ORIGINAL RESEARCH

Randomized Clinical Trial of Oral Probiotic *Streptococcus salivarius* M18 on Salivary *Streptococcus mutans* in Preprimary Children

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Abstract

The oral probiotic *Streptococcus salivarius* M18 (S M18) offers the potential to confer oral health benefits as it produces bacteriocins which are targeting *Streptococcus mutans*.

Aims: The purpose of this clinical trial was to assess the effect of S M18 probiotics on salivary *S. mutans* and to identify the correlation between dental caries with salivary *S. mutans* count.

Materials and methods: This clinical trial was conducted in 40 children between the age-group 3–6 years, subjects were randomly selected and the decay extracted filled (due to caries) (def score) was recorded. The presalivary unstimulated saliva was collected and subjected to microbiological examination for *S. mutans* counts, salivary pH, and buffering capacity. Children were divided into two groups as the control and the experimental group. Bacteriocin like inhibitory substances M18 (M18 is a strain number) (BLIS M18) probiotic was administered in the experimental group, and unsweetened lozenge as a placebo in the control group for 7 days and postsalivary samples were collected and subjected to microbiological examinations for *S. mutans* count, salivary pH, and buffering capacity.

Results: A significant decrease in the *S. mutans* counts were noticed, an increase in buffering capacity, and no significant change in pH was observed after the intervention of S M18. A linear correlation was observed between the def score and *S. mutans* count.

Conclusion: This clinical trial revealed that S M18 in its 7 days regime can lead to the inhibition of *S. mutans* counts favorably in the oral cavity by affecting salivary pH and buffering capacity.

Keywords: Def score, Dental caries, Probiotic, Saliva, *Streptococcus mutans*, *Streptococcus salivarius* M18.

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Introduction

Chemical agents and antibiotics have been proven to be effective against cariogenic bacteria.[1] But it wipes out the entire oral flora and might lead to unwanted negative consequences like antibiotic resistance and the emergence of multiresistant strains. This led scientists to go through history and explore the forgotten concept of using bacteriotherapy/replacement therapy,[2] where adequate amounts of live beneficial organisms are administered to confer a beneficial effect on the host, later termed as probiotics, and has now come to the limelight due to extensive research using modern study designs and methods.[3]

The mechanism of action of probiotics explained is the disruption of plaque biofilm formation through competition for binding sites on host tissues and other bacteria and competition for nutrients or the production of antimicrobial compounds that inhibit oral bacteria, such as organic acids, hydrogen peroxide, low molecular weight antimicrobial compounds, bacteriocins, and adhesion inhibitors produced by lactic acid bacteria.[4] *Lactobacillus reuteri*, *Weissella ciberia*, *L. acidophilus*, and *L. fermentum* are the strains that belong to the category of probiotics.[3] As these organisms have the gastrointestinal tracts as their inherent habitat, the use of these organisms in the oral cavity and their efficacy in the oral context are questionable.[3]

Probiotics bacteriocin like inhibitory substances K12 (K12 is a strain number) (BLIS K12) and BLIS M18 are other strains of microorganisms claimed to be used in the oral cavity. BLIS K12 was first isolated from the saliva of a healthy child and has been utilized as a commercial probiotic for over a decade.[5] These strains are derived from *S. salivarius*, which is a spherical, gram-positive, oxidase negative, and catalase-negative bacterium.[6] It is reported to be a pioneer colonizer of the human oral cavity and predominantly persists throughout life. These bacteria are believed to colonize within 2 days of birth on the dorsum of the tongue and pharyngeal mucosa up to 1×10^7 colony-forming unit (CFU)/mL in saliva. The

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source of these bacteria is their mother.[7] But these colonies make up only <1% of the total bacterial population in these areas.

Another well-studied strain of *S. salivarius* is strain M18, obtained from *S. salivarius*. This strain has shown a markedly different bacteriocin profile compared to strain K12 and effectively secretes the bacteriocins strain number (A2) and antimicrobial proteins (AMPs), which has demonstrated an antibacterial effect against *S. mutans*.[8] Pattern of colonization of S M18 is similar to that of K12 and it is dose-dependent manner.[9] Potential benefits of S M18 as oral probiotics against cariogenic S. *mutans* strain have not been reported extensively in the scientific literature.

