**Effectiveness of the probiotic *Streptococcus salivarius K12* for the treatment and/or prevention of sore throat: a systematic review**

Wilcox CR 1 2, Stuart B 2, Leaver H 2, Lown M 2, Willcox M 2, Moore M 2, Little P 2

1: NIHR Clinical Research Facility, University Hospital Southampton NHS Foundation Trust, Southampton, UK

2: Department of Primary Care and Population Sciences, Aldermoor Health Centre, University of Southampton, Southampton, UK

**Corresponding author**

* Dr Christopher Wilcox
* NIHR Clinical Research Facility, Southampton Centre for Biomedical Research, C Level West Wing, Mailpoint 218, Southampton General Hospital, Tremona Road, Southampton, SO16 6DY
* Email: christopher.wilcox@soton.ac.uk
* Telephone: 02381204956

**Keywords**

Streptococcus salivarius K12; S. salivarius K12; probiotics; sore throat; tonsillitis; pharyngitis

**Abstract**

**Background**

Sore throat secondary to pharyngo-tonsillitis is one of the commonest reasons for primary care consultation and inappropriate antibiotic prescription, and finding effective alternative treatments is important.

**Objectives**

To review the evidence for using the probiotic *Streptococcus salivarius K12 (SsK12)* for the prevention or treatment of pharyngo-tonsillitis.

**Data Sources**

PubMed, EMBASE, CINAHL and Cochrane Library.

**Study eligibility Criteria**

Randomised controlled trials (RCTs)

**Participants**

Adults or children

**Interventions**

*SsK12* as active treatment, or prophylaxis, against pharyngo-tonsillitis.

**Methods**

Literature search

**Results**

Four articles were identified (1846 participants). All were deemed to be of poor quality on Cochrane risk-of-bias assessment. Two trials studied *SsK12* prophylaxis for streptococcal pharyngitis (children without history of recurrence). One compared daily administration of *SsK12* tono treatmentover six months (n=222, aged 33-45 months), reporting significantly lower incidence in the *SsK12* group *(*16.2% vs 48.6%, p<0.01), whereas another placebo-controlled RCT over four school terms (n=1314, 5-14 years) found no significant difference (7.8% vs 8.8%, p=0.34) with *SsK12* (administered on school days). Another trial found daily *SsK12* to significantly protect children (n=250, 6-7 years) against chronic adenoiditis exacerbation over three months, compared to no treatment (71.7% vs 100%, p<0.0001). The one placebo-controlled RCT in adults that studied the use of *SsK12* for acute pharyngotonsillitis (concurrently with penicillin) showed no significant benefit. In all trials *SsK12* was safe and well tolerated.

**Conclusions**

*SsK12* appears safe and well-tolerated, however further RCTs are required to establish its role as a prophylactic therapy, particularly amongst patients experiencing frequent exacerbations of pharyngitis. In the acute setting, *SsK12* is unlikely to be effectiveifgiven concurrentlywith antibiotics, however further RCTs should establish its role as an alternative to antibiotics in non-severe cases, or when prescribed post-antibiotic therapy for the prevention of disease recurrence and/or secondary infection.

**Introduction**

Sore throat due to pharyngeal/tonsillar infection is one of the commonest reasons for primary care consultation, particularly amongst young children, and carries an enormous economic burden 1 2. Whilst usually self-limiting, some patients suffer from frequent debilitating episodes, associated with regular absence from school/work. In the UK, over £400 million is spent annually on consultations and lost productivity associated with sore throat  3.

Whilst most cases are viral, others are caused by pathogenic group A beta-haemolytic streptococci (GABHS), particularly *Streptococcus pyogenes* 4 and, less often, by group C and G streptococci  5. Approximately 90% and 60% of patients diagnosed with tonsillitis and pharyngitis/sore throat will be prescribed broad-spectrum antibiotics acutely by their primary care physician, respectively 6 7, yet these conditions have one of the highest rates of potentially inappropriate prescriptions (46%) of all primary care conditions  8. Furthermore, many with recurrent tonsillitis will eventually undergo surgical removal of the tonsils (adeno-/tonsillectomy) 9. Importantly, whilst patients and healthcare professionals are concerned about complications following infection 10, the risk is low 11, and the key driver for seeking medical attention is effective symptom control 12.

One possible alternative therapy is probiotics. These are live, non-pathogenic bacteria that may act through local inhibition of bacterial adhesion and growth 13 14, modulation of immune responses 15 16, production of anti-viral agents 17, and inhibition of viral attachment to the host-cell receptor 18. Recent trials have demonstrated that probiotics (lactobacilli and bifidobacteria species) may be effective in preventing episodes of acute upper-respiratory tract infection 19, but only *Streptococcus salivarius K12 (SsK12)* has shown potential benefit in the context of pharyngo-tonsillitis 20  21.

