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Review

Streptococcus salivarius K12 in pharyngotonsillitis and acute otitis media – a meta-analysis

Abstract

The oral administration of *Streptococcus salivarius* K12 represents a valid solution for the prevention of pharyngitis or pharyngotonsillitis, of bacterial or viral origin, and/or acute otitis media. In particular, this could be an interesting alternative to antibiotic prophylaxis in patients with relapse or disease recurrence. In this regard, the studies published in the scientific literature are limited, and thus, it was of interest to provide a meta-analysis in order to analyze in more detail the results obtained in this research field.

For the meta-analysis, clinical studies were selected, as identified through PubMed, which examined the relationship between the use of formulations based on *Streptococcus salivarius* K12 and the number of cases or episodes of pharyngitis or pharyngotonsillitis, of bacterial or viral origin, and acute otitis media in children and adults. The *effect size* (ES) was calculated according to Cohen using the fixed effects model (*fixed effects*).

Seven studies met the predefined inclusion criteria and they were included in the meta-analysis. ES values equal to -1.40 [-1.67; -1.13] and -1.01 [-1.19; -0.83] were obtained for the effect of *Streptococcus salivarius* K12 on the prevention of pharyngitis and acute otitis media, respectively. Both values were translated into a "big effect" according to Cohen's scale. Treatment based on this strain is effective for both the prevention of pharyngitis and acute otitis media. In addition, it is further revealed that the strain is more effective in the prevention of pharyngitis.

It is desirable that further clinical investigation continues to expand and update the meta-analysis work and to recommend the use of the strain as a useful prophylactic tool to reduce the events of pharyngotonsillitis and acute otitis media.

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Introduction

Streptococcus salivarius K12 is a strain isolated from a New Zealand child [1]. It is capable of producing salivaricin A2 and salivaricin B, molecules belonging to the lantibiotics [2] family able to counteract effectively in vitro the growth of *S. pyogenes* [3], in addition to inhibiting the growth of other pathogens including *Haemophilus influenzae*, *Moraxella catarrhalis* and *S. pneumoniae*, which are all potentially involved in the aetiopathogenesis of acute otitis media (AOM) [4], and pharyngotonsillitis [5]. The K12 strain proved to also have the ability to inhibit the proliferation of *Micrococcus luteus*, *S. anginosus*, *Eubacterium saburreum* and *Micromonas micros*, involved in the production of volatile sulfur compounds implicated in halitosis development [6, 7]. Jamali *et al* [8] showed that probiotic K12 therapy following oral disinfection with chlorhexidine may reduce the severity of halitosis over longer periods.

The strain also appears to be capable of antiviral actions, mainly attributed to an ability to increase the levels of salivary interferon-γ and to reduce interleukin (IL)-8 release, while leaving the release of IL-1β or tumor necrosis factor-α unaltered [4]. K12 also exhibited potential efficacy and safety as an adjuvant in treating oral candidiasis by enhancing mycological cure and shortening the treatment course of conventional antifungal therapy [9]. Colonization has been demonstrated in the human model from the third day of treatment at the level of the nasopharynx and adenoids [10], persisting up to 32 days after discontinuation [11], and a good overall safety profile in animals [12] and humans [13] has been reported. Several clinical studies have been conducted to investigate the potential of the K12 strain in the prevention of pharyngitis, pharyngotonsillitis and/or AOM of bacterial or viral origin. The aim of this work was to carry out a meta-analysis through which we could assess more thoroughly the results obtained in this research field, comparing the results differentially for pharyngitis and bacterial infections of the ear.

Materials and methods

The studies were sourced from scientific and biomedical journals and through the use of the PubMed database. To be included in the meta-analysis, the studies had to meet the following inclusion criteria: (a) clinical trials; (b) parallel studies; or (c) historical control studies; (d) studies in which the effectiveness of basic formulations of *Streptococcus salivarius* K12 ATCC BAA-1024 was assessed; (e) studies that reported the number of cases or episodes of pharyngitis or pharyngotonsillitis of bacterial or viral infection and/or AOM; (f) studies in which paediatric patients or adults were enrolled.

At first, the inclusion of keywords such as "BLIS K12", "*Streptococcus salivarius* K12", "probiotic *Streptococcus salivarius* K12" and "bacteriocin-like inhibitory substances *Streptococcus salivarius* K12" produced a number of records equal to 103 publications; after eliminating duplicates, this was reduced to 43, of which 28 were screened articles. Finally, there was a total of 21 studies excluded, while seven were included in the meta-analysis. Although one study complied with the predefined inclusion criteria [14], it was not included in the final quantitative analysis because of a characteristic that differed significantly between treated subjects and controls.

