Effects of probiotic drop containing *Lactobacillus rhamnosus, Bifidobacterium infantis,* and *Lactobacillus reuteri* on salivary *Streptococcus mutans* and *Lactobacillus* levels

Maryam Hajnorouzali Tehrani, Najmeh Akhlaghi, Leila Talebian, Jaber Emami[1], Siamak Etzad Keyhani[2]

Abstract

Aims: The aim of the present study was to evaluate the effect of a probiotic drop containing *Lactobacillus rhamnosus*, *Bifidobacterium infantis*, and *Lactobacillus reuteri* on salivary counts of *Streptococcus mutans* (SM) and *Lactobacillus* (LB) in children 3–6 years of age. **Settings and Design:** Sixty-one healthy children were randomly allocated into two parallel blocks in this double-blind, randomized controlled trial (IRCT2014120320202N1) from May to June 2015. **Subjects and Methods:** Finally 53 participants consumed five drops of placebo (n = 23) or probiotic (n = 30) every night for 2 weeks. Before intervention and 1 day after completion of the intervention, unstimulated salivary samples were collected, and microbiologic evaluations were carried out. **Statistical Analysis:** Data were analyzed with descriptive statistical methods Wilcoxon signed ranks, Mann–Whitney, and logistic regression. **Results:** SM level decreased significantly in probiotic group after intervention (P = 0.045), and there were significant differences in salivary SM counts after intervention between two groups (P = 0.04). In probiotic group, LB counts decreased significantly after intervention (P = 0.048); however, there were no significant differences between two groups (P = 0.216). **Conclusions:** Use of this probiotic drop decreased salivary counts of SM; however, LB counts did not change. In addition, use of the drop in children with higher salivary counts appeared to be more effective.

Keywords: Drop, *Lactobacillus*, probiotic, *Streptococcus mutans*

Introduction

In general, there is proper response to probiotic interventions and unfavorable side effects are very rare.[1,2] Although a large number of clinical studies have shown promising results in relation to the effect of probiotics in decreasing caries rate or *Streptococcus mutans* (SM) counts,[1-7] some other studies have not reported a particular effect of probiotics.[8-11] Two studies have been carried out with the use of drops containing *Lactobacillus reuteri* and no differences have been observed between salivary counts of *Lactobacillus* (LB) and SM.[12,13] Regarding the conflicting results of previous studies about the effects of probiotic products on oral health and as well as the importance of oral health, this study was performed.[14]

It is important to know that the efficacy of different species is not the same, and a combination of these species results in an increase in efficacy through synergism.[14,15]

In the present study, an attempt was made to use a product that contains a combination of bacteria, each of which has shown positive results in previous studies.[2,6,7] Therefore, a drop was used that contained *L. reuteri* of *Bifidobacterium infantis* and *Lactobacillus rhamnosus* to transfer probiotics and evaluate their effect on decreasing the counts of cariogenic bacteria. The aim of the present study was to evaluate the effect of this probiotic drop on salivary counts of SM and LB in 3–6-year-old children with initial

Department of Pediatric Dentistry, Dental Research Center, School of Dentistry, Isfahan University of Medical Sciences, [1]Department of Pharmaceutics, Pharmaceutical Research Center, School of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Sciences, Isfahan, [2]Pharmaceutical Incubator Center, Tehran, Iran

Correspondence: Prof. Najmeh Akhlaghi,
Department of Pediatric Dentistry, Dental Research Center,
School of Dentistry, Isfahan University of
Medical Sciences, Isfahan, Iran.
E-mail: n_akhlaghi@dnt.mui.ac.ir

Access this article online	
Quick Response Code:	Website: www.contempclindent.org
	DOI: 10.4103/0976-237X.194102

How to cite this article: Tehrani MH, Akhlaghi N, Talebian L, Emami J, Keyhani SE. Effects of probiotic drop containing *Lactobacillus rhamnosus*, *Bifidobacterium infantis*, and *Lactobacillus reuteri* on salivary *Streptococcus mutans* and *Lactobacillus* levels. Contemp Clin Dent 2016;7:469-74.

dental caries in a double-blind, randomized controlled clinical trial.

