Effect of Probiotic *Lactobacillus reuteri* on Salivary Cariogenic Bacterial Counts among Groups of Preschool Children in Jeddah, Saudi Arabia: A Randomized Clinical Trial

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Objectives: *To evaluate the effect of probiotic Lactobacilli reuteri lozenges on caries-associated salivary bacterial counts (Mutans streptococci and Lactobacillus), dental plaque accumulation, and salivary buffer capacity in a group of preschool children.* ***Study Design:*** *The study group consisted of 178 healthy children (aged 3−6 years). Children were randomly grouped: the experimental group (n = 90) received L. reuteri probiotic lozenges and the control group (n = 88) received placebo lozenges, twice daily, for 28 days. Salivary Mutans streptococci and Lactobacillus counts, and buffer capacity were assessed using chair-side caries-risk test (CRT®) kits. The Simplified Oral Hygiene index (OHI-S) was used to assess dental plaque accumulation at baseline and after 28 days.* ***Results:*** *After 28 days, the experimental group had a statistically significant reduction in Mutans streptococci and lactobacilli (p = 0.000 and p = 0.020, respectively) and both groups had less plaque accumulation than at baseline. While the buffer capacity in the experimental group increased more than in the control group, it was not statistically significant (p = 0.577). Compliance was 90%, with no adverse events.* ***Conclusions:*** *Consumption of probiotic lozenges containing L. reuteri reduces caries-associated bacterial counts significantly. Probiotics consumption may have a beneficial caries-preventive effect.*

Key words: *Child, dental caries, Lactobacillus, probiotics, saliva, Mutans streptococci.*

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INTRODUCTION

Early childhood caries (ECC) is a particularly devastating type of dental caries that can damage the early dentition of toddlers and preschool children.[1] ECC is an infectious multi-factorial disease[2]; however, early infection with *Streptococcus mutans* is reported to be the main risk driver for caries development[1]. Furthermore, *S. mutans* and *Lactobacillus* are considered microbial risk markers for ECC[3].

The contemporary means of caries prevention focuses mainly on host factors, dietary factors, and plaque biofilm removal[4]. Recently, an alternative plan for caries prevention, involving probiotic therapy, has been advocated. Probiotics has emerged as a natural and alternative method to combat infectious disease by displacing and replacing pathogenic microorganisms with non-pathogenic endogenous or commensal bacteria[5]. "Probiotics" refers to the bacteria that are related to beneficial outcomes in humans and animals. According to the World Health Organization/Food and Agriculture Organization of the United Nations report (2002), probiotics are the live microorganisms that promote health if given in an adequate amount[6].

The literature surrounding the usage of probiotics for dental caries shows inconsistent findings in studies conducted in a pediatric population. Some studies reported a reduction in *S. mutans*,

while other studies reported no reduction, which may have been due to differences in the methodology used in the studies. Some studies were conducted with a small sample size and other studies were conducted over a short period of time[7-9]. Further investigations are required to provide scientific evidence about the effect of probiotic therapy in children.

The potential effect of *L. reuteri* probiotics on carciogenic bacteria counts in the saliva, dental plaque, and buffer capacity in children has not yet been reported. Thus, this study aimed to evaluate whether daily consumption of probiotic *L. reuteri* lozenges is effective against caries-associated salivary bacteria (*Mutans streptococci and lactobacilli*), in reducing dental plaque accumulation and, and maintaining the salivary buffer capacity in a group of healthy preschool children. The null hypothesis was that daily consumption of probiotic *L. reuteri* lozenges would have no effect on the salivary bacterial counts of mutans streptococci and lactobacilli, dental plaque accumulation, or salivary buffer capacity.