More precisely, children diagnosed with recurrent streptococcal pharyngitis (mean > three episodes of pharyngotonsillitis during the year before the study) were included in the treatment group, while children without a diagnosis of recurrent disease were enrolled in the control group.

In **Fig. 1** the PRISMA flow diagram or flow chart is depicted; this is an instrument that allows documentation of all stages for the selection and identification of studies to be included in the final analysis [15].

The extraction and processing of data from individual studies then took place (**Table 1**).

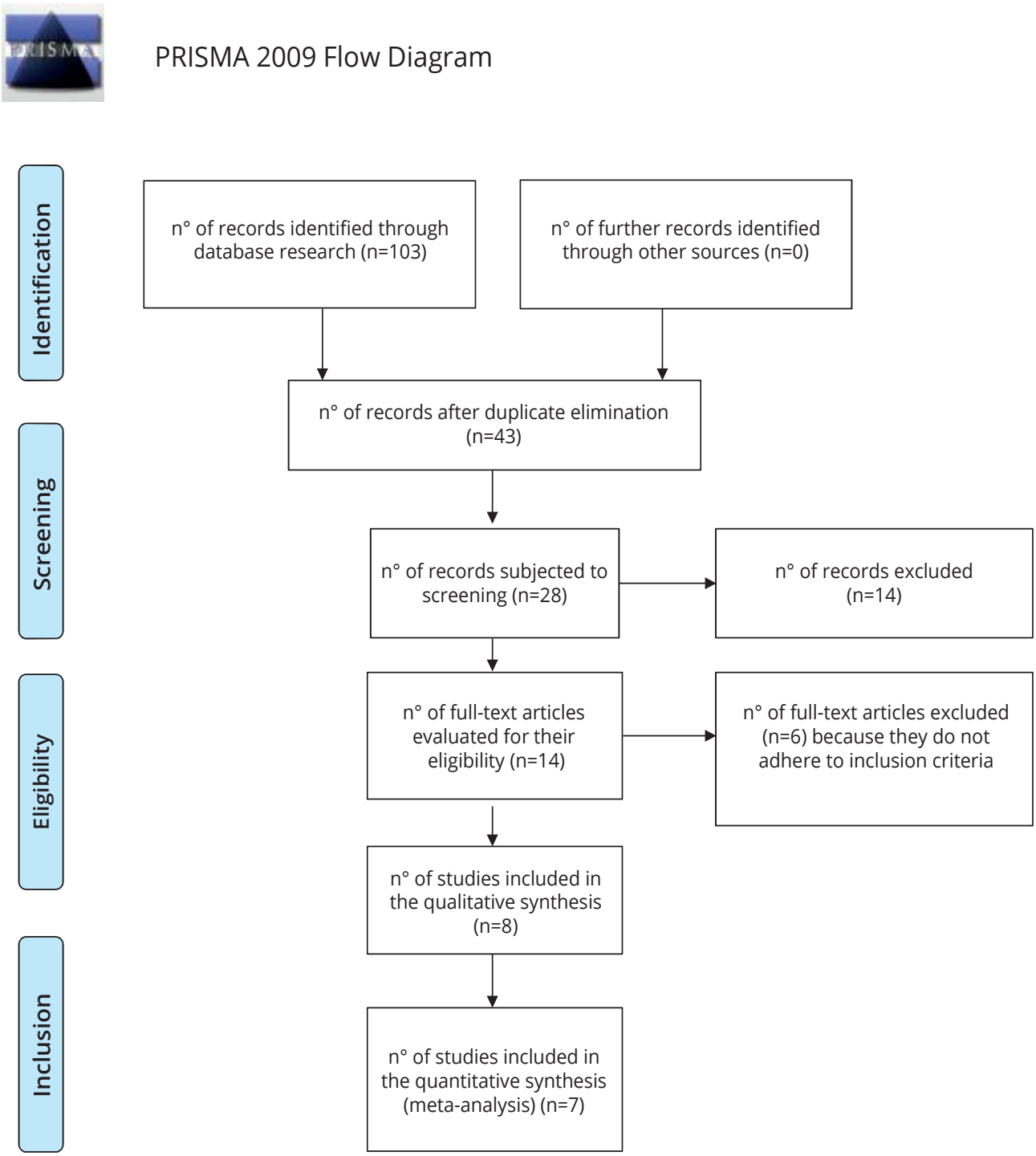

Figure 1 The PRISMA flow diagram of the literature related to BLIS K12 which was examined in order to develop the meta-analysis work

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi:10.1371/journal.pmed1000097
For more information, visit www.prisma-statement.org.