Subjects and Methods

The present double-blind, randomized controlled clinical trial with two parallel groups was carried out after it was registered at IRCT (IRCT2014120320202N1) and was approved by the Research Committee of Faculty of Dentistry, Isfahan University of Medial Sciences, Isfahan, Iran, under the code 393875. The sample size in each group was 28 volunteers at a significance level of 0.05 and a power of 80% ($\alpha = 0.05$, $\beta = 0.20$) using the below formula. This was estimated to show a 40% reduction of SM count in intervention group, 10% in the control, and 50% differences between two groups.[6]

$$n = \frac{(z_{1-\frac{\alpha}{2}} + Z_{1-\beta})^2 \ (\delta_1^2 + \delta_2^2)}{d^2}$$

Participant children

By a public invitation letter that was posted on the bulletin board of Isfahan University's faculties and clinics, volunteers were invited to participate in the study. Examinations and samplings were performed at Pediatric Dentistry Department, Dental School of Isfahan University of Medical Sciences, Isfahan, Iran, from May to June 2015. Written informed consent form was signed by volunteers (parents or caregiver of children). Sixty-one healthy children were recruited for the study. Grouping by randomized, double-blind method, there were 31 and 30 samples for the probiotic and control group, respectively, at the beginning of the study.

The inclusion criteria included 3- to 6-year-old children with initial dental caries and no systemic conditions, who had not received any product containing probiotics, xylitol, corticosteroids, systemic antibiotics, and local fluoride therapy for at least 4 weeks before taking part in the study. The volunteers had no gingivitis or periodontal diseases. Children were excluded from the study if they became ill or used medications. Caries-free children were excluded as well.

The drops for the two groups were randomized according to a computer-generated random table. For blinding purposes, the packaging of the probiotic and placebo drops were dark brown bottles and completely similar and sealed. The bottles were coded A or B by someone who was not involved in the study procedures so that drops were indistinguishable. Neither the researcher nor the volunteers knew which subjects belonged to which group (double-blind). The codes were deciphered at the end of statistical analyses.

The probiotic and placebo drop

The probiotic drop (Pedilact, Zist-Takhmir, Iran) was used, which contained *L. rhamnosus* ATCC 15820 (1×10^{10} colony-forming unit [CFU]/mL), *L. reuteri* ATCC 55730 (2×10^9 CFU/mL), and *Bifidobacterium longum* subsp. *infantis* ATCC 15697 (1.5×10^9 CFU/mL). Each of these bacteria has shown positive results in previous studies.[2,6,7] In addition, the drop contained the prebiotic fructo-oligosaccharide, which helps the growth and activity of probiotics. Its other constituents included sunflower seed oil, medium chain triglyceride oil, silicon dioxide, and a flavoring agent. This drop has been approved by the Center of Pharmaceutical Products, Tehran University of Medical Sciences and is routinely prescribed in Iran for the treatment of diarrhea, flatulence, and colic in infants and children by pediatrician. The placebo consisted of the same constituents except for the probiotics and was manufactured by Zist Takhmir Company too.[16]

Parents were instructed to keep the drop bottle in a refrigerator and given five drops to their children before bedtime for 2 weeks.

It was recommended not to brush their teeth for at least 1 h after taking the drop and refrain from eating and drinking. The parents were asked not to change the children's oral hygiene habits during the 2-week period.[17] To ensure the use of the drop and the volunteers, recalls, phone calls, and short messages were used. In addition, the volunteers were asked to fill out a table for 2 weeks after each time they used the drop. After 2 weeks, salivary samples were once again collected and sent to the microbiologic laboratory.

Clinical examination

Clinical examinations and sampling were carried out by a pediatric dentist (investigator 3). This examiner was trained and calibrated according to the WHO instructions[18] for tooth caries diagnosis. To determine the intraexaminer reliability, 10% of the total samples was reexamined during the data collection (kappa = 0.95).