*Streptococcus salivarius* is the predominant colonizer of the oral cavity 22, and *SsK12* (isolated from healthy individuals) has been shown to produce two class I lantibiotic bacteriocins: salivaricin A2 and salivaricin B 23. Both are strongly antagonistic to the growth of *Streptococcus pyogenes* 24, partially antagonistic to *Moraxella catarrhalis, Haemophilus influenzae* and *Streptococcus pneumoniae*  25, and may also have anti-viral action 26. Furthermore, recent studies have demonstrated that *SsK12* (taken as oral dissolvable tablets) has a good safety profile 27 28, and can achieve persistentcolonisation within the upper respiratory tract 29 22. We therefore set out to summarise the evidence for the use of *SsK12* for the prevention or treatment of sore throat due to pharyngo-tonsillitis.

**Methods**

This review followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 30 and Cochrane Collaboration 31 guidelines, and was registered on the PROSPERO database (CRD42018092990).

**Data sources, search strategy and selection criteria**

Literature searching was conducted through PubMed, EMBASE, CINAHL and the Cochrane Library for articles published before 29/04/2018. Search terms were developed in collaboration with a librarian, and adapted for each database (see supplementary information). The search strategy is displayed as a PRISMA diagram in Figure 1. After removal of duplicates, two authors (CW, HL) independently screened the titles and abstracts of all articles identified by the search criteria. Full-text copies of any articles that appeared eligible were obtained and assessed for inclusion, and their reference lists were screened for additional eligible articles. Any disagreement was resolved upon discussion with senior authors (PL and MM).

Eligible articles reported on either randomised controlled trials (RCTs) or quasi-RCTs investigating the use *SsK12* for the active treatment, or the prophylaxis, of pharyngo-tonsillitis/sore throat. Possible comparators included no treatment, placebo and alternative treatment. Diagnoses may have included pharyngitis, tonsillitis, pharyngotonsillitis, pharyngotonsillar infection, nasopharyngitis, rhinopharyngitis and adenoiditis. Infection may have been bacterial or viral in origin, participants could be of any age and recruited from any setting, and articles published in any language. The primary outcomes of interest for acute and prophylactic studies were time-to-recovery and incidence of pharyngo-tonsillitis, respectively. Secondary outcomes included symptom severity, incidence of reinfection/reconsultation/complications, compliance and tolerance of treatment, and change in clinical or laboratory parameters.

**Data extraction & quality assessment**

Data extraction and risk-of-bias assessment was undertaken independently and in duplicate by two authors (CW and HL) using a standardised form and the Cochrane Collaboration’s risk of bias tool 32. Any differences in bias assessment between reviewers was resolved upon discussion with a senior author (BS). Overall certainty of evidence was assessed using the Grading of Recommendations, Assessment, Development and Evaluations (GRADE) approach 33. Intention-to-treat data was reported in one study (Doyle 2017). Authors were also asked via email for their trial registration details and protocol, in order to assess for selective reporting. Doyle 2017 provided their registration details and protocol, Gilbey 2015 provided registration details only, and the others did not respond.

**Statistical analysis**

For one article (Karpova *et al* 2015) statistical analysis was not reported. We therefore carried out statistical analysis of their reported data using GraphPad QuickCalcs (https://www.graphpad.com/quickcalcs). Pearson’s chi squared test was performed to compare the proportion of participants in each group who had >1 and >3 exacerbations of chronic adenoiditis by day 30 and day 90, and p-values <0.05 were considered statistically significant.

**Results**

We identified 239 articles of potential interest after removal of duplicates. Full-text review was undertaken for nine articles, and four met the inclusion criteria 21,34–36

**Study characteristics**

The characteristics of the four articles are summarised in Table 1. Together they reported on 1846 participants, and publication date ranged from 2015-2017. Three articles (from New Zealand, Israel and Italy) were published in the English language, and one (from Russia) in Russian. Three paediatric studies assessed the use of *SsK12* as prophylactic treatment, and one adult study assessed use of *SsK12* for acute pharyngotonsillitis (in conjunction with antibiotics). All studies used the same formulation of *SsK12* (oral dissolvable tablets). All studies were deemed to be of poor quality for having two or more areas of high/unclear risk of bias (see Table 2). The overall certainty of evidence was very low for all outcomes on GRADE assessment (see Table 3).

**Study outcomes**

*Incidence of GABHS-positive sore throat*

Two studies investigated the use of *SsK12* as a prophylactic treatment to protect children (without a history of recurrence) against GABHS-positive sore throat.