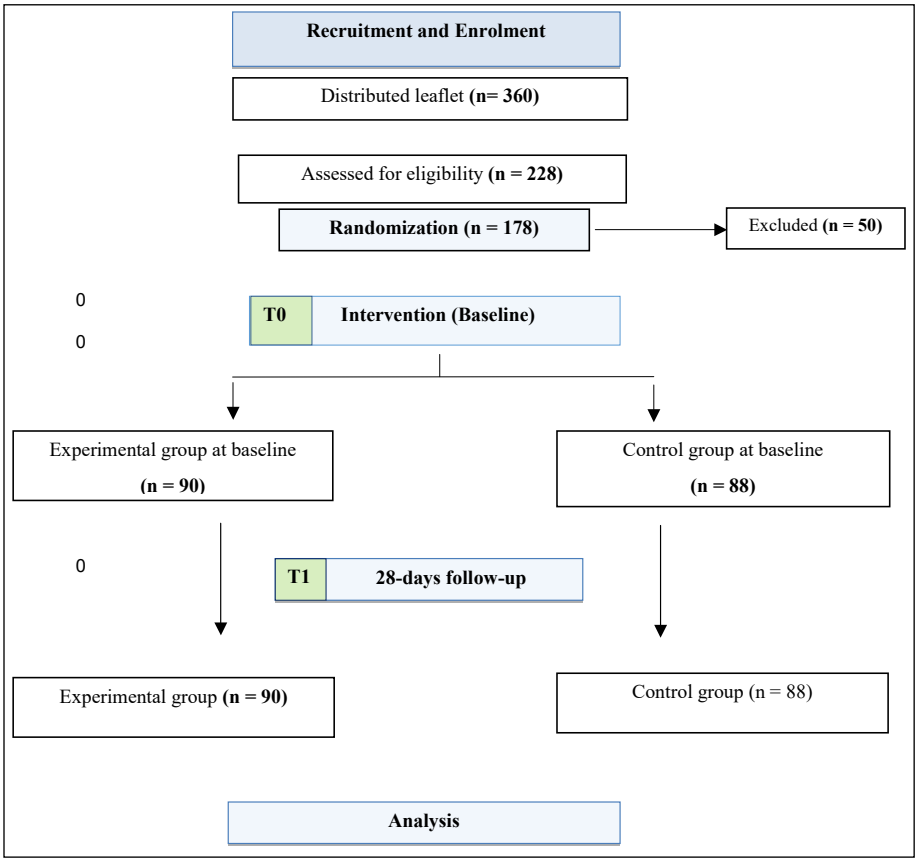
MATERIALS AND METHOD

The study protocol (proposal no. 031-41) was reviewed and the ethical approval was granted by the King Abdul-Aziz University Research Ethics Committee, Faculty of Dentistry. The trial was registered at www.clinicaltrials.gov (NCT NCT01601145). Prior to the clinical trial, children's parents were informed and their consent was obtained.

The sample size estimation was based on the assumption that the intervention would reduce *mutans streptococci* counts. Based on previous studies[10,11], it was assumed that a reduction of *mutans streptococci* would occur in 30% of the subjects in the intervention group and in 10% of the controls. A free-ware web-based operating system, OpenEpi version 3, was used for sample size calculation.[12] Eighty subjects per group were required to show a statistically significant difference between the groups at the 5% significance level, with 80% power. After screening 228 children, a total of 178 children (aged 3−6 years) were found eligible for participation in the study. The children were recruited from the pediatric dentistry clinic at King Abdul-Aziz University.

Children were allocated to two groups randomly, in a double-blind method, using a random number generator on www.graphpad.com to create a list of random numbers. Inclusion criteria were the following: healthy children with no history of any systemic condition, having a full set of primary dentition, high counts of salivary *Mutans streptococci* ($\geq 10^5$ CFU/mL), a decayed, missing, and filled teeth (dmft) index score ≥1, with no history of recent antibiotic administration. The exclusion criteria were as follows: lack of consent, medically compromised children, children who had used topical fluoride 4 weeks before the baseline time-point, except for fluoride in toothpaste, and use of xylitol chewing gums. Figure 1 demonstrates the number of children recruited and randomized to the experimental (90 children) and control groups (88 children).

Figure 1. Participant flow chart showing the number of subjects in the experimental and control groups at different time points during the study.