The volunteers received an oral examination before the study using the oral mirror and Community Periodontal Index probe under the dental unit light and according to the WHO criteria. A questionnaire including demographic features and oral health data was completed. The number of decayed/missing/filled teeth (dmft/DMFT) was recorded for all teeth (inclusion criteria of 1–3 with initial stages of dental caries). DMFT was recorded in the 6-year-olds for permanent teeth. It should be noted that radiographic examinations for proximal caries detection could not be performed due to limitations and lack of Ethics Committee approval.

Collection of salivary samples

Salivary samples were collected by one operator twice for each volunteer: Before the intervention and 1 day after the end of intervention. The parents were asked to prevent the child from eating, drinking, and brushing before each sampling procedure from the time of waking up until the sampling procedure was completed. Due to different flow rates of saliva during the day, the salivary samples were collected during the early hours of the morning (7:30–8:30).

In addition, to prevent the effect of individual differences during the stimulation of salivary flow in the present study, unstimulated salivary samples were collected.[19] Each child was asked to evacuate his/her saliva (minimum 1 mL) into a sterile container. The sample was transferred to the microbiology laboratory within a maximum of 45 min and culturing was carried out on the same day.

Culturing of salivary samples

For SM count, 20 µl of saliva sample was spread on Mitis Salivarius Agar (Difco Detroit, MI, USA) supplemented with 0.2 units/ml bacitracin and sucrose (20% w/v) and incubated aerobically at 37°C for 24 h. In addition, 20 µl of saliva sample was spread on Man Rogosa Agar (MRS agar, Unipath, Basingstoke, UK) for the count of total LB. Plates were incubated anaerobically (85% N_2, 5% CO_2, and 10% H_2) into chambers at 37°C for 2 days. The CFUs were identified by morphology, size, and color and were counted using a stereomicroscope (Vision Engineering, Surrey, UK).

To confirm the diagnosis of colonies in mitis salivarius agar plates, Gram-staining, sugar fermentation, and catalase tests were performed. For sugar fermentation test, SM from each sample incubated for 24 h at 37°C in tryptic soy broth (TSB) contained (phenol red, mannitol 1%, TSB) and (phenol-red, sorbitol 1%, TSB) as well. Gram-positive cocci, negative catalase, positive mannitol, and sorbitol were reported as SM.

To confirm the diagnosis of colonies in MRS agar Gram-staining, oxidase and catalase diagnostic tests were performed. Gram-positive coccobacilli, negative catalase, and oxidase were reported as LB. SM and LB concentration in saliva was expressed as CFU/ml.

Statistical methods

The analyses were processed by SPSS software (version 20 Chicago, IL, USA). Posttreatment and pretreatment values within each regimen were compared with the Wilcoxon signed ranks test. To compare changes in the bacterial levels during the intervention between two groups, Mann–Whitney test was used. In each block, volunteers were divided into two groups according to their first SM or LB counts ($\geq 10^4$ or $>10^4$). These binary groups considered as dependent variables. Other variables (dmft/DMFT, age, gender, probiotic/placebo consumption) were considered as independent variables. Backward logistic regression with a step-wise selection procedure was utilized to investigate the influence of factors to the outcome of salivary bacterial levels.

Results

Of the –61 volunteers participating in the study, finally 53 volunteers (28 girls and 25 boys, mean age 4.6 ± 1.2, dmft 3.4 ± 2.2) including 30 volunteers in the probiotic group and 23 in control group completed the study [Figure 1]. Mann–Whitney U-test showed that dmft/DMFT values were not statistically significant between the two groups ($P = 0.324$). The compliance of volunteers was good as reported by parents. Based on the report of colony counts by the laboratory, the volunteers were placed in one of the following three groups:

- I: <1000 CFU/mL
- II: 1000–10,000 CFU/mL
- III: >10,000 CFU/mL.

Streptococcus mutans

Table 1 shows the numbers (percentages) of volunteers with different SM counts at before and after 2-week consumption of probiotic and placebo drop.