Based on the research hypothesis, increasing the colonization of S M18 in the oral cavity can lead to competitive inhibition of *S. mutans* which affects the salivary buffering capacity and pH. Based on the above hypothesis, our primary objective of this clinical trial was to assess the effect of S M18 on *S. mutans* count and its effect on salivary buffering capacity and pH. The secondary objective was to correlate the relation between caries experience and salivary *S. mutans* count.

Materials and Methods

The randomized parallel single blinded clinical trial study was conducted between 15th March 2018 and 15th March 2019 after obtaining ethical clearance from the Institutional Review Board [IRB no: IRB/CIDS/130/2017] and registering in the Clinical Trials Registry–India [CTRI/2018/01/011207]. In the current study, data were analyzed using appropriate statistical methods, *t*-test, and correlation analysis were done between pre and postsalivary samples. The $p < 0.05$ is considered statistically significant.

Procedure

This trial comprised 40 subjects, of which 20 in each group was estimated using G*Power version 3.1.9.2 with effective size = 0.5, α error = 0.05, power (1 – β) = 0.46, allocation rate $n = 1$, and degree of freedom (df) = 38. A total of 40 healthy children in the age-group between 3 and 6 years were selected for the study after obtaining informed consent from their parents. Inclusion criteria were children in primary dentition who had no history of antibiotic consumption in the last 3–4 months.

Selected children were screened for caries under broad light using a mouth mirror and probe and scored using def criteria, and presalivary samples were collected.

Presalivary Sample Collection Method

Unstimulated whole saliva was collected after 1 hour of the breakfast with the head tilted down. Saliva was collected from the floor of the mouth and then spit into a sterile vial at regular intervals.[9]

The procedure was repeated until a sufficient sample was collected. About 1 mL of saliva was pipetted into a labeled, sterile eppendorf tube for microbiological assay, and 2 mL of saliva was pipetted into another labeled, sterile eppendorf tube for pH and buffering capacity estimation. The samples were immediately plated in both brain heart infusion (BHI) and mitis salivarius agar (MSA) plates, and the samples for pH and buffering capacity estimation were stored deep freezer at –20°C for further evaluation.

Administration of Probiotics BLIS M18 and Placebo (Unsweetened lozenge) in Selected Children

Children were randomly divided into two groups (20 children in the control group and 20 children in the experimental group).

Participants were blinded of the medicaments, probiotics BLIS M18, and placebo (unsweetened lozenge). The probiotic BLIS M18 was available as lozenges; therefore, unsweetened lozenges were selected as a placebo and there was no direct association.

Children in the experimental group were given BLIS M18 probiotic [Biopro]; children were instructed to take it once daily for 1 week by slowly dissolving it in the mouth after half an hour of consumption of food. Unsweetened lozenges were given to the children in control group. They were also instructed to take it once daily, half an hour after food consumption, by slowly dissolving it in the mouth for 1 week.

The postsalivary samples were collected after 1 week and were immediately plated for microbiological assay and stored at –20°C for evaluating the pH and buffering capacity.

Pre and Postsalivary *Streptococcus* Count Assay

Mitis salivarius agar (MSA) and BHI agar were brought to room temperature before use. Using a sterile cotton swab, the saliva sample was streaked onto the entire surface of the agar plate for lawn culture and was incubated for 24 hours at 37°C in an aerobic incubator. After 24 hours, the colonies were counted using a manual colony counter. A gram staining test was done to confirm the presence of characteristics of gram-positive organisms (*Streptococcus* and *Staphylococcus).* Followed by a catalase test to distinguish between staphylococci and streptococci. Thus, the catalase test confirmed CFU in MSA as *S. mutans*.

Pre and Postsalivary pH and Buffering Capacity Estimation

The stored saliva samples were brought to room temperature and 2 mL of it was pipette using an automated micropipette into a graduated sterile tube with a conical end, and the pH was estimated and recorded. The buffering capacity was estimated using Ericsson Method.[10] A toal of 5 mmol/L of hydrochloric acid (HCl) was prepared by adding 0.044 mL of HCl into 100 mL of water. After initial pH calculation, 1.5 mL of 5 mmol/L HCl was added into 0.5 mL of saliva and was shaken vigorously using an apparatus called vortex mixer. It was centrifuged for 1 minute and then allowed to stand for 10 minutes in the test tube rack. Then the pH was estimated using a pH meter, and the values were recorded.

Results

The baseline data obtained from presalivary and postsalivary samples with individuals with S M18 and unsweetened placebo were depicted in line graphs (Figs 1 and 2).