Test products

Probiotic lozenges consisted of a minimum of 200 million live *L. reuteri* (*L. reuteri* DSM 17938 and *L. reuteri* ATCC PTA 5289) (*L. reuteri* Prodentis®) (Biogaia, Stockholm, Sweden). The size, look, and taste of probiotic and placebo lozenges were similar, but there were no probiotic bacteria in the placebo. The lozenges were packed in white plastic bottles; the examiners and contributors were unable to distinguish between probiotic and placebo lozenges. BioGaia AB, (Stockholm, Sweden) provided the lozenges after assessing the study protocol.

Study design

This was a double-blind clinical trial, randomly grouped, and controlled using two parallel groups: Group A (Experimental group, n = 90) consumed probiotic lozenges consisting of a minimum of 200 million live *L. reuteri* for 28 days and Group B (control group, n = 88) consumed placebo lozenges, which did not contain any live microorganisms, also for 28 days. At the beginning of the study, a questionnaire was filled out about the baseline characteristics of each subject. The parents were instructed to give the probiotic lozenges or placebo to their children twice daily, at the same time. Parents were instructed that participants should take their first lozenges in the morning and the second in the evening after brushing teeth. During the intervention period, the parents were strongly encouraged to perform regular dental hygiene for their children using a "pea-size" amount of toothpaste with 500 ppm fluoride twice daily. Both toothbrushes and toothpaste were provided to the parents. All parents were also instructed to inform the investigators immediately regarding any adverse effects resulting from lozenge consumption.

Clinical examination

Intraoral examinations for the children were performed by two pediatric dentists after performing inter- and intra-examiner calibration before the study. The dental caries status at the baseline was assessed using the dmft index according to the modified WHO criteria for diagnosis of dental caries.[13] In addition to oral examination, bitewing radiographs were performed for every participant to analyze proximal caries. The modified version of the simplified Oral Hygiene index (OHI-S) was used to assess oral hygiene; this index had been amended to be applicable to primary dentition.[14] However, the system used for scoring was almost the same as the original OHI-S.[15] Assessment of oral hygiene was carried out at baseline (T0) and after 28 days (T1) from the beginning of the trial for every participant.

Salivary microbial and buffer capacity evaluation

To assess the salivary Mutans streptococci and lactobacilli counts, and the buffer capacity, a simple chair-side caries risk test (CRT®; Ivoclar Vivadent AG, Bendererstrasse 2, FL-9494 Schaan, Liechtenstein) was used according to the manufacturer's instructions. For the salivary mutans streptococci and lactobacilli counts, the data obtained were scored as either "low" ($<10^5$ CFU/mL) or "high" ($>10^5$ CFU/mL), on the scale recommended by the manufacturer. The readings were collected by one investigator for all children, using the model chart. The buffer capacity BC was recorded as high, medium, or low, according to the strip color. A blue color was recorded as a "high", a green color indicated "medium BC", and a yellow color meant a "low BC". These assessments were collected at baseline (T0) and after 28 days (T1).

Compliance assessment

Two weeks' supply of lozenges was handed out to the parents. To assess compliance, the number of remaining lozenges returned by each child's parent was counted to confirm the missed consumption of lozenges. Furthermore, a compliance calendar chart was given to the parents to document the brushing frequency and intake of lozenges. Parents were instructed to mark one box for each lozenge taken per day, but if a lozenge was not taken, the corresponding box was left unticked. Moreover, a text message was sent weekly as a reminder for the parents/guardians.

Examiner calibration

To achieve good intra- and inter-examiner reliability, examiners were calibrated prior to baseline registration. Ten children were examined by two of the examiners to assess their caries and plaque. One examiner was responsible for evaluating salivary microbial counts and buffer capacity. Subjects were re-examined the next day and the level of agreement between corresponding readings was assessed.

Statistical analysis of data

Statistical Package for Social Sciences (version 20 Inc., Chicago, IL, USA) software was used to analyze all variables. Numerical data were described using mean and standard deviation. Qualitative data were described in the form of frequencies and percentages. Internal consistency was calculated using Cronbach's Alpha. In order to perform inter- and intra-examiner reliability, the inter-class correlation (ICC) test was used. A *t*-test was applied for comparisons of quantitative variables and chi-square for qualitative variables. The variation in the inter-group salivary buffer capacity, salivary lactobacilli counts, and salivary *S. mutans* counts were evaluated using the Wilcoxon's signed-rank test. Statistical significance was set at a p-value < 0.05.