Mann–Whitney test did not reveal any significant difference in the salivary SM counts before intervention between the placebo and probiotic groups ($P = 0.054$); however, there were significant differences in the salivary SM counts of after intervention between the placebo and probiotic groups ($P = 0.04$). Wilcoxon signed ranks test showed a significant decrease in SM colony counts in probiotic group after intervention ($P = 0.045$), with no significant changes SM colony counts after intervention in placebo group ($P = 0.139$). Logistic regression showed a higher rate of decrease in SM colony counts with an increase in the initial counts before intervention. However, such a relationship was not noted in the placebo group (odds ratio = 11.6, 95% confidence interval: 1.6–78.1, $P = 0.043$).

Lactobacillus

Table 2 shows the numbers (percentages) of volunteers with different LB counts at before and after 2-week consumption of probiotic and control drop. Mann–Whitney test did not reveal any significant difference in LB salivary colony counts of before intervention between the placebo and probiotic groups ($P = 0.58$). In the probiotic group, LB colony counts decreased significantly after intervention ($P = 0.04$). In the placebo group, changes in LB colony counts were not significant ($P = 0.22$). Mann–Whitney test did not reveal any

Table 1: Numbers and percentages of volunteers with different salivary *Streptococcus mutans* scores before and after intervention

	Streptococcus mutans score, *n* (%)		
	I	II	III
Probiotic group (*n*=30)			
Before intervention	4 (13.3)	14 (46.7)	12 (40.0)
After intervention	16 (53.3)	12 (40.0)	2 (6.7)
Control group (*n*=23)			
Before intervention	2 (8.7)	9 (39.1)	12 (52.2)
After intervention	3 (13.0)	11 (47.8)	9 (39.1)

I: <1000 CFU/ml, II: 1000-10,000 CFU/ml, III: >10,000 CFU/ml.
CFU: Colony-forming unit

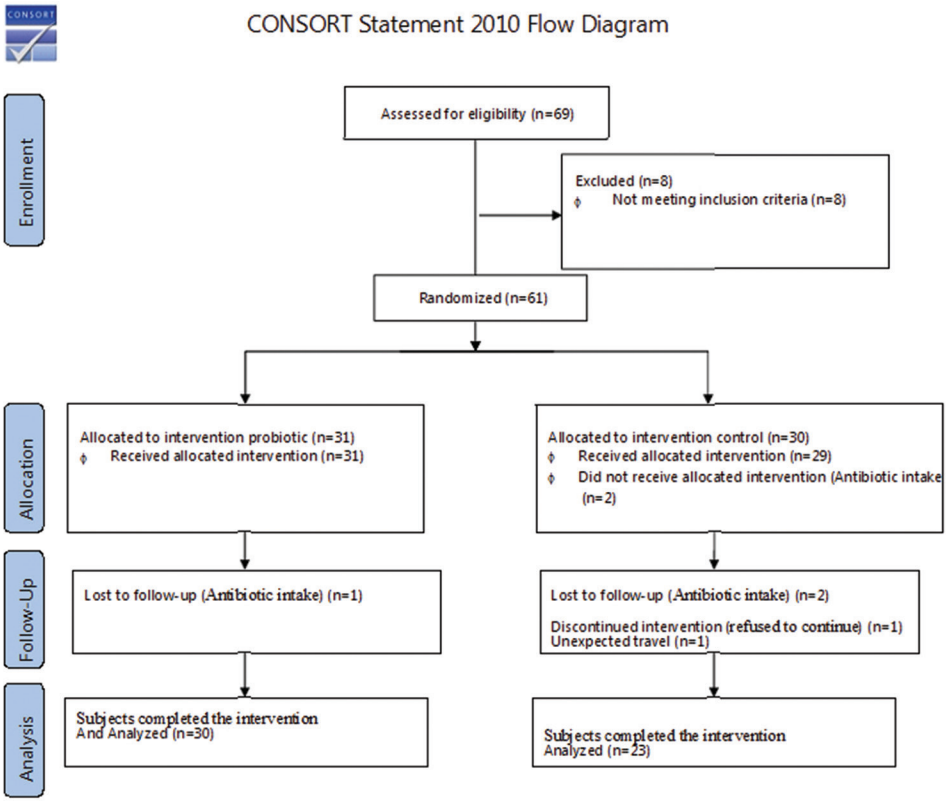

Figure 1: The CONSORT form

Table 2: Numbers and percentages of volunteers with different salivary *Lactobacillus* scores before and after intervention