Authors and year	Locations	Sample age	N° of subjects	N° of experimental subjects	N° of control subjects	Duration	Doses	Outcomes
Di Pierro *et al* (2016)	Milan, Italy	3-year-old children	222 children	111	111	180 days of treatment + 3 months of follow-up for 29 children of each group	1 daily pill of Bactoblis® containing not less than 1 billion CFU of *Streptococcus salivarius* K12 ATCC BAA-1024	Tolerance to the treatment, onset of collateral effects or symptoms of toxicity during administration of the product, efficacy of BLIS K12 in the prevention of *Streptococcus pyogenes* infections and number of events of streptococcal pharyngitis, tonsillitis, scarlet fever and the efficacy of BLIS K12 in reducing the occurrence of acute otitis media
Di Pierro *et al* (2012)	Cuneo, Brescia, Verona, Novara, Turin, Italy	3-12-year-old children (mean age: 7 years old)	78 children. Initially, 82 children; 65 with a diagnosis of at least 3 events of recurring pharyngitis and/or tonsillitis in the previous year and 17 without recurring events. During the study, 4 children with the diagnosis were excluded for failed adherence to the experimental therapy	41 (with recurring pathology)	37 (20 with recurring disease and 17 without recurring disease)	90 days of treatment + 6 months of follow-up for 30 children (16 of the treated subjects and 14 of the 20 controls with recurring infections)	1 daily pill of Bactoblis® containing 5 billion CFU of *Streptococcus salivarius* K12 ATCC BAA-1024	Episodes of pharyngitis, tonsillitis, acute otitis media in recidivist patients, not treated and not-recidivist-not-treated-groups during 90 days of treatment with the product and during the 6 months of follow-up. Results of pharyngeal swab and signs of acute otitis media; tolerability, compliance and collateral effects of the product during the 90 days of treatment
Di Pierro *et al* (2014)	Milan, Italy	3-13-year-old children (mean age: 8 years)	60 children. Initially 61. Only one subject immediately abandoned the study for the mouthfeel unpleasantness of the product	30	30	90 days of treatment	1 daily pill of Bactoblis® containing not less than 1 billion CFU of *Streptococcus salivarius* K12 ATCC BAA-1024	The efficacy of Bactoblis® in the prevention of pharyngotonsillitis in group A streptococcus or *S. pyogenes* groups, in reducing infections of pharyngotonsillitis of viral origin during the period of study. The occurrence of collateral effects and toxicity during the administration of the product. Collection of information on the use of antibiotic therapy, on antipyretic treatment, working days lost by parents and absences at school (or at kindergarten for children below 6 years of age)
Gregori *et al* (2016)	Piacenza, Italy	3-7-year-old children (mean age: 5 years old)	130 children	76	54	90 days of treatment + 9 months of follow-up	1 daily pill of Bactoblis® containing not less than 1 billion CFU of *Streptococcus salivarius* K12 ATCC BAA-1024	Retrospectively evaluate if the use of *Ss* K12 in paediatric patients with RPTI could significantly reduce the occurrence of streptococcal pharyngotonsillitis relapses during the treatment period and the following 9 months compared to the period of 6 to 12 months immediately before the beginning of the antibiotic treatment, significantly reduce the occurrence of pharyngotonsillitis relapses during the treatment period and during the following 9 months when compared with a control group of children who experienced RPTI but who were not treated with *Ss* K12. Evaluate if subjects treated with *Ss* K12 had significant differences in the occurrence of bronchitis, otitis, sinusitis and bronchial pneumonia
Di Pierro *et al* (2018)	Genova, Italy	3–14-year-old children (mean age: 8 years old)	133 children	133 children	133 children (previous year)	90 days of treatment (October to December 2015) + 90 days of treatment (April to June 2016)	1 daily pill of Bactoblis® containing not less than 1 billion CFU of *Streptococcus salivarius* K12 ATCC BAA-1024	Preventive role of BLIS K12 in streptococcal or viral pharyngotonsillitis and acute otitis media. Collateral effects, tolerance, doses and number of days in which children were subjected to an antibiotic and antipyretic therapy, number of absences at school for children and at work for parents
Karpova *et al* (2015)	Russia	6-7-year-old children	219 children	113 (probiotics + nasal shower)	106 nasal shower)	30 days of treatment	1 daily pill of *Streptococcus salivarius* K12 ATCC BAA-1024 + nasal shower with NaCl solution at 0.9%. Doses not specified	Efficacy of *Ss* K12 in reducing worsening effects related to chronic adenoiditis (acute otitis media); frequency of diagnosed otitis; necessity of an anti-inflammatory therapy; frequency of adenoiditis complications development (acute otitis media and acute rhinosinusitis). Necessity of using systemic antibacterial drugs
Di Pierro *et al* (2013)	Merano, Verona, Piacenza, Italy	Adults aged between 18–65 years (mean age: 42 years old)	40 adults	20	20	90 days of treatment + 6 months of follow-up	1 daily pill of Bactoblis® containing 5 billion CFU of *Streptococcus salivarius* K12 ATCC BAA-1024	Evaluation, through visits and pharyngeal swabs, of the number of episodes of pharyngitis and/or tonsillitis of bacterial origin in treated and not-treated groups during 90 days of treatment with the product and the 6th month of follow-up. Tolerability, compliance and collateral effects during 90 days of treatment