RESULTS

In assessment of intra-examiner reliability for each examiner for recording dmft, the Intra Class Correlation (ICC) was 0.920 and 0.934, respectively, while the ICC for inter-examiner reliability was 0.965, indicating an almost perfect level of agreement. For the debris component (DI-S) of the simplified OHI-S measurement, the inter-examiner reliability was 0.819, indicating a strong level of agreement. The intra-rater reliability for caries-associated salivary bacterial counts (*Mutans streptococci* and *Lactobacillus*) using the CRT® test was determined using the kappa test of agreement, which was 0.83. Figure 1 shows the participant flow chart. The baseline characteristics of children are shown in Table 1. Baseline data on the subjects' OHI-S and salivary parameters are given in Table 2. Changes in the counts of Mutans streptococci and lactobacilli, and in buffer capacity, after 28 days' consumption of probiotic lozenges are shown in Table 3.

After 28 days of lozenge consumption, the experimental group showed significantly lower microbial counts compared to the control group (Table 3), for both salivary Mutans streptococci and lactobacilli P= 0.000, P=0.020 respectively. However, there was no statistically significant difference between the experimental and the control groups in terms of the buffer capacity (p = 0.577) Moreover, there was also no statistically significant difference between

Table 1. Baseline characteristics of children at the baseline of the study.

Baseline characteristic		Experimental (n = 90)	Control (n = 88)	Total (n = 178)	p value*
Sex (%)	Male	38 (42.2%)	32 (36.4%)	70 (39.32%)	0.446
	Female	52 (57.8%)	56 (63.6%)	108 (60.68%)	
Mean Age (Years)		4.90 (± 1.012)	4.98 (± 0.950)		0.617
Mean dmft at baseline		6.83 (± 2.94)	7.28 (± 3.728)		0.353

dmft, decayed, missing, and filled teeth

Table 2. Percent distribution of *Mutans streptococci* and *Lactobacillus* counts, buffer capacity, and mean debris component (DI-S) of the OHI-S score between the experimental and control groups at baseline.

	Experimental N = 90	Control N = 88	p value
	n1 (%)	n2 (%)	
***Mutans streptococci* (CFU/mL)**			
High (≥10^5)	90 (100)	88 (100)	NA*
Low (<10^5)	0 (0)	0 (0)	
***Lactobacilli* (CFU/mL)**			
High (≥10^5)	75 (83.3)	77 (83.3)	0.526*
Low (<10^5)	15 (16.7)	11 (16.7)	
Buffer capacity			
High	45 (50)	50 (45)	0.151*
Medium	27 (30)	30 (34)	
Low	18 (20)	20 (9)	
Debris component (DI-S) of (OHI-S)			
Mean (± S.D)	1.6 (± 0.4)	1.6 (± 0.49)	0.979**

*p-value using chi-square test

**p-value using t-test

n1 = number of children in experimental group.

n2= number of children in control group.

N = total number of children.

the experimental and the control groups in terms of OHI-S for the Debris Component (DI-S) P=0.451. However, intragroup evaluation revealed that probiotic lozenge administration resulted in a significantly improved buffer capacity after 28 days as compared to the baseline levels (P = 0.000; Table 4). There were also decreases in the oral hygiene indexes in both groups, but these were not statistically significant. All parents reported that lozenge consumption compliance of their children exceeded 90% over the 28 days, indicating perfect compliance. There were no significant differences in the number of lozenges consumed between the two groups (p = 0.590).

Table 3. Percent distribution of *Mutans streptococci* and *Lactobacillus* counts, buffer capacity, and mean debris component (DI-S) of the OHI-S score between the experimental and control groups at the end of the study.