	Lactobacillus score, *n* (%)		
	I	II	III
Probiotic group (*n*=30)			
Before intervention	4 (13.30)	17 (56.70)	9 (30.0)
After intervention	12 (40.0)	18 (60.0)	0
Control group (*n*=23)			
Before intervention	6 (26.10)	10 (43.50)	7 (30.40)
After intervention	4 (17.40)	15 (65.20)	4 (17.40)

I: <1000 CFU/ml, II: 1000-10,000 CFU/ml, III: >10,000 CFU/ml.
CFU: Colony-forming unit

significant difference in LB colony counts after intervention between the placebo and probiotic groups ($P = 0.21$). Logistic regression did not show a relationship between the initial colony counts of LB and the amount of decrease in colony counts in the probiotic and placebo groups.

Discussion

The present study showed that the use of a drop containing *L. rhamnosus*, *L. reuteri*, and *B. infantis* for 2 weeks decreased the salivary counts of SM in children 3–6 years of age. However, it had no effect on LB counts. The results of the present study in relation to SM counts are consistent with several studies.[2,5-7,19] However, some other studies have not shown any difference in SM counts.[8,12,13,20] Several studies have shown a relationship between dental caries and SM counts.[21] It is very difficult to compare the results of studies because of different study designs, duration of intervention, the parameters evaluated, and the type of probiotics used, combination of different species, and their vehicles.

To our knowledge, two studies have been carried out with the use of a drop containing *L. reuteri* and have reported results different from those of the present study. Cildir reported that use of this drop for 25 days in 4–12-year-old children with cleft lip/palate did not result in any difference in SM and LB counts.[13] However, the same composition in a study by Caglar *et al*. with the use of gum and lozenge resulted in a significant decrease in SM counts.[22,23] Cildir *et al*. believed one possible explanation was the complex interaction of probiotics with the oral cavity microflora of children with cleft lip/palate and also the small sample size.[13]

Stensson *et al*.[12] evaluated the effect of such a drop by pregnant mothers during the last month of pregnancy and by their newborns up to 1 year of age on the prevalence of dental caries in deciduous teeth at 9 years of age. Although no significant differences were found in salivary counts of SM and LB, the rate of caries-free state increased and the rate of

proximal caries decreased. In that study, colony counts were determined 8 years after administration of probiotics. The possible effect of this drop on caries without any change in bacterial counts might be attributed to an increase in SIgA in the probiotic group, resulting in the inhibition of the activity of SM, which can be considered another mechanism of probiotics.

One of the important reasons for differences between the results of the present study might be the use of a combination of three different species of probiotics and their synergistic effect on the inhibition of SM.

The exact mechanisms of action of probiotics are unknown.[3] *Lactobacilli* showed better adherence to "saliva-coated hydroxyapatite blocks" than *bifidobacteria* in an *in vitro* study.[24] Antibacterial substances production and competition with pathogenic microorganisms for adhesion sites and/or substrates are suggested roles of probiotics in reducing oral pathogenic germs.[1,3,14,24]

The effects of *L. reuteri* and *L. rhamnosus* on changing SM counts have been evaluated in various studies.[2,6] To our knowledge, the effect of *B. infantis* on combination with other LB on salivary SM counts has not been evaluated.

Another factor is the use of different vehicles such as dairy products, chewing gum, and drops to transfer probiotics. The probiotic vehicles are suitable for all the ages, especially for young children.[1] The concentrations of probiotics might decrease in dairy products over time, and the process of food preparation might affect the viability of bacteria and decrease the number of viable bacteria to below the recommended levels.[25,26] A drop was selected in the present study because it is safe, available, and used routinely in Iran for the treatment of diarrhea, flatulence and colic, and prevention of various allergies (respiratory and cutaneous) in newborns and children as an over-the-counter medication. The concentration of bacteria in this drop is consistent with those in previous studies. Use of a drop in younger children is easier than the use of mouthwashes, chewing gum, and dairy products and its effect on orodental health has not been evaluated to date. In the present study, the use of the probiotic drop for 2 weeks resulted in a decrease in LB salivary counts; however, the difference was not significant compared to the control group.