Table 1 Data extracted from individual studies relating to: authors, year, places, age of the enrolled subjects, number of treated subjects, number of controls, duration of treatment with *S. salivarius* K12, dose and outcomes; RPTI = recurrent pharyngo-tonsillar infections

As we can see from **Table 1**, the studies were mainly carried out in Italy and in subjects of paediatric age, and they looked at the role of *Streptococcus salivarius* K12 in the prevention and reduction of occurrences of typical childhood diseases, such as pharyngotonsillitis of bacterial or viral origin and AOM. Specifically, six studies involved children aged between 3 and 14 years, while the study "Di Pierro *et al* (2013)" [16] is the only one that involved adults aged between 18 and 65 years. In "Di Pierro *et al* (2018)" [17] 133 children were enrolled and the number of episodes of illness during the period of treatment with *Streptococcus salivarius* K12

was compared with the number recorded in the same 133 children in the previous year where treatment was not given. The absence of a control group treated with placebo, of randomization and of blinded conditions are all limitations shared by some of the studies included in the meta-analysis.

Oral formulations containing between 1 and 5 billion CFU of *Streptococcus salivarius* K12 ATCC BAA-1024 were administered to the patients, although the study "Karpova *et al*" [18] does not specify the used dose. The duration of treatment varied from 30 to 180 days. Generally, during the period of treatment, children with a positive throat swab test for *S. pyogenes* received antibiotics. After the discontinuation of antibiotic therapy, the treatment was resumed and brought to an end. Viral infections, throat/laryngeal pain and/or fever were treated with acetaminophen or ibuprofen or according to the reference guidelines.

Subsequently, in order to obtain a single final estimate, we focused on the number of enrolled subjects with or without episodes of disease in the treated group and the control group, only considering the period of treatment with formulations based on *S. salivarius* K12.

The meta-analyses were performed using the statistical software ProMeta (version 3.0) with the adoption of the fixed effects model or *fixed effects*.

Results

The results of the individual studies and the overall estimate of the meta-analysis, associated with the 95% confidence interval (CI), are shown in a type of graph called a forest plot. Specifically, two forest plots were obtained on the basis of the outcomes "n° pharyngitis cases" and "n° otitis cases" that were attributed respectively to six and four of the included studies.

In **Fig. 2**, the forest plot regarding the effect of the strain on the prevention of pharyngitis is depicted. From the observation of the forest plot of the "Number of pharyngitis cases" (**Fig. 2**), it is clear that all studies included in the meta-analysis are in favour of the treatment examined and none are in favour of the controls; this is apparent from the fact that all estimates fall to the left of the insignificance line.