	Experimental N = 90	Control N = 88	p value*
	n1 (%)	n2 (%)	
***Mutans streptococci* (CFU/mL)**			
High (≥10^5)	46 (51.1)	79 (89.8)	0.000*
Low (<10^5)	44 (48.9)	9 (10.2)	
***Lactobacilli* (CFU/mL)**			
High (≥10^5)	57 (63.3)	70 (79.5)	0.020*
Low (<10^5)	33 (36.7)	18 (20.5)	
Buffer capacity			
High	58 (64.4)	50 (56.8)	0.577*
Medium	24 (26.7)	28 (31.8)	
Low	8 (8.9)	10 (11.4)	
Debris component (DI-S) of (OHI-S)			
Mean (± S.D)	1.12 (± 0.43)	1.20 (± 0.48)	0.451**

*p-value determined using chi-square test.

**p-value determined using *t*-test

n1 = number of children in Experimental group.

n2 = number of children in Control group.

N = total number of children.

DISCUSSION

The prevalence of dental caries was gauged to be roughly 80% for the initial dentition, using a mean dmft of 5.0 and 70% for children's primary dentition, using a mean dmft of 3.5.[16] According to these data, dental caries is considered to be a serious public health problem in Saudi Arabia. Dental caries treatment is costly, necessitating the use of various preventative strategies. Thus, we examined whether the daily consumption of probiotic *L. reuteri* lozenges is effective in reducing caries-associated salivary bacterial counts and dental plaque accumulation, and refining salivary buffer capacity, in a high-risk group for caries, i.e., healthy preschool children in Jeddah, Saudi Arabia.

We found that *mutans streptococci* counts were significantly decreased in the experimental group as compared to the control group by the end of the study period. *L. reuteri* secretes an antimicrobial compound, reuterin, which may partly be responsible for this positive effect.[17] This finding was corroborated by many previous studies that evaluated *mutans streptococci* levels in saliva after oral probiotic therapy, in both adults and children, using different probiotic stains.[9-11, 18-24] Four of those studies used the *L. reuteri* probiotic strain in different forms; all the studies were conducted in adult's populations.[11, 21-22, 25] The results of these studies showed that products containing *L. reuteri* resulted in an apparent decline in salivary *mutans streptococci*. Conversely, some studies contradicted the findings of this study, as they reported no changes in salivary *mutans streptococci* levels post-probiotic administration.[8, 26-31] One of these studies examined the impact of *L. reuteri* as a probiotic in the form of oral drops on the salivary mutans streptococci and lactobacilli

Table 4. Distribution of children according to changes in salivary *Mutans streptococci* counts, lactobacilli counts, and buffer capacity from baseline in the experimental and control groups.

Changes in salivary counts	n	Experimental group			Control group		
		Mean Rank	P-value*	n	Mean Rank	P-value*	
Mutans streptococci	Negative Ranks	44	22.50		9	5.00	
	Positive Ranks	0	0.00		0	.00	
	Ties	46			79		
	Total	90		0.000	88		0.003
Lactobacilli	Negative Ranks	20	11.50		11	8.00	
	Positive Ranks	2	11.50		4	8.00	
	Ties	68			73		
	Total	90		0.000	88		0.071
Buffer capacity	Negative Ranks	0	11.00		5	10.00	
	Positive Ranks	21	0.00		11	7.00	
	Ties	69			72		
	Total	90		0.000	88		0.166

*p-value determined using Wilcoxon's signed-rank test.

n = total number of children in each group

Table 5: Comparison of mean and standard deviation of number of lozenges taken after 28 days between the experimental and control groups.

Study groups	Number of lozenges taken Mean (± S.D.)	P-value*
Experimental group (n = 90)	53.84 (± 9.380)	0.590
Control group (n = 88)	54.49 (± 6.275)	

*p-value determined using *t*-test.

counts in children with cleft lip/palate, aged 4−12 years.[8] The results of that clinical trial showed no statistically significant decrease in salivary mutans streptococci and lactobacilli after consumption of probiotic or placebo drops. The authors explained their results as follows. First, the expected effect of probiotics and their interactions on the oral microflora of this group of children may be complex. Secondly, oral drops may not be a suitable form for administration of probiotics. Lastly, the limited number of subjects in the study (n = 19) may have confounded the results.