Contrary to the majority of studies which have shown a decrease in SM counts,[2,4,6,7,24,25] in relation to LB the majority of studies have not shown any change in LB counts.[7,8] A lack of change in LB counts has been observed in various studies in association with a decrease in SM counts,[7,13] which might be attributed to various mechanisms of action of probiotics in the oral cavity. Some probiotics exert their oral effects by colonizing the oral cavity or other mechanism similar to the explanation provided by Stensson *et al*.[12]

In a study, LB counts increased in *L. reuteri* group, with no changes in *L. rhamnosus* group.[27] Others reported that LB counts increased.[11,27] The increase in LB counts during the study period might be considered colonization of LB in the oral cavity, rather than an increase in caries risk because as discussed previously not all the LB increase caries risk.[27] However, complexities of the interaction of biofilm and interactions between bacteria found in the oral cavity might explain such diversities in the results in relation to LB.[28] It should be pointed out that popularity and acceptance of drops were high among patients. In this study, culturing techniques were used to determine salivary bacterial courts. Although use of chair-side kits is easier, they are semi-quantitative and the gold standard in this field is the culture technique.[14]

To increase control and the study power, the double-blind, controlled, and placebo design was used. In addition to the determination of inclusion and exclusion criteria, logistic regression was used to compare changes in SM between the two groups with high and low concentrations of SM by controlling other similar factors. It was shown, similar to previous studies, that in the probiotic group the rate of decrease in colony counts increased with an increase in the initial counts of SM.[8] It might be concluded that it appears probiotic drop is more effective in individuals with higher SM counts. Therefore, it is advisable, in future studies, to determine salivary SM counts in subjects in the first step and include subjects with high SM counts.

It does not seem that permanent establishment of probiotics occurs after discontinuation. Therefore, daily consumption of probiotics seems necessary to achieve its potential impact;[1] however due to limitations 2 weeks study period was considered in this study. Due to some limitations, different species of salivary lactobacillus were not considered in the present study which may affect results and a follow-up study after the drop withdrawal would be interesting as well.

Conclusions

The use of a drop containing a combination of *Bifidobacterium* and LB for 2 weeks resulted in a decrease in salivary counts of SM; however, the salivary counts of LB did not change. In addition, it appears use of the drop was more effective in subjects with higher SM counts. However, further studies are required with different combinations of probiotics in the form of a drop so that a conclusion can be reached in relation to the efficacy of this form of administration. It seems that a combination of different probiotic species results in an increase in efficacy through synergism as well.

Acknowledgments

The authors are grateful to Dr. Bahram Soleimani for his valuable aids in statistical analyses and study design, Prof. A Talebi, Ms. Dehdashti and Amini for microbiological

analyses, the staff of Pediatric Dentistry Department for their supports during sample collection and intervention period and all volunteers who cheerfully participated in the study. This study was based on a thesis to be submitted to Dental School, in partial fulfillment of the requirements of Specialist in Pediatric Dentistry.

Financial support and sponsorship

This study was funded by Vice Chancellery for Research (Grant number 393875, Isfahan University of Medical Sciences).

Conflicts of interest

There are no conflicts of interest.