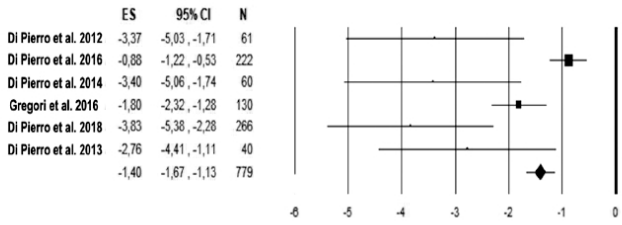

Figure 2 Forest plot of the "Number of pharyngitis cases"

The no-significance line is not crossed by the confidence intervals corresponding to individual studies, which means they are all significant. The studies "Di Pierro *et al* (2012)" [19], "Di Pierro *et al* (2014)" [20], "Di Pierro *et al* (2018)" [17] and "Di Pierro *et al* (2013)" [16] are more significant; they respectively show the values of the ES and the corresponding confidence intervals as being -3.37 [-5.03; -1.71], -3.40 [-5.06; 1.74], -3.83 [-5.38; -2.28] and -2.76 [-4.41; -1.11]. Considering Cohen's scale, these ES values are translated into "great effect". The studies just mentioned were carried out in male and female subjects with or without diagnosis of recurrent disease aged between 3 and 14 years, except for the study "Di Pierro *et al* (2013)" [16] which involved adults aged between 18 and 65 years.

One tablet per day containing not less than 1 billion CFU of *Streptococcus salivarius* K12 ATCC BAA-1024 was administered to the patients in "Di Pierro *et al* (2014)" [20] and "Di Pierro *et al* (2018)" [17] and one tablet per day containing 5 billion CFU of *Streptococcus salivarius* K12 ATCC BAA-1024 in "Di Pierro *et al* (2012)" [19] and "Di Pierro *et al* (2013)" [16].

For each study, the duration of treatment was 90 consecutive days. In relation to the number of participants and events, less weight (W) was attributed to these studies as seen by the square that rests on the horizontal line; it represents the confidence interval and indicates the degree of uncertainty of the study, potentially attributable solely to the effect of chance. The "Di Pierro *et al* (2016)" [21] and "Gregori *et al* (2016)" [22] studies are less uncertain. Both studies were conducted on a greater number of children aged between 3 and 7 years old, with or without a diagnosis of recurrent disease. One tablet per day containing not less than 1 billion CFU of *Streptococcus salivarius* K12 ATCC BAA-1024 was administered to the patients in "Di Pierro *et al* (2016)" [21] for 180 days and for 90 days in "Gregori *et al* (2016)" [22]. The ES values and confidence intervals correspond to -0.88 [-1.22; -0.53] for "Di Pierro *et al* (2016)" [21] and -1.80 [-2.32; -1.28] for "Gregori *et al* (2016)" [22]. As for the studies mentioned above, both translated as "big impact" on the Cohen scale. From the quantitative combination of results, the overall ES and the confidence intervals were obtained, and they are represented respectively by the diamond core and the amplitude of its sides. In numerical terms, the overall effect estimate is -1.40 [-1.67; -1.13], indicative of a "great effect" of the treatment based on the strain in the prevention of pharyngitis or pharyngotonsillitis. As the available studies were not numerous, the total value is closer to "more important studies" which, as expected, impacted more strongly on the analysis.

In **Fig. 3** the forest plot regarding the effect of the strain on the prevention of AOM is represented. Observing the forest plot for "n° otitis cases" (**Fig. 3**) it is evident that the studies show a preference for treatment based on *S. salivarius* K12, as the individual estimates are placed on the left side of the graph. The line of significance is not crossed by the confidence intervals of the individual studies, which is why the latter studies are all considered significant.

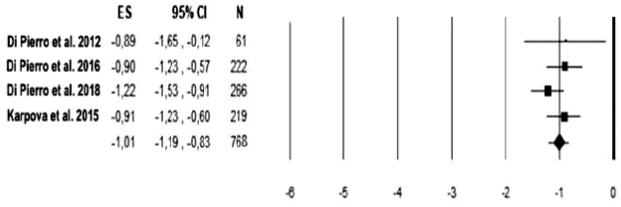

Figure 3 Forest plot for "N. otitis cases"

Among the studies, the most significant is "Di Pierro *et al* (2018)" [17], the most recent and also the most significant in the previous analysis. According to Cohen, the ES has a "great effect", assuming a value of -1.22 with confidence intervals of [-1.53; -0.91]. To carry out the study, using historical controls, boys and girls aged 3 to 14 years diagnosed with at least one episode of AOM and/or pharyngitis in the year prior to the study were enrolled. One tablet per day containing not less than 1 billion CFU of *Streptococcus salivarius* K12 ATCC BAA-1024 was administered for 180 days; or for 90 consecutive days in two separate quarters. In addition, the software gave more weight to this study.