Di Pierro *et al* (2016) reported a non-placebo-controlled RCT in which 222 children (aged 33-45 months) were randomised (1:1) to receive either *SsK12* once daily, or no treatment, for 180 days. Children reporting a sore throat during the study period underwent a throat swab in clinic to test for presence of GABHS. Significantly fewer children in the probiotic group were diagnosed with a with an episode of GABHS-positive pharyngo-tonsillitis during the treatment period (16.2% vs 48.6%; p<0.01*).* From each group, 29 participants also underwent a further 3-month follow-up after the end of the treatment phase, however there was no significant difference in incidence of GABHS-positive sore throat during this period.

Doyle *et al* (2017) reported a pragmatic placebo-controlled double-blinded quasi-RCT based across 12 schools in New Zealand, involving 1314 children aged 5-14. Participants were randomised (1:1, by odd/even birthday) to receive *SsK12* or placebo, administered once daily by school staff on school days over one year (maximum of 209 days). Any children reporting a sore throat during the study period underwent a throat swab to test for presence of GABHS. There was a non-significant reduction in GAS positive throat swabs amongst the probiotic group (7.8% vs 8.8%; p=0.34). It should be noted that duringthis trial, trace amounts of *SsK12* were also discovered in the placebo lozenges due to factory error. However, the authors state that the latter issue was unlikely to have had an effect on the results as the rates of GABHS-positive swabs were similar before and after the study (data not shown).

*Time-to-recovery in acute sore throat*

One article (Gilbey *et al* 2015) reported on the use of *SsK12* for acute pharyngotonsillitis. This was a double-blinded randomised placebo-controlled trial, in which *SsK12* (or placebo, randomisation ratio 1:1) was prescribed concurrently with intravenous penicillin for 10 days in 60 hospitalised adults. The results showed no significant difference in blood inflammatory markers, body temperature, or volume of fluids consumed. Mean pain scores (visual analogue scale) in the probiotic group were significantly higher on day 4, however exact figures were not reported. Further subanalysis of GABHS-positive and –negative participants revealed no significant differences for any outcomes.

*Exacerbation of chronic adenoiditis*

One study (Karpova *et al* 2015) investigated the use of *SsK12* as a prophylactic treatment to protect children with chronic adenoiditis against episodes of exacerbation. This was a non-placebo-controlled RCT involving 250 children aged 6-7 years. Children were randomised to receive either *SsK12* once nightly, or no treatment, in addition to daily nasal irrigation. By day 30 and day 90, significantly fewer children in the treatment group had had an exacerbation of adenoiditis than the control group (49.6% vs 88.7%, and 71.7% vs 100%; both p<0.0001). Furthermore, by day 90, significantly fewer children in the treatment group had had > 3 exacerbations (20.4% vs 62.2%; p<0.0001), and required intranasal steroids (46.9% vs 93.4%; p<0.0001).

*Side effects*

Only one article (Karpova 2015) reported side effects*.* Three participants in the treatment group (3%) developed an urticarial rash, and were therefore moved to the control group. However, the authors report that all three had a history of food allergy, and the parents of two of the children couldn’t exclude whether these symptoms may have been diet-related.

*Tolerability and compliance*

Two out of the three studies which investigated the use of *SsK12* as a prophylactic

agent reported on tolerability and compliance with treatment. Doyle *et al* 2017 found that the lozenges provided in schools during their study were well tolerated, and only two children (0.2%) refused to take the treatment regularly. The mean lozenge adherence was 72% (out of a maximum of 209 days). Older children were significantly more adherent to treatment. Children ≤6 years were less adherent than 7-9 year olds (69.3% vs 71.9%, p=0.007) and 10 year olds (72.9%, p=0.0002). Di Pierro *et al* 2016 reported that compliance with treatment was “very good” and that no children withdrew from the study, however actual adherence values were not reported. Karpova *et al* 2015 didn’t report on compliance, and excluded any children with intolerance to the probiotic from the trial, however the number of children excluded for this reason was not reported. The one study that reported on the use of *SsK12* for acute pharyngotonsillitis (Gilbey 2015) excluded three patients (10%) from the probiotic group and four (13%) from the placebo group due to lack of compliance with treatment. Reasons for lack of compliance were not reported.**Discussion**

This was a systematic review of four RCTs studying the use of *SsK12* to either prevent or treat sore throat in adults and children.The results support previous studies demonstrating that *SsK12* is safe and well-tolerated  27,28,37 , however its role as a prophylactic and acute therapyremain uncertain due to the poor quality of the trials to-date, and high risk of bias.

