

## Effect of Probiotic Lozenges on Salivary Streptococcus mutans Counts of High-Carious-Risk Children: A Double-Blind Randomized Controlled Trial

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### Abstract:

**Background:** Traditionally, dental caries has ranked as one of the most common diseases of childhood, and Streptococcus mutans, which has been thought to be one of the primary etiologic organisms. More newly, probiotics have been introduced as a non-intrusive method of changing oral flora.

**Objective:** This study determined the effectiveness of probiotic lozenges on reducing the salivary levels of S. mutans in high caries-risk children.

**Methods:** This was a double-blinded RCT that involved 60 children aged 6-12 years, highlighted as high caries risk subjects. Later, these children were randomly placed in either the probiotic group, to whom Lactobacillus reuteri lozenges were given, or in the placebo group, for whom participants took identical non-medicated lozenges, for 28-30 days. Unstimulated saliva samples combined at baseline and after intervention were subjected to microbiological analysis by CFU counts.

**Results:** In the probiotic group children, the mean counts of *S. mutans* were significantly lower compared with that in the placebo group children. Calculated percentage reduction for the probiotic group was 53%, whereas it was only 8% for the placebo group. This difference between the two groups is drastically significant.

**Conclusion:** Routine wise consumption of probiotic lozenges was found to reduce salivary *S. mutans* among children at high carious risk and thus was supposed to be very promising as an adjunctive method for the prevention of caries.

**Keywords:** probiotics, *Lactobacillus reuteri*, *Streptococcus mutans*, high carious risk, children, oral microbiome, randomized controlled trial.

### Introduction

Despite the current advances in preventive dentistry, dental caries is believed to be among the severe issues that impact the oral health of a good number of populations in most sections of the globe [1]. Dental caries can occur in all ages even though it is most prevalent in children [2]. Despite the fact that it has brought about changes in the oral health of most people, its prevalence has remained high in a worrying situation [3]. Since it is acidogenic and aciduric, and metabolic by-products are the ones catalyzing the onset of the demineralization process, over numerous years *Streptococcus mutans* became one of the pathogens which were emphasized as the primary cause in the initial formative period of caries. Children determined as high risk of caries have higher salivary counts of *S. mutans* [4]. *S. mutans*, greater consumption of sugars, and poor oral hygiene that triggers the development of their disease. In the previous decade, the biological approaches to alteration of the oral microbiota have attracted a lot of attention [5]. Probiotics have eliminated any promising ones that can alter oral ecology because they can strike a health benefit on the host assuming a proper dose delivery as live micro-organisms. Different probiotics resume has different mechanisms of action, with some competing with pathogenic bacteria, adhesion inhibitory properties, anti-microbial production, among others [6]. The reasoning behind the administration of probiotics in lozenge form is convenience and kid-friendliness, therefore, facilitating permanent oral colonization [7]. Conversely, numerous studies have been carried out to determine the properties of probiotics in the dental practice, evidence is still inconsistent, and no quality randomized controlled trials have been conducted on a population of children [8]. The second area that requires further investigation is that the trials should be done in relation to the effect of the probiotics on the children and this can be categorically defined as being at high caries risk [9]. It is arguing that such a population would be more indicative of clinical benefits due

to their increased pathogens [10]. It would examine the common intake of a probiotic lozenge and its potential outcomes on the salivary *S. mutans* in high caries-risk children, and in a randomized, controlled pilot study, double-blind way [11]. The findings of this type can inspire the incorporation of probiotics into the regular caries prevention programmers.

### **Methodology**

In the actual study design, a double-blind RCT was held on 60 children aged 6-12 years who attended the pediatric dental clinic. An increased risk was determined clinically in the presence of  $\geq 3$  active carious lesions along with salivary *S. mutans* levels and dietary/hygiene habits. Randomization and Interventional: Then, 30 participants each were randomly distributed to two groups. The probiotic group received *Lactobacillus reuteri* DSM 17938 and ATCC PTA 5289 in lozenges. Placebo: Patients received identically appearing lozenges devoid of active probiotic cultures. The instruction given was to dissolve one lozenge in the mouth at bedtime for a period of 28 days. Compliance was measured by checklists filled out by the parents and follow-up calls once a week. Saliva Sampling and Microbial Analysis The unstimulated saliva samples were collected at the baseline and day 28. Cultivation on Mitis-Salivarius agar with bacitracin was done for the samples. Counting of CFU/mL of *S. mutans* was performed by using standard microbiological procedures. Statistical Analysis The paired and independent t-tests were used to make the contrast among and between the groups for mean CFU values. All obtained probabilities were evaluated at the level of  $p < 0.05$ .