When assessing the effect of *L. reuteri* probiotic therapy on lactobacilli levels, a statistically significant reduction in *Lactobacillus* level was recorded in the experimental group, as compared to the control group, similar to the findings of other studies. A study performed by Cogulu *et al* found a significant decline in both salivary mutans streptococci and lactobacilli after multistrain probiotic-kefir consumption[24]. Additionally, two studies performed by Caglar *et al* in 2005 [5] and 2006 [11] found a reduction of lactobacilli after probiotic consumption; however, this reduction did not reach statistical significance. In this study, the reduction in *Lactobacillus* levels observed in the experimental group may have been due to the improved oral hygiene care for children by their parents.

Additionally, it was observed that the buffer capacity value did not reduce from high to low or medium in any of the cases in either group. Most of the children with low or medium buffer capacity increased their buffer capacity, although all the children with high buffer capacity retained their high values. While buffer capacity in the experimental group increased more than in the control group, there was no statistically significant difference among the experimental and the control groups. This suggests that certain changes in buffer capacity might be related to improvement in OHI-S, and that the changes are not related to probiotic therapy alone.

Plaque accumulation was measured by the debris component of the OHI-S, which has been accepted for measuring oral hygiene in large epidemiological studies .[32] Both experimental and control groups experienced improvement in the debris component of the OHI-S by the end of the study period. One potential reason for this improvement in children in the control group may be the improved oral hygiene care and plaque removal due to being observed as a participant in this study. This so-called Hawthorne effect (which is also known as observer influence) is well documented in clinical trial literature.[33] Specifically, the parents in this study were counseled that they were participating in a trial about probiotic lozenges to decrease salivary cariogenic bacteria and plaque levels of their children. Therefore, knowledge about the targeted outcome may have consciously or subconsciously caused these parents to take greater care in their children's oral hygiene routines, as they knew beforehand which outcomes were being evaluated.

To date, the impact of *L. reuteri* probiotic in lozenge form has not been reported for a group of preschool children with high caries risk. Furthermore, the effect of probiotic lozenges was evaluated on different oral health aspects, i.e., caries-associated salivary bacterial counts (*Mutans streptococci* and *Lactobacillus*), dental plaque accumulation, and salivary buffer capacity. We showed that *L. reuteri* probiotic lozenges can be used safely in children and that lozenges are an easy and acceptable vehicle for probiotic administration, as demonstrated by the high compliance to the study protocol.

In the present study, there were a few unavoidable limitations. An inherent limitation of this study was the confounding variables. Although randomization was performed so that the effect of confounding variables would be evenly distributed between both study groups, there is a chance that the groups were not identical. Some potential confounding variables that may have affected the outcome are diet, fluoridate toothpaste, and the frequency of eating. Each of these variables exert an influence on plaque build-up and cariogenic bacteria levels as well.[34] The other limitation is that the result of the probiotic lozenges related to the incidence of the caries itself has not been evaluated, as dental caries incidence require a long-term follow-up period, and this will be costly, since other parameters evaluated in the study should be included as well. The intention to treat analysis was not performed, as no subject was excluded during the trial.

Finally, the body of literature regarding the use of oral probiotics is currently growing; however, the scientific evidence is still not well established. Further studies on oral probiotics are required to evaluate the clinical significance of probiotic therapy. More investigations should be undertaken to define the optimal dose and time intervals of administration for each strain and delivery vehicle, as the existing literature does not provide any beneficial insights in this regard.

CONCLUSION

We conclude that consumption of a probiotic lozenge containing *L. reuteri* significantly reduces caries-associated bacterial counts. The probiotic lozenge was also found to be effective in reducing plaque accumulation. Probiotic consumption may have a beneficial effect on salivary buffer capacity. Further clinical trials are needed to assess the different methods of probiotic administration in the oral cavity and to make recommendations regarding long-term and short-term use, and the frequency and dose of oral probiotic consumption required.