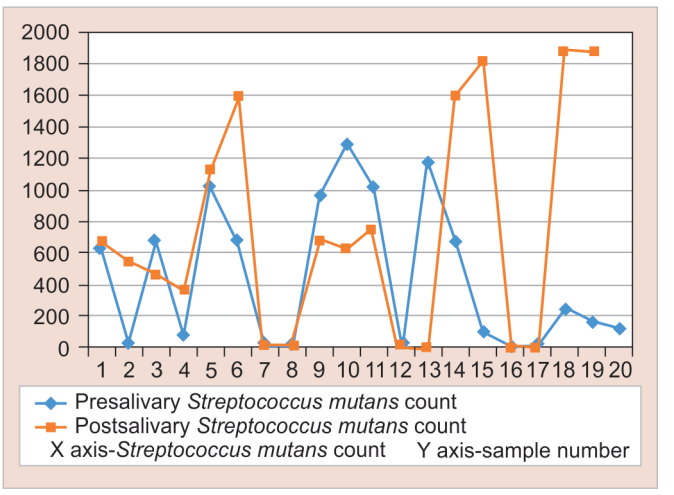

Fig. 1: Control group (placebo)

Salivary *S. mutans* Count after Administration of Unsweetened Placebo and S M18

In children administered with unsweetened placebo, a marginal nonsignificant increase in salivary *S. mutans* count was observed ($p = 0.4193$), data were represented in (Fig. 3), in contract children administered with S M18 probiotic showed a significant marked decrease in salivary *S. mutans* count ($p = 0.04965$) (Table 1).

Salivary Buffering Capacity after Administration of Unsweetened Placebo and S M18

In children administered with vitamin C there was a marginal decrease in salivary buffering capacity ($p = 0.1699$), on the contrary, S M18 exhibited a significant increase in buffering capacity ($p = 0.04965$) (Table 2).

Salivary pH after Administration of Unsweetened Placebo and S M18

A marginal decrease in salivary pH was observed in children administered with vitamin C ($p = 0.07712$). In contract, an increase in salivary pH was observed in children administered with S M18 ($p = 0.974$) (Table 3).

Correlation of Caries Experience (Def Score) and *S. mutans* Count

The correlation coefficient analysis revealed that there was a significant correlation established between caries experience and organism count (0.9012; $p = 2.256e\text{-}15$) and infers to have a significant correlation (Table 4). The linear correlation between def score and *S. mutans* count showed a direct correlation (Fig. 4).

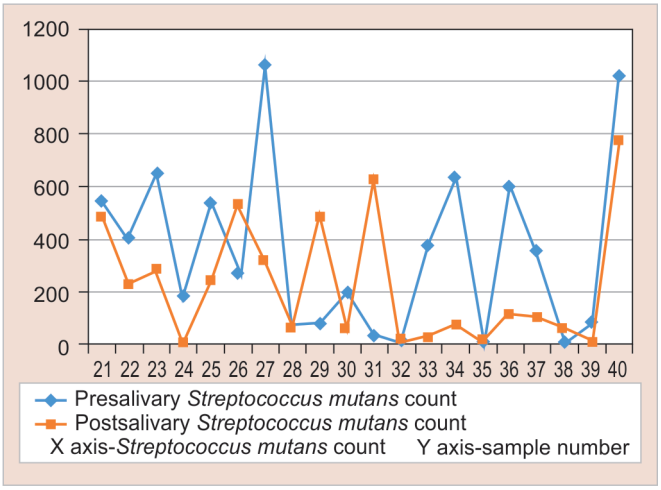

Fig. 2: Experimental group (S M18)

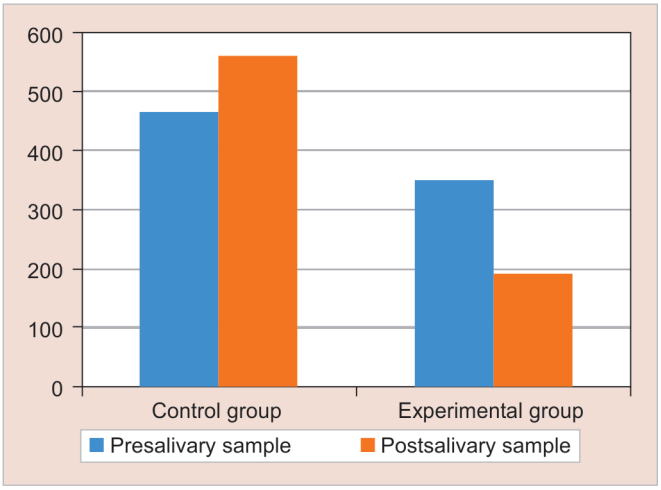

Fig. 3: Bar diagram for colony count in MSA agar

Table 1: The difference between the colony count in pre and postsalivary samples in the control group and experimental group