The benefit of using *SsK12* as prophylaxis against streptococcal sore throat remains unclear, as whilst Di Pierro’s trial demonstrated a significant reduction in episodes, Doyle’s trial did not. Differences in study methodology make comparison of these two trials difficult. Unlike the daily administration in Di Pierro’s trial, Doyle *et al’s* study design meant that lozenges were only administered on school days, and this may be partly responsible for the lack of protective effect observed. There was also a large discrepancy in the overall incidence of GABHS-positive cases between trials, and the incidence for both differ somewhat from a recent international meta-analysis in which the pooled prevalence of GAS amongst children presenting with sore throat was 37% (95% CI: 32-43%) and 24% (95% CI: 21-26%) for those of any age and for children under five years, respectively 38. Doyle *et al* do suggest that their study population was probably atypical with respect to levels of GABHS, as their trial was undertaken in conjunction with a national rheumatic fever prevention program. The routine swabbing and antibiotic treatment recommended by this program over four years prior to the trial commencing may have significantly reduced GAS prevalence in this community, as a similar program in New Zealand was seen to reduce rates by almost 50%  39. Limitations to Di Pierro’s trial include its open-label design, and a significant conflict of interest, as the first author (Francesco Di Pierro) is the main formulator of *SsK12* and sits on the scientific council of the marketing company.

It is worth noting that a number of non-RCTs (including retrospective analyses and controlled trials lacking randomisation), ineligible for inclusion in this review, have supported the use of *SsK12* as prophylactic treatment to prevent streptococcal pharyngitis when administered once daily (see Table 4 – supplementary information). These studies have all demonstrated significantly reduced rates of exacerbation amongst both children 26,40–42 and adults 43 with a prior history of recurrent streptococcal pharyngitis by up to ~90%, as well as healthy children without a history of recurrence 40 44. One of these studies (Di Pierro *et al* 2014) has also suggested that *SsK12* may have anti-viral action, showing a significant decrease (80%) in the incidence of viral pharyngitis, as diagnosed by cases of pharyngitis in the absence of symptoms, signs and swab result consistent with bacterial infection 26. However, a major conflict of interest is that Francesco Di Pierro was the lead author of all but one of these studies.

The one RCT investigating the use of *SsK12* to prevent against chronic adenoiditis exacerbation in children demonstrated a significant protective effect, however the article is very limited in its detail as to how children were diagnosed. Chronic adenoiditis is not a well-recognised clinical entity, and may actually be indistinguishable from sinusitis in clinical practice 45. Unlike typical pharyngitis/tonsillitis, the pathogens most often implicated in chronic adenoiditis are *Staphylococcus aureus, Haemophilus influenzae, Streptococcus pneumoniae* and *Moraxella catarrhalis*, against which *SsK12* has been shown to have partial antagonistic effect  25.

Finally, the one RCT by *Gilbey et al* that compared *SsK12* with placebo as acute therapy for adults hospitalised with pharyngo-tonsillitis, in conjunction with penicillin, showed no significant benefit of SsK12. It is worth noting that the study’s small sample size means that the groups may not have been comparable in terms of baseline risk factors, despite randomisation. Hospitalisation for pharyngotonsillitis amongst adults is also rare  46 47, and so these participants are likely to represent a severe subset of patients. Furthermore, this result is not surprising given that *SsK12* is known to be sensitive to a number of antibiotics used for treatment of upper respiratory tract infection, including penicillin 48 49. It was therefore likely to have been eradicated before it could successfully reach the oral mucosa and achieve colonisation, negating any protective effect 50.

**Suggestions for future research**

There is a clear need for well-conducted RCTs to establish the role of *SsK12* as a prophylactic therapy. Future trials should also identify the optimal target population, and consider cost-effectiveness, as *SsK12* may provide more effective prophylaxis amongst younger patients, or those experiencing frequent exacerbations of pharyngo-tonsillitis. To-date this subgroup has only been studied in non-randomised trials. With regards to the acute setting, whilst the use of *SsK12* in conjunction with antibiotics is unlikely to be effective, studies should explore whether *SsK12* might have a role in acute infection as an alternative to antibiotics in non-severe cases, or when administered post-antibiotic therapy to prevent recurrence and/or secondary infection. Finally, future studies would also benefit from documenting the baseline colonisation rates of GABHS and *Streptococcus salivarius*, and studying the effect of *SsK12* treatmenton the oral microbiota.

**Strengths and limitations**

Although every effort was made to retrieve papers relevant to our research questions, the lack of standardised keywords and MeSH terms means that some eligible articles may have been missed. Additionally, conflicts of interest (and possibly publication bias) exist in the literature. Finally, the small number of studies, and heterogeneity of study populations, interventions and outcome assessment, meant that drawing comparisons was difficult.

**Conclusions**

Further high-quality RCTs are required to establish the role of *SsK12* as prophylaxis, particularly amongst patients experiencing frequent exacerbations of pharyngo-tonsillitis. In the acute setting, *SsK12* is unlikely to be effectiveifgiven concurrentlywith antibiotics, however further RCTs should establish its role as an alternative to antibiotics in non-severe cases, or when prescribed post-antibiotic therapy for the prevention of disease recurrence and/or secondary infection.

