interfering" alpha streptococci on recurrences of acute and secretory otitis media in children: randomized placebo controlled trial. *BMJ*. 2001;322:210–212.
- Tano K, Olofsson C, Grahn-Hakansson E, Holm SE. In vitro inhibition of *S. pneumoniae*, nontypable *H. influenzae* and *M. catharralis* by alpha-hemolytic streptococci from healthy children. *Int J Pediatr Otorhinolaryngol*. 1999;47:49–56.

International Journal of General Medicine**Dovepress****Publish your work in this journal**

The International Journal of General Medicine is an international, peer-reviewed open-access journal that focuses on general and internal medicine, pathogenesis, epidemiology, diagnosis, monitoring and treatment protocols. The journal is characterized by the rapid reporting of reviews, original research and clinical studies across all disease areas.

Submit your manuscript here: <http://www.dovepress.com/international-journal-of-general-medicine-journal>

A key focus is the elucidation of disease processes and management protocols resulting in improved outcomes for the patient. The manuscript management system is completely online and includes a very quick and fair peer-review system. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.