Group	*Salivary sample*	*Mean*	*Standard deviation (SD)*	*t-test value*	*df*	*p-value*	*Conclusion*
Control group	Pre	465.35	454.322	−0.8260	19	0.4193	Not significant
	Post	563.05	451.3566				
Experimental group	Pre	349.6667	338.9334	2.1134	17	0.04965	Significant
	Post	191.6667	231.4014				

Table 2: The difference in buffering capacity between the pre and postsalivary samples in the control group and experimental group

Group	*Salivary sample*	*Mean*	*SD*	*t-test value*	*df*	*p-value*	*Conclusion*
Control group	Pre	5.87	0.6382	1.4266	19	0.1699	Not significant
	Post	5.5535	0.8952				
Experimental group	Pre	5.7466	0.9517	−2.1643	17	0.04497	Significant
	Post	6.3155	0.8497				

Table 3: The difference in pH between the pre and postsalivary samples in control group and experimental group

Group	*Salivary sample*	*Mean*	*SD*	*t-test value*	*df*	*p-value*	*Conclusion*
Control group	Pre	6.745	0.6784	1.869	19	0.07712	Not significant
	Post	6.403	0.7837				
Experimental group	Pre	7.0039	0.4065	−0.0331	17	0.974	Not significant
	Post	7.0094	0.7580				

Table 4: Correlation between presalivary *S. mutans* count and def score

Variables	*Mean*	*SD*	*Correlation coefficient*	*t-value*	*df*	*p-value*	*Conclusion*
Mutant count	405.625	393.0515	0.9012	12.819	38	2.256e-15	Significant
def score	2.55	2.5914					

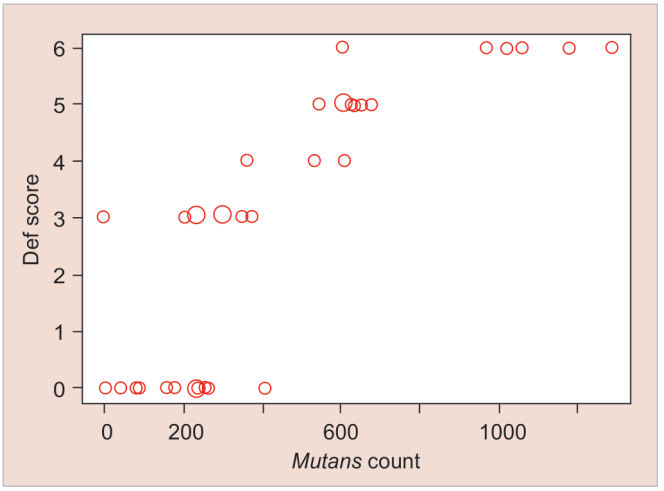

Fig. 4: Correlation between presalivary *S. mutans* count and def score–scatter plot

Discussion

Research findings indicate that most carious lesions reflect a sucrose-dependent *S. mutans* infection and has indicated that S. *mutans* are involved with the initiation of decay.[11] The growth and metabolism of these pioneer species changes local environmental conditions (e.g., pH, co-aggregation, and substrate availability), thereby enabling more fastidious organisms to further colonize after them, forming dental plaque.[12] Numerous studies have demonstrated the association of *S. mutans* involved in decay with the observation of reduced caries experience with decreased salivary *S. mutans* count.[13–24] In the present clinical trial a significant linear correlation was observed between the def and presalivary *S. mutans* count.