### **Results:**

The comparison of salivary *Streptococcus mutans* between the groups after 28 days with intercede showed a clear, clinically meaningful difference. Starting almost with similar baseline counts of bacteria, the probiotic group ended the study period with a statistically significant decrease in the level of *S. mutans* by as much as 53%. This reduction, besides being clinically linked, was systematically significant to show the efficiency of the probiotic lozenges in suppressing cariogenic bacterial load. The placebo group had just an 8% reduction, which was not statistically significant. However, when directly linked with the placebo group, the reduction in the probiotic group stands out to be significant as well as superior. Evidence points out the potential of probiotics as a useful adjunct in the strategies against caries.

### **Discussion:**

This presented, double-blind RCT established that the normal probiotic lozenges containing *Lactobacillus reuteri* resulted in a demonstrable cut of the salivary *Streptococcus mutans* counts among the children at

high caries risk [12]. This was much higher compared to the placebo group with a particular support of the hypothesis that probiotics could be involved in a positive modulation of the oral microbiome of pediatric populations [13]. There are a number of biological plausible events in which *L. reuteri* can be capable of exerting their antimicrobial effect: the strain synthesizes reuterin, which is a broad-spectrum antimicrobial agent that acts against pathogenic bacteria. The probiotic organisms are also able to compete with the *S. mutans* as regards to adhesion sites [14]. The lozenge form is likely to raise the time of contact in the oral cavity, as well as enhance the interaction time of the probiotic organism and the oral biofilm. Findings of the current research are consistent with the majority of the past researches that have reported the decrease of *S. mutans* in children who are under probiotic supplementation [15]. Study results are however not always uniform as observed in literature but this could be attributable in part to the various probiotic strains, doses and delivery vehicles and other groups of study may also be a cause of such variation [16]. It is worth mentioning that, the current research targeted high risk children, in other words, the population benefiting the most with biological caries-preventive strategies [17]. The significant decreases indicated imply that probiotics could be used as a useful supplement to the use of fluoride, as well as dietary counselling and mechanical plaque control. The present study has a strength in that it is done in a double-blind randomized design that reduces selection and observer bias [18]. Adherence was strictly observed and microbial evaluation was done according to standard laboratory protocols. In addition to that, there are several limitations, which must be taken into account. The time taken was relatively little and further studies should be conducted on longer duration to determine whether the decreases in *S. mutans* are sustained on longer periods [19]. But no clinical outcome involving a change in caries incidence - had been quantified so the long-term preventive effect still has to be determined. More research is required to discover the optimization of dosing frequency and the probiotic strains. Collectively, these results indicate how probiotic lozenges could be viable and palatable methods in the process of limiting the cariogenic bacterial load. These preparations are a promising addition or adjunct in pediatric caries prevention with high-risk children since they are non-invasive and are easy to administer, and they have a good safety profile.

**Conclusion:**

The effect of a 28-day course of a probiotic *Lactobacillus reuteri* lozenge versus placebo as an adjunct to daily salivary streptococci mutans counts was significantly lower in high caries-risk children. Therefore, probiotic use could become a new promising adjunctive method of caries prevention in children, but longitudinal studies should be provided with the description of clinical outcomes.

## References:

1. Olczak-Kowalczyk, D., Turska-Szybka, A., Twetman, S., Gozdowski, D., Piekoszewska-Ziętek, P., Góra, J., & Wróblewska, M. (2025). Effect of tablets containing a paraprobiotic strain and the cranberry extract on caries incidence in preschool children: A randomized controlled trial. *Dent. Med. Probl*, 62, 209-215.
2. Chen, W. J., Sharma, L. A., Shao, P., Griffith, T., Love, R., Jain, R., ... & Sharma, A. (2025). Adjunctive use of *Streptococcus salivarius* M18 probiotic in the treatment of periodontitis: a randomized controlled trial. *3 Biotech*, 15(6), 192.
3. Campus, G., Cagetti, M. G., Lehrkinder, A., Alshabeeb, A., Caimoni, N., & Lingström, P. (2025). The Probiotic Effects of *Lactobacillus brevis* CD2 on Caries Related Variables of Dental Plaque Biofilm. *International Dental Journal*, 75(3), 1662-1671.
4. Fu, D., Shu, X., Yao, L., Zhou, G., Ji, M., Liao, G., ... & Zou, L. (2025). Unveiling the dual nature of *Lactobacillus*: from cariogenic threat to probiotic protector—a critical review with bibliometric analysis. *Frontiers in Oral Health*, 6, 1535233.
5. Pachava, S., Jayavaram, N., & Grandhi, P. (2026). Effect of Xylitol-Containing Chewing Gums on Oral Microflora: A Systematic Review and Meta-Analysis. *Pesquisa Brasileira em Odontopediatria e Clínica Integrada*, 26, e240028-e240028.
6. Liang, N. L., Luo, B. W., Sun, I. G., Chu, C. H., & Duangthip, D. (2024). Clinical effects of sugar substitutes on cariogenic bacteria: a systematic review and meta-analysis. *International Dental Journal*, 74(5), 987.
7. Giray, F. E., Özşahin, C. G., Topcuoğlu, N., & Kargul, B. (2024). Investigation of Antifungal Effects of Different Remineralization Agents on Salivary *Candida* amount in children with Early Childhood Caries. *Cumhuriyet Dental Journal*, 27(4), 245-251.
8. Gloria-Garza, M. A., Reyna-Martínez, G. R., Jiménez-Salas, Z., Campos-Góngora, E., Kačániová, M., Aguirre-Cavazos, D. E., ... & Elizondo-Luevano, J. H. (2025). Medicinal Plants Against Dental Caries: Research and Application of Their Antibacterial Properties. *Plants*, 14(9), 1390.
9. Pørksen, C. J., Keller, M. K., Damholt, A., Frederiksen, A. K. S., Ekstrand, K. R., Markvart, M., ... & Bakhshandeh, A. (2023). The effect of a lozenge combining prebiotic arginine and probiotics on caries increment in children during 10–12 months, a randomized clinical trial. *Journal of Dentistry*, 135, 104599.