**Acknowledgements**

We would like to thank Paula Sands from the University Hospital Southampton library for her assistance with developing the search terms used in this review.

**Author contributions**

CW drafted the manuscript. CW, BS, ML, MW, MM and PL contributed to the protocol design. CW, HL and BS undertook the search and data extraction process. All authors critically revised the manuscript and approved the final version.

**Funding**

No funding was obtained for this review.

**Conflicts of interest**

ML, MM & PL are co-investigators for the Probiotics to Reduce Infections iN Care homE reSidentS (PRINCESS) trial investigating the use of probiotics to reduce infections in care home residents (ISRCTN16392920). The other authors report no conflicts of interest.

**Figure captions:**

Figure 1: Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) diagram detailing the search process undertaken in this review.

**References**

 1. Pfoh, Wessels, Goldmann & Lee. Burden and Economic Cost of Group A Streptococcal Pharyngitis. *PEDIATRICS* **121,** 229–234 (2008).

2. Wessels, M. Streptococcal Pharyngitis. *New England Journal of Medicine* **364,** 648–655 (2011).

3. Burns, R. M. *et al.* Economic analysis of oral dexamethasone for symptom relief of sore throat: the UK TOAST study. *BMJ Open* **8,** e019184 (2018).

4. Shulman, S. T. *et al.* Clinical practice guideline for the diagnosis and management of group A streptococcal pharyngitis: 2012 update by the Infectious Diseases Society of America. *Clin. Infect. Dis.* **55,** 1279–82 (2012).

5. Little, P. *et al.* PRImary care Streptococcal Management (PRISM) study: in vitro study, diagnostic cohorts and a pragmatic adaptive randomised controlled trial with nested qualitative study and cost-effectiveness study. *Health Technol Assess* **18,** vii–xxv, 1–101 (2014).

6. Pouwels, K. B., Dolk, C. F., Smith, D. R., Robotham, J. V. & Smieszek, T. Actual versus ‘ideal’ antibiotic prescribing for common conditions in English primary care. *Journal Of Antimicrobial Chemotherapy* **73,** 19–26 (2017).

7. Gulliford, MC, Dregan, A, Moore, MV & open, A.-M. Continued high rates of antibiotic prescribing to adults with respiratory tract infection: survey of 568 UK general practices. *BMJ open* (2014). at <http://bmjopen.bmj.com/content/4/10/e006245.short>

8. Unknown. OUP accepted manuscript. *J Antimicrob Chemoth* (2017). doi:10.1093/jac/dkx500

9. Burton, M. J., Glasziou, P. P., Chong, L. Y. & Venekamp, R. P. Tonsillectomy or adenotonsillectomy versus non-surgical treatment for chronic/recurrent acute tonsillitis. *Cochrane Database Syst Rev* CD001802 (2014). doi:10.1002/14651858.cd001802.pub3

10. Kumar, S., Little, P. & Britten, N. Why do general practitioners prescribe antibiotics for sore throat? Grounded theory interview study. *BMJ* **326,** 138 (2003).

11. Little, P. *et al.* Predictors of suppurative complications for acute sore throat in primary care: prospective clinical cohort study. *Bmj* **347,** f6867 (2013).

12. Van Driel, M. L. *et al.* Are sore throat patients who hope for antibiotics actually asking for pain relief? *Ann Fam Med* **4,** 494–9 (2006).

13. Guglielmetti, S. *et al.* Oral Bacteria as Potential Probiotics for the Pharyngeal Mucosa. *Appl Environ Microb* **76,** 3948–3958 (2010).

14. Taverniti, V. *et al.* In Vitro Functional and Immunomodulatory Properties of the Lactobacillus helveticus MIMLh5-Streptococcus salivarius ST3 Association That Are Relevant to the Development of a Pharyngeal Probiotic Product. *Applied and Environmental Microbiology* **78,** 4209–4216 (2012).

15. Tapiovaara, L., Pitkaranta, A. & Korpela, R. Probiotics and the Upper Respiratory Tract - A Review. *Pediatric Infect Dis Open Access* **01,** (2016).

16. Wan, Chen, Shah & El-Nezami. Modulation of Intestinal Epithelial Defense Responses by Probiotic Bacteria. *Critical Reviews in Food Science and Nutrition* **56,** 2628–2641 (2015).

17. Kassaa, A., Hober, Hamze, Chihib & Drider. Antiviral Potential of Lactic Acid Bacteria and Their Bacteriocins. *Probiotics Antimicro* **6,** 177–185 (2014).

18. Salminen, S. *et al.* Interaction of probiotics and pathogens--benefits to human health? *Curr. Opin. Biotechnol.* **21,** 157–67 (2010).