Probiotics have the potential to modify the oral microbiota and are being investigated to prevent or treat diseases of the oral cavity, such as dental caries and the periodontal diseases.[25] The world's first two *S. salivarius* commercial probiotic strains, K12 and M18, were employed in the treatment of pharyngitis for its ability to colonize in oral cavity by adhering, co-aggregate, and inhibit the growth of various strains of *S. pyogenes*.[26] The administration of S M18 has claimed to colonize oral region and have some local changes in oral cavity.[5]

An *in vivo* analysis of plaque biofilm and persistence of S M18 in oral cavity has demonstrated the chances of development of new dental caries in children in presence of S M18 were low.[27] S M18 was found to reduce *S. mutans* count in 90 days regime. In this clinical trial, 7 days regime of S M18 significantly reduced *S. mutans* count. The probable reasons that can be attributed is its mechanism of action is that of the following:

- Release of M is a series coding number (salivaricin M) proteins from S M18.
- The release of dextranase and urease, wherein the plaque get solubilized due to its dextranase activity and urease increases pH of the oral cavity by breaking down urea to carbon dioxide and ammonia.[27]
- Reducing the plaque formation by acting directly on the formation or by competing and interfering with bacteria—bacteria attachments.[28]

Lacticaseibacillus rhamnosus and *Bifidobacterium longum* was found to increase the salivary buffering capacity consistently during its 9 months regime.[17] Significant increase in salivary buffering capacity was observed after 7 days of S M18 regime in this clinical trial. Increase in salivary buffering capacity can be considered favorable in neutralizing the acids produced by substrates and microorganism during carious process.[27]

Minimal change in salivary pH toward alkalinity was observed, though not statistically significant after 7 days of administration of S M18. This may be due to reduced number of acidogenic bacteria, *S. mutans,* after S M18 administration and resulting less acid production similar finding was reported in preschool children after 9 months of milk supply with probiotics containing *L. rhamnosus* 5×10^6 and *B. longum* 3×10^6.[20]

Children administrated with unsweetened lozenges as placebo in this clinical trial exhibited a nonsignificant marginal increase in *S. mutans* count, decreased salivary pH, and reduced buffering capacity. S M18 can be recommended in caries risk individuals and reduce colonization of acidogenic microorganisms, *S. mutans*.

Conclusion

Streptococcus salivarius M18 (S M18) in its 7 days regime, can lead to inhibition of *S. mutans* counts in the oral cavity by affecting salivary pH and buffering capacity favorably. A linear correlation between caries experience and salivary *S. mutans* count was observed. Potential benefits of probiotics S M18 can be reaped in replacement therapy or bacteriotherapy of caries in preprimary children. Further studies in large sample size are warranted to use probiotics as a beneficial agent in the dental sciences.

Clinical Significance

Streptococcus mutans have a key role in the caries experience of a child. In this research, S M18 demonstrated inhibition of salivary *S. mutans* count by affecting salivary pH and buffering capacity. Potential benefits of probiotics S M18 can be reaped in replacement or bacteriotherapy of caries management in preprimary children.