Less weight was attributed to "Di Pierro *et al* (2012)" [19], considering the highest variance and smaller sample size. In addition, "Di Pierro *et al* (2012)" [19] is the most uncertain study as observed by the length of the confidence intervals. The study was conducted by enrolling 61 children of both sexes, aged between 3 and 12 years. One tablet per day containing 5 billion CFU of *Streptococcus salivarius* K12 ATCC BAA-1024 was administered for 90 consecutive days to the patients. In contrast to the previous study, it shows the lowest ES obtained, -0.89 [-1.65; -0.12], but this is still translatable into a "great effect".

The overall ES was -1.01 [-1.19; -0.83] which means "great effect" through the reading of Cohen's scale. In conclusion, treatment with the strain in the prophylaxis of AOM is effective.

Fig. 4 depicts a forest plot which summarizes the previous two plots. In both graphs, the diamond is not placed close to

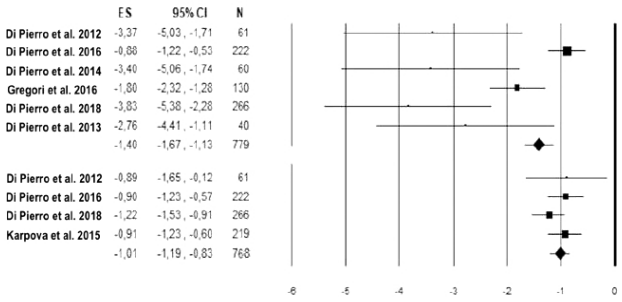

Figure 4 Forest plot for "Number of pharyngitis cases" (upper section) and "Number of otitis cases" (lower section)

the line of insignificance, so the analysis is considered significant for the two studied variables. Considering the global data for "Number of pharyngitis cases" (N=779), the ES is -1.40 and ranges from -1.67 to -1.13.

On the other hand, considering the global data for "Number of otitis cases" (N=768), the ES is -1.01 and ranges from -1.19 to -0.83. According to Cohen, the values represent a "great effect". This confirms the effectiveness of the treatment under investigation in the prevention of pharyngitis or pharyngotonsillitis and/or AOM. Further data that emerged from the ANOVA Q test is that treatment with the strain appears to be more effective in the prevention of pharyngitis than in the prevention of AOM.

Finally, the software provides the statistical heterogeneity and variability among studies, which according to Higgins (I2) was 85.35% and 0.00%, respectively, for the studies to which the outcomes "N° pharyngitis cases" and "N° otitis cases" were attributed. The heterogeneity of 85.35% is considered "significant", while the heterogeneity of 0.00% is defined as "not important". The clinical and methodological heterogeneity are added to the statistical heterogeneity and they were evaluated during the selection phase and identification of the studies.

Discussion

Pharyngitis or pharyngotonsillitis and AOM are among the diseases that most frequently require the support of a paediatrician or general practitioner and the use of prescription medications, including antibiotics and non-steroidal anti-inflammatory drugs (NSAIDs). Moreover, both for pharyngotonsillitis and AOM, events of recurrence and relapse often involving long antibiotic prophylaxis are documented. On the other hand, it is now known that excessive use of antibiotics increases the risk of appearance of resistant microbes, both at the individual level and at the community level. Finally, in order to try to improve the quality of patients' lives, the diagnosis of chronic or recurrent pharyngotonsillitis can identify the need for tonsillectomy, or the surgical removal of the tonsils, as a last resort, and the final result of this action has not been demonstrated in random controlled studies as of yet.

Streptococcus salivarius K12 or BLIS K12 is a component of the human oral microbiota naturally present in a small percentage of the population, that with a lower incidence of pharyngitis, tonsillitis and otitis. After it was isolated from the throat of a healthy child [1], it was demonstrated in vitro to produce two bacteriocins, salivaricin A2 and salivaricin B, with strong inhibitory action towards *Streptococcus pyogenes* and other pathogenic strains [3–5], some of which promote symptoms of halitosis [6, 7]. Moreover, the strain has antifungal [23], anti-inflammatory and antiviral properties.

In particular, it seems to significantly reduce the levels of IL-8, which explains the treatment effect observed in infections of viral origin, as well as its positive action when administered to children with periodic fever, adenitis, pharyngitis, aphthous ulcer (PFAPA) syndrome [24]. Recently, the efficacy of K12 in the prevention of pharyngo-tonsillar infections, the decreased use of antibiotics and the improvement of overall quality-of-life was confirmed, with a decreased number of absences from school and fewer patients undergoing surgery [25].