References

1. Meurman JH, Stamatova I. Probiotics: Contributions to oral health. Oral Dis 2007;13:443-51.
2. Näse L, Hatakka K, Savilahti E, Saxelin M, Pönkä A, Poussa T, *et al.* Effect of long-term consumption of a probiotic bacterium, *Lactobacillus rhamnosus* GG, in milk on dental caries and caries risk in children. Caries Res 2001;35:412-20.
3. Stamatova I, Meurman JH. Probiotics: Health benefits in the mouth. Am J Dent 2009;22:329-38.
4. Stecksén-Blicks C, Sjöström I, Twetman S. Effect of long-term consumption of milk supplemented with probiotic lactobacilli and fluoride on dental caries and general health in preschool children: A cluster-randomized study. Caries Res 2009;43:374-81.
5. Jindal G, Pandey RK, Agarwal J, Singh M. A comparative evaluation of probiotics on salivary mutans streptococci counts in Indian children. Eur Arch Paediatr Dent 2011;12:211-5.
6. 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 2004;95:219-23.
7. Caglar E, Kuscu OO, 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 2008;66:154-8.
8. Mortazavi S, Akhlaghi N. Salivary *Streptococcus mutans* and lactobacilli levels following probiotic cheese consumption in adults: A double blind randomized clinical trial. J Res Med Sci 2012;17:57-66.
9. 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 four years of age. Caries Res 2013;47:364-72.
10. Hasslöf P, West CE, Videhult FK, Brandelius C, Stecksén-Blicks C. Early intervention with probiotic *Lactobacillus paracasei* F19 has no long-term effect on caries experience. Caries Res 2013;47:559-65.
11. Montalto M, Vastola M, Marigo L, Covino M, Graziosetto R, Curigliano V, *et al.* Probiotic treatment increases salivary counts of lactobacilli: A double-blind, randomized, controlled study. Digestion 2004;69:53-6.
12. Stensson M, Koch G, Coric S, Abrahamsson TR, 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 2014;48:111-7.
13. 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 2012;49:369-72.
14. 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 Investig 2014;18:1539-52.
15. Cagetti MG, Mastroberardino S, Milia E, Cocco F, Lingström P, Campus G. The use of probiotic strains in caries prevention: A systematic review. Nutrients 2013;5:2530-50.
16. Tehran: NB_Store_ProductList. Available from: http://www.zisttakhmir.com/english/product/tabid/150/ProdID/43/Pedilact.aspx/. [Last updated on 2016 Jul 09; Last cited on 2016 Aug 08].
17. 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 Orthod 2009;31:407-11.
18. Petersen PE, Baez RJ, World Health Organization (WHO). Basic principles of clinical oral health surveys, reliability and validity of data, estimating reproducibility of recordings. Oral Health Surveys: Basic Methods. 5th ed. Switzerland: World Health Organization; 2013. p. 25-7.
19. Petti S, Tarsitani G, D'Arca AS. A randomized clinical trial of the effect of yoghurt on the human salivary microflora. Arch Oral Biol 2001;46:705-12.
20. Singh RP, Damle SG, Chawla A. Salivary mutans streptococci and lactobacilli modulations in young children on consumption of probiotic ice-cream containing *Bifidobacterium lactis* Bb12 and *Lactobacillus acidophilus* La5. Acta Odontol Scand 2011;69:389-94.
21. Chuang LC, Huang CS, Ou-Yang LW, Lin SY. Probiotic *Lactobacillus paracasei* effect on cariogenic bacterial flora. Clin Oral Investig 2011;15:471-6.
22. Caglar 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 2008;18:35-9.
23. Caglar E, Kavaloglu SC, Kuscu OO, Sandalli N, Holgerson PL, Twetman S. Effect of chewing gums containing xylitol or probiotic bacteria on salivary mutans streptococci and lactobacilli. Clin Oral Investig 2007;11:425-9.
24. Haukioja A, Loimaranta V, Tenovuo J. Probiotic bacteria affect the composition of salivary pellicle and streptococcal adhesion *in vitro*. Oral Microbiol Immunol 2008;23:336-43.
25. Twetman S, Stecksén-Blicks C. Probiotics and oral health effects in children. Int J Paediatr Dent 2008;18:3-10.
26. Moeiny P, Jamei N, Mohamadi M, Shafizadeh N, Valaei N, Rahbar M, *et al*. Effect of a probiotic yogurt produced in Iran on the salivary counts of *Streptococcus mutans*. Res Dent Sci 2013;10:73-82.
27. Marttinen A, Haukioja A, Karjalainen S, Nylund L, Satokari R, Öhman C, *et al.* Short-term consumption of probiotic lactobacilli has no effect on acid production of supragingival plaque. Clin Oral Investig 2012;16:797-803.
28. Caufield PW, Li Y, Dasanayake A, Saxena D. Diversity of lactobacilli in the oral cavities of young women with dental caries. Caries Res 2007;41:2-8.