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REFERENCES

1. Berkowitz RJ. Causes, treatment and prevention of early childhood caries: a microbiologic perspective. J Can Dent Assoc 69(5): 304-307, 2003.
2. Babu Jose B, King MNM. Early childhood caries lesions in preschool children in Kerala, India. Pediatr Dent 25(6): 594-600, 2003.
3. Kanasi E, Johansson I, Lu SC, Kressin NR, Nunn ME, Kent R, et al. Microbial risk markers for childhood caries in pediatricians' offices. J Dent Res 89(4): 378-383, 2010.
4. Laleman I, Detailleur V, Slot DE, Slomka V, Quirynen M, Teughels W. Probiotics reduce mutans streptococci counts in humans: a systematic review and meta-analysis. Clin Oral Invest 18(6): 1539-1552, 2014.
5. Caglar E, Kargul B, Tanboga I. Bacteriotherapy and probiotics' role on oral health. Oral Diseases 11(3): 131-137, 2005.
6. FAO/WHO. Health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria—Joint Food and Agricultural Organization of the United Nations and World Health Organization Expert Consultation Report, Córdoba, Argentina. 2002.
7. Aminabadi N, Erfanparast L, Ebrahim, A, Oskouei S. Effect of chlorhexidine pretreatment on the stability of salivary lactobacilli probiotic in six-to twelve-year-old children: a randomized controlled trial. Caries Res 45(2): 148-154, 2011.
8. Cildir SK, Sandalli N, Nazli S, Alp F, Caglar E. A novel delivery system of probiotic drop and its effect on dental caries risk factors in cleft lip/palate children. Cleft Palate Craniofac J 49(3): 369-372, 2012.
9. Juneja A, Kakade A. Evaluating the effect of probiotic containing milk on salivary mutans streptococci levels. J Clin Ped Dent 37(1): 9-14, 2012.
10. Ahola A, Yli-Knuuttila H, Suomalainen T, Poussa T, Ahlström A, Meurman JH, et al. Short-term consumption of probiotic-containing cheese and its effect on dental caries risk factors. Arch Oral Biol 47(11): 799-804, 2002.
11. Caglar E, Kavaloglu Cildir S, Ergeneli S, Sandalli N, Twetman S. Salivary mutans streptococci and lactobacilli levels after ingestion of the probiotic bacterium *Lactobacillus reuteri* ATCC 55730 by straws or tablets. Acta Odontol Scandin 64(5): 314-318, 2006.
12. Dean AG, Sullivan KM, Soe MM. Epi Info™ and OpenEpi In Epidemiology and Clinical Medicine. Health Applications of Free Software Chapter 15, 2010. http://www.epiinformatics.com/DownloadFiles/EpiBook.pdf
13. World Health Organization. Oral health surveys: basic methods. Fourth Edition ed. Geneva, Switzerland: World Health Organization; 40-45, 1997.
14. Miglani D, Beal J, James P, Behari S. The assessment of dental cleanliness status of the primary dentition using a modification of the simplified oral hygiene index (OHIS-M). J Ind Dent Assoc 45(12): 385, 1973.
15. Greene JG, Vermillion JR. The simplified oral hygiene index. J Am Dental Assoc 68(1): 7-13, 1964.
16. Al Agili DE. A systematic review of population-based dental caries studies among children in Saudi Arabia. Saudi Dent J 25(1): 3-11, 2013.
17. Speck ML, Dobrogosz WJ, Casas IA. Lactobacillus reuteri in food supplementation. Food Technol 47.7: 90-94, 1993.
18. Ashwin D, Vijayaprasad K, Taranath M, Ramagoni NK, Nara A, Sarpangala M. Effect of probiotic containing ice-cream on salivary mutans streptococci (SMS) levels in children of 6−12 years of age: A randomized controlled double blind study with six-months follow up. J Clin Diagnost Res 9(2): ZC06, 2015.
19. Caglar E, Kavaloglu S, Kuscu O, Sandalli N, Holgerson P, Twetman S. Effect of chewing gums containing xylitol or probiotic bacteria on salivary mutans streptococci and lactobacilli. Clin Oral Invest 11(4): 425-429, 2007.