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References

1. John SA, Shantala BM, Rao VN. Salivarius K12 as a probable probiotic. Res J Pharm, Biol Chem Sci 2013;4(4):1056–1061.
2. Anderson MH, Shi W. A probiotic approach to caries management. Pediatr Dent 2006;28(2):151–153.
3. Meurman JH, Stamatova I. Probiotics: health benefits in mouth. Am J Dent 2009;22(6):329–338.
4. Chen F, Wang D. Novel technologies for the prevention and treatment of dental caries: a patent survey. Expert Opin Ther Pat 2010;20(5):681–694. DOI: 10.1517/13543771003720491
5. Stowik TA. Contribution of probiotics *S. salivarius* strains K12 and M18 to oral health in humans: a review. Honors Scholar Theses 2016;488.
6. Burton JP, Chilcott CN, Wescombe PA, et al. Extended safety data for the oral cavity probiotic *S. salivarius* K12. Probiotics Antimicrob Proteins 2010;2(3):135–444. DOI: 10.1007/s12602-010-9045-4
7. Burton JP, Wescombe PA, Macklaim JM, et al. Persistence of the oral probiotic *S. salivarius* M18 is dose dependent and megaplasmid transfer can augment their bacteriocin production and adhesion characteristics. PLoS One 2013;8(6):e65991. DOI: 10.1371/journal.pone.0065991
8. Di Pierro F, Adami T, Rapacioli G, et al. Clinical evaluation of the oral probiotic *S. salivarius* K12 in the prevention of recurrent pharyngitis and/or tonsillitis caused by streptococcus pyogenes in adults. Expert Opin Biol Ther 2013;13(3):339–343. DOI: 10.1517/14712598.2013.758711
9. Dawes C. Factors influencing salivary flow rate and composition. Saliva and Oral Health Duns Tew: Stephen Hancocks Limited 2012.
10. Schrezenmeir J, de Vrese M. Probiotics, prebiotics, and synbiotics—approaching a definition. Am J Clin Nutr 2001;73(2):361s–364s. DOI: 10.1093/ajcn/73.2.361s
11. Baron S. Microbiology of dental decay and periodontal disease. Medical Microbiology 1996;4:99.
12. Vinogradov AM, Winston M, Rupp CJ, et al. Rheology of biofilms formed from the dental plaque pathogen *S. mutans*. Biofilms 2004;1(1):49–56. DOI: 10.1017/S1479050503001078
13. Pannu P, Gambhir R, Sujlana A. Correlation between the salivary *S. mutans* levels and dental caries experience in adult population of Chandigarh, India. Eur J dent 2013;7(2):191–195. DOI: 10.4103/1305-7456.110169
14. Sakeenabi B, Hiremath SS. Dental caries experience and salivary *S. mutans*, lactobacilli scores, salivary flow rate, and salivary buffering capacity among 6-year-old Indian school children. J Int Soc Prev Community Dent 2011;1(2):45–51. DOI: 10.4103/2231-0762.97697
15. Katiyar A, Nayak UA, Mishra N, et al. The correlation between salivary mutans streptococcus count and dental caries experience among preschool children and their parents. Int J Oral Care Res 2018;6(2):64–68.
16. Srilatha A, Doshi D, Kulkarni S, et al. Determination and comparison of dermatoglyphic patterns and salivary *S. mutans* counts and its correlation with dental caries among 3 to 6 year old children. Oral Health Prev Dent 2018;16(3):291–297. DOI: 10.3290/j.ohpd.a40720
17. Villavicencio J, Villegas LM, Arango MC, et al. Effects of a food enriched with probiotics on *S. mutans* and Lactobacillus spp. salivary counts in preschool children: a cluster randomized trial. J Appl Oral Sci 2018;26:e20170318. DOI: 10.1590/1678-7757-2017-0318
18. Ghazal TS, Levy SM, Childers NK, et al. Mutans streptococci and dental caries: A new statistical modeling approach. Caries Res 2018;52(3):246–252. DOI: 10.1159/000486103
19. Al-Otaibi SM, Rizk H, Riyaz MA. Prevalence of dental caries, salivary Streptococcus Mutants, Lactobacilli count, pH level and buffering capacity among children with Down's syndrome in Al-Qassim region, KSA. Int J Contemp Med Res 2016;3(9):2793–2797.
20. Edelstein BL, Ureles SD, Smaldone A. Very high salivary *S. mutans* predicts caries progression in young children. Pediat Dent 2016;38(4):325–330.
21. Chokshi A, Mahesh P, Sharada P, et al. A correlative study of the levels of salivary *S. mutans*, lactobacilli and Actinomyces with dental caries experience in subjects with mixed and permanent dentition. J Oral Maxillofac Pathol 2016;20(1):25–28. DOI: 10.4103/0973-029X.180916
22. Hegde PP, Kumar BA, Ankola VA. Dental caries experience and salivary levels of *S. mutans* and Lactobacilli in 13-15 years old children of Belgaum city, Karnataka. J Ind Soc Pedod Prev Dent 2005;23(1):23–26. DOI: 10.4103/0970-4388
23. Seibert W, Farmer-Dixon C, Bolden T, et al. *S. mutans* levels and caries prevalence in low-income school children. J Tenn Dent Assoc 2002;82(1):19–22.
24. Granath LA, Cleaton-Jones PE, Fatti LP, et al. Prevalence of dental caries in 4- to 5-year-old children partly explained by presence of salivary mutans streptococci. J Clin Microbiol 1993;31(1):66–70. DOI: 10.1128/jcm.31.1.66-70.199
25. Allaker RP, Stephen AS. Use of probiotics and oral health. Curr Oral Health Rep 2017;4(4):309–318. DOI: 10.1007/s40496-017-0159-6
26. Macdonald KW. The role of *S. salivarius* as a modulator of homeostasis in the oral cavity. Electron Thesis Diss Repos 2015;1–94.
27. Di Pierro F, Zanvit A, Nobili P, et al. Cariogram outcome after 90 days of oral treatment with *S. salivarius* M18 in children at high risk for dental caries: results of a randomized, controlled study. Clin Cosmet Investig Dent 2015;7:107–113. DOI: 10.2147/CCIDE.S93066
28. Reddy JJ, Sampathkumar N, Aradhya S. Probiotics in dentistry: review of the current status. Rev Clin Pes Odontol 2010;6(3):261–267.