19. Hao, Q., Dong, B. R. & Wu, T. Probiotics for preventing acute upper respiratory tract infections. *Cochrane Database Syst Rev* CD006895 (2015). doi:10.1002/14651858.CD006895.pub3

20. Little, P. *et al.* Probiotic capsules and xylitol chewing gum to manage symptoms of pharyngitis: a randomized controlled factorial trial. *Can Med Assoc J* **189,** E1543–E1550 (2017).

21. Di Pierro, F. *et al.* 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 (2016).

22. Horz, H.-P. P., 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.* **22,** 126–30 (2007).

23. Hyink, O. *et al.* 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,** 1107–13 (2007).

24. Wescombe, P. A. *et al.* Megaplasmids encode differing combinations of lantibiotics in Streptococcus salivarius. *Antonie Van Leeuwenhoek* **90,** 269–80 (2006).

25. Wescombe, P. A., Hale, J. D., Heng, N. C. & Tagg, J. R. Developing oral probiotics from Streptococcus salivarius. *Future Microbiol* **7,** 1355–71 (2012).

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

27. Burton, J., Chilcott, C., Wescombe, P. & Tagg, J. Extended Safety Data for the Oral Cavity Probiotic Streptococcus salivarius K12. *Probiotics Antimicrob Proteins* **2,** 135–144 (2010).

28. Burton, J. P. *et al.* 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 (2011).

29. Power, Burton, Chilcott, Dawes & Tagg. 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* **27,** 1261 (2008).

30. Moher, D. *et al.* Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. *Syst Rev* **4,** 1 (2015).

31. Higgins, J. P. & Green, S. *Cochrane Handbook for Systematic Reviews of Interventions: Version 5.1.0*. (The Cochrane Collaboration, 2011).

32. Higgins *et al.* The Cochrane Collaboration’s tool for assessing risk of bias in randomised trials. *BMJ* **343,** d5928–d5928 (2011).

33. Guyatt, G. H. *et al.* GRADE: an emerging consensus on rating quality of evidence and strength of recommendations. *BMJ* **336,** 924–6 (2008).

34. Karpova, E. P., Karpycheva, I. E. & Tulupov, D. A. [Prophylaxis of chronic adenoiditis in the children]. *Vestn. Otorinolaringol.* **80,** 43–45 (2015).

35. Gilbey, P., Livshits, L., Sharabi-Nov, A., Avraham, Y. & Miron, D. Probiotics in addition to antibiotics for the treatment of acute tonsillitis: a randomized, placebo-controlled study. *European J Clin Microbiol Infect Dis* **34,** 1011–1015 (2015).

36. Doyle, H. *et al.* The Effect of the Oral Probiotic Streptococcus salivarius (K12) on Group A Streptococcus Pharyngitis: A Pragmatic Trial In Schools. *Pediatric Infect Dis J* **Publish Ahead of Print,** 1 (2017).

37. Burton, J. P., Wescombe, P. A., Moore, C. J., Chilcott, C. N. & Tagg, J. R. Safety assessment of the oral cavity probiotic Streptococcus salivarius K12. *Appl. Environ. Microbiol.* **72,** 3050–3 (2006).

38. Shaikh, N., Leonard, E. & Martin, J. M. Prevalence of streptococcal pharyngitis and streptococcal carriage in children: a meta-analysis. *Pediatrics* **126,** e557–64 (2010).

39. Lennon, D., Stewart, J., Farrell, E., Palmer, A. & Mason, H. School-Based Prevention of Acute Rheumatic Fever: A Group Randomized Trial in New Zealand. *Pediatric Infect Dis J* **28,** 787 (2009).

40. Pierro, F. *et al.* 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 Medicine* **Volume 5,** 991–997 (2012).

41. Gregori, G. *et al.* 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 (2016).

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

43. Di Pierro, F., Adami, T., Rapacioli, G., Giardini, N. & Streitberger, C. 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,** 339–43 (2013).

44. DI Pierro, F. *et al.* Use of Streptococcus salivarius K12 to reduce the incidence pharyngo-tonsillitis and acute otitis media in children: a retrospective analysis in not-recurrent pediatric subjects. *Minerva Pediatr.* (2018). doi:10.23736/S0026-4946.18.05182-4

45. Marzouk, H., Aynehchi, B., Thakkar, P., Abramowitz, T. & Goldsmith, A. The utility of nasopharyngeal culture in the management of chronic adenoiditis. *Int. J. Pediatr. Otorhinolaryngol.* **76,** 1413–5 (2012).

46. Little, P. *et al.* Antibiotic prescription strategies for acute sore throat: a prospective observational cohort study. *Lancet Infect Dis* **14,** 213–9 (2014).

47. John, L. J., Cherian, M., Sreedharan, J. & Cherian, T. Patterns of antimicrobial therapy in acute tonsillitis: A cross-sectional hospital-based study from UAE. *An. Acad. Bras. Cienc.* **86,** 451–7 (2014).