Finally, it is considered safe for human

use [12, 13] and is able to colonize and persist in the oral cavity, the nasopharynx and adenoids [10].

Within the present work, clinical studies aimed at investigating the role of *Streptococcus salivarius* K12 ATCC BAA-1024 in the prevention of pharyngitis or pharyngotonsillitis and/or AOM were selected, in order to conduct a meta-analysis able to assess in more detail the results obtained in this research field.

For the development of the meta-analysis, a small number of studies were identified and those included were clinical trials, non-randomized, non-placebo-controlled and not blinded, carried out mainly in Italy and in paediatric subjects. In a preliminary study only the role of the strain was evaluated in 40 adult subjects diagnosed with pharyngitis or recurring pharyngotonsillitis [16]. To the treated subjects enrolled in several studies, oral formulations were administered for a variable period from 30 to 180 days, which contained between 1 and 5 billion CFU of *Streptococcus salivarius* K12 ATCC BAA-1024.

From the results obtained from the meta-analysis, it is concluded that the treatment based on BLIS K12 is effective for the prevention of pharyngitis or pharyngotonsillitis, of viral or bacterial origin, and/or AOM, with or without a diagnosis of recurrent disease. In fact, ES values corresponding to -1.40 and -1.01, respectively, for the prevention of pharyngitis and AOM were obtained. According to Cohen's scale, both are translated into "great effect". In addition, it was found that the strain appears to be even more effective in the prevention of pharyngitis than the prevention of AOM.

Conclusions

The results obtained from the meta-analysis, integrated with present knowledge, suggest that formulations based on *Streptococcus salivarius* K12 may be a viable therapeutic solution in the prevention of pharyngitis or pharyngotonsillitis, of viral or bacterial origin, and/or AOM, with or without a diagnosis of recurrent disease. Studies, including those in the meta-analysis, show a significant reduction in the number of episodes of illness that is accompanied by a significant reduction in the use of antibiotics, antipyretics and anti-inflammatories, following oral administration of the strain [17, 18, 21]. In addition, there is a reduction in the number of days lost with respect to preschool or school for children and work for their parents [17, 19]. Similar results were obtained in a study aimed to evaluate the role of Streptococcus in PFAPA [24].

In conclusion, it is desirable that further clinical investigation continues to expand and update the work of the meta-analysis and to recommend the use of *S. salivarius* K12 as a useful prophylactic tool to reduce the events of pharyngotonsillitis and AOM, the clinical manifestations derived therefrom, the use of antibiotics in the hope of fighting on another front antibiotic resistance, the use of antipyretics and anti-inflammatories and to avoid surgical operations or tonsillectomies in cases of recurring pharyngitis.

References

1. Tagg JR (2004) Prevention of streptococcal pharyngitis by anti-Streptococcus pyogenes bacteriocin-like inhibitory substances (BLIS) produced by Streptococcus salivarius. Indian J Med 119:13–16

2. Hyink O, Wescombe PA, Upton M, Ragland N, Burton JP, Tagg JR (2007) 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 73:1107–1113

3. Sharma S, Verma KK (2001) Skin and soft tissue infection. Indian J Pediatr 68:S46–S50

4. Wescombe PA, Hale JD, Heng NC, Tagg JR (2012) Developing oral probiotics from Streptococcus salivarius. Future Microbiol 7:1355–1371

5. van Zon A, van der Heijden GJ, van Dongen TM, Burton MJ, Schilder AG (2012) Antibiotics for otitis media with effusion in children. Cochrane Database Syst Rev 9:CD009163

6. Burton JP, Chilcott CN, Tagg JR (2005) The rationale and potential for the reduction of oral malodour using Streptococcus salivarius probiotics. Oral Dis 11:29–31

7. Burton JP, Chilcott CN, Moore CJ, Speiser G, Tagg JR (2006) A preliminary study of the effect of probiotic Streptococcus salivarius K12 on oral malodour parameters. J Appl Microbiol 100:754–764

8. Jamali Z, Aminabadi NA, Samiei M, Sighari Deljavan A, Shokravi M, Shirazi S (2016) Impact of chlorhexidine pretreatment followed by probiotic Streptococcus salivarius strain K12 on halitosis in children: a randomised controlled clinical trial. Oral Health Prev Dent 14:305–13