20. Çaglar E, Kuscu OO, Cildir SK, Kuvvetli SS, Sandalli N. A probiotic lozenge administered medical device and its effect on salivary mutans streptococci and lactobacilli. Int J Paediatr Dent 18(1): 35-39, 2008.
21. Çaglar E, Onder Kuscu O, Selvi Kuvvetli S, Kavaloglu Cildir S, Sandalli N, Twetman S. Short-term effect of ice-cream containing *Bifidobacterium lactis* Bb-12 on the number of salivary mutans streptococci and lactobacilli. Acta Odontol Scand 66(3): 154-158, 2008.
22. Çaglar E, Sandalli N, Twetman S, Kavaloglu S, Ergeneli S, Selvi S. Effect of yogurt with *Bifidobacterium* DN-173 010 on salivary mutans streptococci and lactobacilli in young adults. Acta Odontol Scandin 63(6): 317-320, 2005.
23. Cildir SK, Germec D, Sandalli N, Ozdemir FI, Arun T, Twetman S, et al. Reduction of salivary mutans streptococci in orthodontic patients during daily consumption of yoghurt containing probiotic bacteria. Eur J Orthodont 31(4): 407-411, 2009.
24. Cogulu D, Topaloglu-Ak A, Caglar E, Sandalli N, Karagozlu C, Ersin N, et al. Potential effects of a multistrain probiotic-kefir on salivary *Streptococcus mutans* and *Lactobacillus* spp. J Dent Sci 5(3): 144-149, 2010.
25. Nikawa H, Makihira S, Fukushima H, Nishimura H, Ozaki Y, Ishida K, et al. *Lactobacillus reuteri* in bovine milk fermented decreases the oral carriage of mutans streptococci. Int J Food Microbiol 95(2): 219-223, 2004.
26. Burton JP, Drummond BK, Chilcott CN, Tagg JR, Thomson WM, Hale JD, et al. Influence of the probiotic *Streptococcus salivarius* strain M18 on indices of dental health in children: a randomized double-blind, placebo-controlled trial. J Med Microbiol 62(6): 875-884, 2013.
27. Gizani S, Petsi G, Twetman S, Caroni C, Makou M, Papagianoulis L. Effect of the probiotic bacterium *Lactobacillus reuteri* on white spot lesion development in orthodontic patients. Eur J Orthodont 38(1): 85-89, 2016.
28. Keller M, Hasslöf P, Dahlén G, Stecksén-Blicks C, Twetman S. Probiotic supplements (*Lactobacillus reuteri* DSM 17938 and ATCC PTA 5289) do not affect regrowth of mutans streptococci after full-mouth disinfection with chlorhexidine: a randomized controlled multicenter trial. Caries Res 46(2): 140-146, 2012.
29. Stensson M, Koch G, Coric S, Abrahamsson T, Jenmalm MC, Birkhed D, et al. Oral administration of *Lactobacillus reuteri* during the first year of life reduces caries prevalence in the primary dentition at 9 years of age. Caries Res 48(2): 111-117, 2013.
30. Taipale T, Pienihäkkinen K, Alanen P, Jokela J, Söderling E. Administration of *Bifidobacterium animalis* subsp. lactis BB-12 in early childhood: A post-trial effect on caries occurrence at 4 years of age. Caries Res 47(5): 364-372, 2013.
31. Toiviainen A, Jalasvuori H, Lahti E, Gursoy U, Salminen S, Fontana M, et al. The impact of orally administered lozenges with *Lactobacillus rhamnosus* GG and *Bifidobacterium animalis* subspecies lactis BB-12 on the number of salivary mutans streptococci, the amount of plaque, gingival inflammation and the oral microbiome in healthy adults. Clin Oral Investig 19(1): 77-83, 2015.
32. Broadbent JM, Thomson WM, Boyens JV, Poulton R. Dental plaque and oral health during the first 32 years of life. J Am Dental Assoc;142(4): 415–426, 2011.
33. McCarney R, Warner J, Iliffe S, van Haselen R, Griffin M, Fisher P. The Hawthorne Effect: a randomised, controlled trial. BMC Med Res Methodol 7(1): 30, 2007.
34. Mejàre I, Axelsson S, Dahlén G, Espelid I, Norlund A, Tranæus S. Caries risk assessment. : A systematic review. Acta Odontol Scand ;72(2):81-91, 2014.

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