48. Barbour, A. & Philip, K. Variable characteristics of bacteriocin-producing Streptococcus salivarius strains isolated from Malaysian subjects. *PLoS ONE* **9,** e100541 (2014).

49. Santagati, M., Scillato, M., Patanè, F., Aiello, C. & Stefani, S. Bacteriocin-producing oral streptococci and inhibition of respiratory pathogens. *FEMS Immunol. Med. Microbiol.* **65,** 23–31 (2012).

50. Klaenhammer, T. & Kullen, M. Selection and design of probiotics. *Int J Food Microbiol* **50,** 45–57 (1999).

**Table 1:** Summary of the included studies

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Author** | **Study design** | **Setting** | **Funding** | **Subjects** | **N (male)** | **Mean age** | **Intervention** | **Comparator** | **Outcomes** | **Assessment** | **Results** |
| Gilbey 2015 | RCT | Hospital, Israel | No financial support obtained. Probiotic and placebo supplied by SupHerb, Israel | Adults admitted with acute pharyngo-tonsillitis | 60 (UNK) randomised:IG: 30 (UNK)CG: 30 (UNK)53 (28) completed and included in analysis:IG: 27 (14)CG: 26 (14) | Of those completing study:IG: 31.5+11.0 yrsCG: 31.8+11.4 yrs | *SsK12* administered BD concurrently with penicillin and analgesia for 10 days  | Identical placebo administered over same time period | (1) Pain(2) Temperature (3) Fluids consumed4) Serum inflammatory markers | - Measurement by blinded non-study staff of: (1) pain visual analogue scale on analgesia request, (2 & 3) other clinical measurements taken OD, and (4) bloods taken at admission and discharge- Differences in categorical variables assessed using Fisher's exact test and Pearson's chi square test. T-test used to compare continuous variables | - Pain score significantly higher in IG on day 4 (exact figure not reported, p<0.05), but no other differences between groups. - 7 subjects (3 in IG and 4 in CG) subjects excluded following randomisation due to ‘lack of compliance’ |
| Karpova 2015 | RCT | Outpatient, Russia | No source of financial support or probiotic supply acknowledged | Children with chronic adenoiditis | 250 (119) randomised:IG: 128 (UNK)CG: 122 (UNK)219 (UNK) completed follow-up and were included in analysis, and 3 children transferred to control group after developing urticaria:IG: 113CG: 106 | Of those completing study: Range: 6-7 yrs | *SsK12* taken OD for 30 days, plus nasal irrigation OD | OD nasal irrigation only | (1) Percentage of patients experiencing > 1 or >3 adenoiditis exacerbations over study period(2) Need for antibiotics/anti-inflammatories(3) Occurrence of side-effects | - Physical examination in clinic and retrospective assessment of medical records at days 30 and 90 by non-blinded study team- Retrospective statistical analysis carried out by us (the reviewer authors) using Pearson's chi square | - Significantly fewer of the IG had had >1 episode of exacerbation by day 30 [56/113 (49.6%) vs 94/106 (88.7%), p<0.0001] and day 90 [81/113 (71.7%) vs 106/106 (100%), p<0.0001]- Significantly fewer of the IG had had > 3 exacerbations by day 90 [23/113 (20.4%) vs 66/106 (62.2%), p<0.0001]- Significantly fewer of the IG required intranasal steroids [53/113 (46.9%) vs 99/106 (93.4%), p<0.0001] - 3 of the IG experienced urticaria following randomisation and were moved the to the CG, however the possibility of food allergy in these patients couldn’t be excluded  |
| Di Pierro 2016 | RCT | Outpatient, Italy | No source of financial support acknowledged. Probiotic provided by Omeopiacenza, Italy. | Healthy children | [1] Treatment phase222 (106) randomised:IG: 111 (50)CG: 111 (56)No drop-outs reported[2] Follow-up phase58 (UNK) included:IG: 29 (UNK)CG: 29 9UNK) | Of those in treatment phase:IGM: 36+3.2 mthsF: 34+3.0 mthsCGM: 35+3.0 mthsF: 35+3.6 mths | *SsK12* taken ONfor 180 day treatment phase(*SsK12* suspended if taking antibiotics), and 90 follow-up phase | Untreated control group monitored over same time period | (1) Percentage of patients experiencing >1 pharyngo-tonsillitis episode (associated with throat swab positive for streptococcus(2) Occurrence of side effects | - Participants instructed to attend for immediate clinical review and throat swab (performed by study team) upon experiencing symptoms of sore throat- Number of streptococcal-positive episodes between groups compared using two-tailed Wilcoxon-Mann-Whitney test, and Fisher's exact test. | - Significantly fewer children in IG had >1 streptococcal pharyngo-tonsillitis episode during treatment phase [18/111 (16.2%) vs 54/111 (48.6%), p<0.01)] - Significantly fewer total number of episodes in IG [21 vs 67, p<0.01]- No significant difference in number of children having >1 episode between IG and CG during follow-up phase [5/29 (17.2%) vs 8/29 (27.6%)]- No SEs and compliance “very good”. |
| Doyle 2017 | QRCT | 12 schools, NZ | Funded by The Health Research Council of NZ, Cure Kids, The NZ Heart Foundation and the NZ Ministry of Health. Probiotic and placebo provided by BLIS technologies Ltd, NZ. | Healthy children | 1314 (647) quasirandomised:IG: 666CG: 6481137 (UNK) completed:IG: 584CG: 648 | Of those randomsied:IG154: 5-6 yrs275: 7-9 yrs237: 10-14 yrsCG186: 5-6 yrs256: 7-9 yrs206: 10-14 yrs | *SsK12* taken OD (on school days only) over 4 school terms, usually witnessed by school staff | Identical placebogiven over same period | (1) Proportion of GAS-positive throat swabs(2) Adherence / tolerability of treatment | - Participants who reported a sore throat underwent throat swabbing, either by blinded study nurses who visited schools twice weekly, or by non-study staff at GP or ED - Treatment adherence monitored by school staff.- Pearson chi square with Yates correction used to compare groups- ITT analysis | - Non-significant reduction in proportion of positive throat swabs between IG and CG [199/1525 (7.8%) vs 124/1402 (8.8%), p=0.34)] - All but 2 children accepted treatment. - Mean adherence 72.2% (38% if included non-school days). - Children < 6 years significantly less adherent (69.3%) than those aged 7-9 (71.9%, p=0.007) and 10 (72.9%, p=0.0002) |