9. Hu LJ, Mao QH, Zhou PR, Lv X, Hua H, Yan ZM (2019) Effects of Streptococcus salivarius K12 with nystatin on oral candidiasis – RCT. Oral Dis. doi:10.1111/odi.13142

10. Power DA, Burton JP, Chilcott CN, Dawes PJ, Tagg JR (2008) Preliminary investigations of the colonization of the upper respiratory tract tissues of infants using a pediatric formulation of the oral probiotic Streptococcus salivarius K12. Eur J Clin Microbiol Infect Dis 27:1261–1263

11. Horz HP, Meinelt A, Houben B, Conrads G (2007) 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 22:126–130

12. Burton JP, Wescombe PA, Moore CJ, Chilcott CN, Tagg JR (2006) Safety assessment of the oral cavity probiotic Streptococcus salivarius K12. Appl Environ Microbiol 72:3050–3053

13. Burton JP, Cowley S, Simon RR, McKinney J, Wescombe PA, Tagg JR (2011) Evaluation of safety and human tolerance of the oral probiotic Streptococcus salivarius K12: a randomized, placebo-controlled, double-blind study. Food Chem Toxicol 49:2356–2364

14. Di Pierro F, Colombo M, Zanvit A, Rottoli AS (2016) Positive clinical outcomes derived from using Streptococcus salivarius K12 to prevent streptococcal pharyngotonsillitis in children: a pilot investigation. Drug Healthc Patient Saf 8:77–81

15. Moher D, Liberati A, Tetzlaff J, Altman DG (2009) Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. J Clin Epidemiol 62:1006–1012. doi:10.1016/j.jclinepi.2009.06.005

16. Di Pierro F, Adami T, Rapacioli G, Giardini N, Streitberger C (2013) Clinical evaluation of the oral probiotic Streptococcus salivarius K12 in the prevention of recurrent pharyngitis and/or tonsillitis caused by Streptococcus pyogenes in adults. Expert Opin Biol Ther 13:339–343

17. Di Pierro F, Risso P, Poggi E, Timitilli A, Bolloli S, Bruno M, Caneva E, Campus R, Giannattasio A (2018) 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. Minerva Pediatr 70:240–245

18. Karpova EP, Karpycheva IE, Tulupov DA (2015) Prophylaxis of chronic adenoiditis in the children. Vestn Otorinolaringol 80:43–45

19. Di Pierro F, Donato G, Fomia F, Adami T, Careddu D, Cassander C, Albera R (2012) 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. Int J Gen Med 5:991–997

20. Di Pierro F, Colombo M, Zanvit A, Risso P, Rottoli AS (2014) Use of Streptococcus salivarius K12 in the prevention of streptococcal and viral pharyngotonsillitis in children. Drug Healthc Patient Saf 6:15–20

21. Di Pierro F, Colombo M, Giuliani MG, Danza ML, Basile I, Bollani T, Conti AM, Zanvit A, Rottoli AS (2016) Effect of administration of Streptococcus salivarius K12 on the occurrence of streptococcal pharyngo-tonsillitis, scarlet fever and acute otitis media in 3 years old children. Eur Rev Med Pharmacol Sci 20:4601–4606

22. Gregori G, Righi OR, Risso P, Boyars G, Demuru G, Ferzetti TO, Galli TO, Ghisoni TO, Lenzini S, Marenghi C, Walls C, Bags R, Suzzani L (2016) Reduction of group A beta-hemolytic streptococcus pharyngo-tonsillar infections associated with use of the oral probiotic Streptococcus salivarius K12: a retrospective observational study. Ther Clin Risk Manag 12:87–92

23. Ishijima SA, Kazumi M, Burton JP, Reid G, Okada M, Matsushita Y, Abe S (2012) Effect of Streptococcus salivarius K12 on the in vitro growth of Candida albicans and its protective effect in an oral candidiasis model. Appl Environ Microbiol 78:2190–2199

24. Di Pierro F, Campana A, Panatta ML, Antenucci V, De Vincentiis G (2016) The use of Streptococcus salivarius K12 in attenuating PFAPA syndrome, a pilot study. Altern Integr Med 5:222

25. Marini G, Sitzia E, Panatta ML, De Vincentiis GC (2019) Pilot study to explore the prophylactic efficacy of oral probiotic Streptococcus salivarius K12 in preventing recurrent pharyngo-tonsillar episodes in pediatric patients. Int J Gen Med 12:213–217