10. Lai, S., Lingström, P., Cagetti, M. G., Cocco, F., Meloni, G., Arrica, M. A., & Campus, G. (2021). Effect of Lactobacillus brevis CD2 containing lozenges and plaque pH and cariogenic bacteria in diabetic children: a randomised clinical trial. *Clinical Oral Investigations*, 25(1), 115-123.
11. Olczak-Kowalczyk, D., Turska-Szybka, A., Twetman, S., Gozdowski, D., Piekoszewska-Ziętek, P., Góra, J., & Wróblewska, M. (2025). Effect of tablets containing a paraprobiotic strain and the cranberry extract on caries incidence in preschool children: A randomized controlled trial. *Dent. Med. Probl*, 62, 209-215.
12. Gedam, K. Y., & Katre, A. N. (2022). Efficacy of probiotic, chlorhexidine, and sodium fluoride mouthrinses on mutans streptococci in 8-to 12-year-old children: a crossover randomized trial. *Lifestyle Genomics*, 15(1), 35-44.
13. Elshennawy, S. M. (2023). *In-Vitro Characterization of the Effects of a Developing Oral Probiotic Against Dental Caries in Orthodontic Patients (Streptococcus salivarius LAB813, a Probiotic Against Dental Caries)* (Master's thesis, University of Toronto (Canada)).
14. Amargianitakis, M., Antoniadou, M., Rahiotis, C., & Varzakas, T. (2021). Probiotics, prebiotics, synbiotics and dental caries. new perspectives, suggestions, and patient coaching approach for a cavity-free mouth. *Applied Sciences*, 11(12), 5472.
15. Kavitha, M., Prathima, G. S., Anusha, D., Kengadaran, S., Gayathri, K., & Vinothini, V. (2022). Evaluation of the efficacy of plaque reduction and gingival health among 6-12 years old school children before and after a short term daily intake of probiotic lozenge-A comparative study. *Indian Journal of Dental Research*, 33(2), 184-187.
16. Chen, W. J., Sharma, L. A., Shao, P., Griffith, T., Love, R., Jain, R., ... & Sharma, A. (2025). Adjunctive use of Streptococcus salivarius M18 probiotic in the treatment of periodontitis: a randomized controlled trial. *3 Biotech*, 15(6), 192.
17. Zhang, Q., Shan, B., Xu, X., Mao, B., Tang, X., Zhao, J., ... & Chen, W. (2023). Lactiplantibacillus Plantarum CCFM8724 reduces the amounts of oral pathogens and alters the oral microbiota in children with dental caries: A randomized, double-blind, placebo-controlled trial. *Journal of the American Nutrition Association*, 42(4), 361-370.
18. Tafti, S. D., Parisay, I., Mehrabkhani, M., Sabbagh, S., Seddigh, S., Ghazvini, K., & Daghestani, N. (2023). Effects of probiotic yogurt, casein phosphopeptide-amorphous calcium phosphate, and

xylitol chewing gums on the salivary count of Streptococcus mutans: A single-blinded randomized controlled clinical trial. *Dental Research Journal*, 20(1), 117.

19. Srinivasan, M., Ms, N., & Poorni, S. (2022). Comparing the effect of probiotic Streptococcus salivarius K12 and M18 on the Streptococcus mutans Count, salivary pH and buffer capacity: a randomized double blinded clinical trial. *Cumhuriyet Dental Journal*, 24(4), 346-354.