Abbreviations: N = number of participants; NZ = New Zealand; RCT = randomised controlled trial; QRCT = Quasi-randomised controlled trial IG = intervention group; CG = control group; *SsK12* = *Streptococcus salivarius K12;* OD = once per day; BD = twice per day; ON = Once at night; GP = general practitioner; ED = emergency department; SEs = side effects; UNK = unknown; Yrs = years; Mths = Months

**Table 2:** Risk of bias in the included studies, as assessed using the Cochrane Collaboration’s Risk of Bias Tool

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Study** | **Random sequence generation** | **Allocation concealment** | **Selective reporting** | **Blinding (participants and personnel)** | **Blinding (outcome assessment)** | **Incomplete outcome data** | **Other bias** | **Overall** |
| Gilbey 2015 | Low risk(Computer generated random sequences) | Low risk(Randomisation in advance and with unmarked bottles) | High risk(Follow-up results presented over four days didn’t match online study registration plan of ten days. No protocol provided.) | Low risk(Double blinded)  | Low risk(Data collection done by blinded staff members) | High risk(No intention-to-treat analysis and participants excluded for non-compliance) | Unclear risk | High risk |
| Karpova 2015 | Unclear risk(Simple randomisation, but no further information) | Unclear risk | Unclear risk(No registration details/protocol provided by authors) | High risk(Non-blinded subjects) | High risk(Non-blinded outcome assessors) | Low risk(88% followed-up) | Unclear risk | High risk |
| Di Pierro 2016 | Low risk(Coin toss) | Unclear risk | Unclear risk(No registration details/protocol provided by authors) | High risk(Non-blinded subjects) | High risk(Non-blinded outcome assessors) | Low risk(All subjects followed-up for the primary outcome) | High risk (Significant conflict of interest) | High risk |
| Doyle 2017 | High risk(Randomised based on odd/even date of birth) | High risk(Based on odd/even date of birth) | Low risk(Reporting matched plan set out in online registration/protocol) | Unclear risk(Blinded subjects but unclear whether school personnel blinded) | Low risk(Swabs taken by personnel independent of the study) | Low risk(87% followed up and loss to follow-up does not appear differential | Unclear risk | High risk |

**Table 3:** Grading of Recommendations, Assessment, Development and Evaluations (GRADE) table summarising the certainty of evidence available from the included studies

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Outcome** | **Studies** | **Risk of bias** | **Inconsistency** | **Indirectness** | **Imprecision** | **Publication bias** | **Large effect** | **Overall certainty of evidence** |
| Prevention of GABHS pharyngitis | 2 RCTs | Very serious | Very serious | Not serious | Very serious | Undetected | No | Very low |
| Time-to-recovery in acute sore throat | 1 RCT | Serious | Very serious | Not serious | Very serious | Undetected | No | Very low |
| Prevention of chronic adenoiditis exacerbation | 1 RCT | Very serious | Very serious | Not serious | Very serious | Undetected | No | Very low |

GABHS = Group A beta-heamolytic streptococcus; RCT = Randomised Controlled Trial