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Evaluation of probiotics' efficiency on cariogenic bacteria: randomized controlled clinical study

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Abstract

Background Probiotics are live beneficial bacteria to human health and their efficiency on oral health is still being investigated. The purpose of this study was to evaluate the level of *Streptococcus mutans* and *Lactobacillus* species with and without the use of probiotics for six-months after the treatment of all dental caries under general anesthesia.

Methods Fifty-eight pediatric patients without any systemic diseases, whose dental treatments were completed under general anesthesia (GA), were included in the study. The patients were recruited in two-groups; Group A: Patients started using probiotics after GA and Group B: Patients did not use probiotics after GA. Saliva samples were taken from all patients on the day before GA (T0), at one-month (T1), three-month (T2) and six-month (T3) follow-up after GA. The counts of cariogenic bacteria were determined by the analysis of saliva samples using real-time polymerase chain reaction. Statistical significance level was accepted as $p < 0.05$.

Results There was statistically significant difference between Group A and B for T0, T1, T2 and T3 regarding *S. mutans* ($p = 0.001$, $p = 0.04$, $p = 0.04$, $p = 0.03$; $p < 0.05$). However, there was no statistically significant difference between groups regarding *Lactobacillus* species ($p \geq 0.05$).

Conclusions Probiotic use and treatment of all caries significantly reduced the level of *S. mutans* but not *Lactobacillus* species. Furthermore, *S. mutans* decreased after cessation of probiotics, but it was not statistically significant.

Trial registration Study was registered as "Effects of Probiotics on *Streptococcus mutans* and *Lactobacillus species*" with the registration number of NCT05859646 (16/05/2023) at https://www.clinicaltrials.gov Protocol Registration and Results System.

Keywords *Lactobacillus* species, Oral flora, Probiotics, *Streptococcus mutans*, Dental caries

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Background

Oral pathogens have the capacity to establish themselves in the oral environment, proliferate, adhere to tooth surfaces and gingival epithelium, and ultimately lead to the development of oral diseases such as dental caries or gingival disease [1]. The traditional microbial risk markers for early childhood caries include acidogenic-aciduric bacterial species, such as *Streptococcus mutans* and *Lactobacillus* species (spp.) [2]. The colonization of cariogenic bacteria, particularly *S. mutans*, which is the primary bacterium involved in caries formation, increases, thereby supporting the proliferation of *Lactobacillus* spp. The colonization of these cariogenic bacteria on the tooth surface establishes the fundamental structure of the dental biofilm, which then leads to dental caries through demineralization of the tooth surface caused by the acidic pH of the mature dental biofilm structure [3, 4]. In recent years, the modification of nutritional products and the consumption of foods that will prevent caries formation by reducing the colonization of oral pathogens have gained importance, particularly for use in pediatric population as a complement to pharmacological products [5, 6].

Probiotics, defined as live bacteria with beneficial effects on human health, are non-pharmacological products that are recommended for use with the purpose of enhancing oral health and general body health. In the early 20th century, the concept of utilizing beneficial microorganisms to address medical conditions or support immune system was introduced. In this context, treatments employing probiotics were introduced with the term bacteriotherapy [7]. Probiotics utilized in bacteriotherapy have been defined as dietary supplements containing potentially beneficial bacteria or yeast, and their efficacy in promoting human health has been demonstrated in a range of contexts [5, 7, 8].

The capacity of probiotics to influence the immunological response of the host, inhibit the activity of pathogenic microbes, or compete with pathogenic microorganisms for adhesion sites is contingent upon their action against microorganisms [9]. It can thus be concluded that probiotics can be employed in the field of oral health to prevent and treat dental caries, periodontal disease and halitosis by reducing the concentration of harmful bacteria [10]. However, dental caries is a multifactorial disease, and the composition of the oral microbiota is a critical factor in its development. Consequently, the results of the efficiency of probiotics on tooth caries and the presence of cariogenic bacteria are inconsistent [11].

The purpose of this study was to evaluate the level of *S. mutans* and *Lactobacillus* spp. with and without the use of probiotics for six-months follow-up after the treatment of all dental caries under general anesthesia. This study was designed to test the null hypothesis that there would be no difference between the groups who used probiotics and those who did not with regard to the numbers of *S. mutans* and *Lactobacillus* spp.

Methods

Ethical consideration and priori statistical analysis

In accordance with the ethical standards set forth in the Declaration of Helsinki, the research was conducted with approval from the Başkent University Institutional Review Board and Ethics Committee (project number D-KA19/41). Furthermore, written informed consent was obtained from the parents of each participating child prior to the collection of the initial saliva sample and the administration of general anesthesia. The sample size of the study was determined at least 48 patients as 24 patients for each group with 85% power, 0.8 effect size and 5% significance level. In consideration of the 10% drop-off probability, 58 patients were included in two independent groups with a 1:1 allocation ratio in this clinical study, as illustrated in Fig. 1.

Inclusion and exclusion criteria

The study population consisted of children aged of 2–12 years old, without any systemic, physical, physiological, or allergic conditions, who were referred for general anesthesia for dental treatments. The important criteria for inclusion were that every included child had at least 8 teeth with caries. The children who underwent dental treatment under general anesthesia were included in the study to ensure that all participants had zero (0) caries and facilitate a six-month follow-up period. Patients who were unable to provide saliva samples due to elevated anxiety or insufficient saliva production were excluded from the study.

Study design

PICO is an abbreviation that stands for Patient/Population, Intervention, Comparison and Outcomes. In this randomized clinical study, these characteristics are defined as: P: Children aged 2–12 years old, without any systemic, physical, physiological, or allergic conditions; I: Probiotics; C: No probiotics; O: A reduction in the levels of *S. mutans* and *Lactobacillus* spp. Patients who were referred to the Pediatric Dentistry Clinic, Faculty of Dentistry, Başkent University, Ankara, Türkiye, were randomly allocated to groups using block randomization. This was conducted in advance using a table of random numbers, with the number of groups being the multiple of the total number of participants, in order to ensure a similar number of individuals in each group to provide blindness for the patient distribution. Participating patients were distributed into two-groups that use probiotics or not use probiotics after their dental treatments were completed under general anesthesia. Decayed,

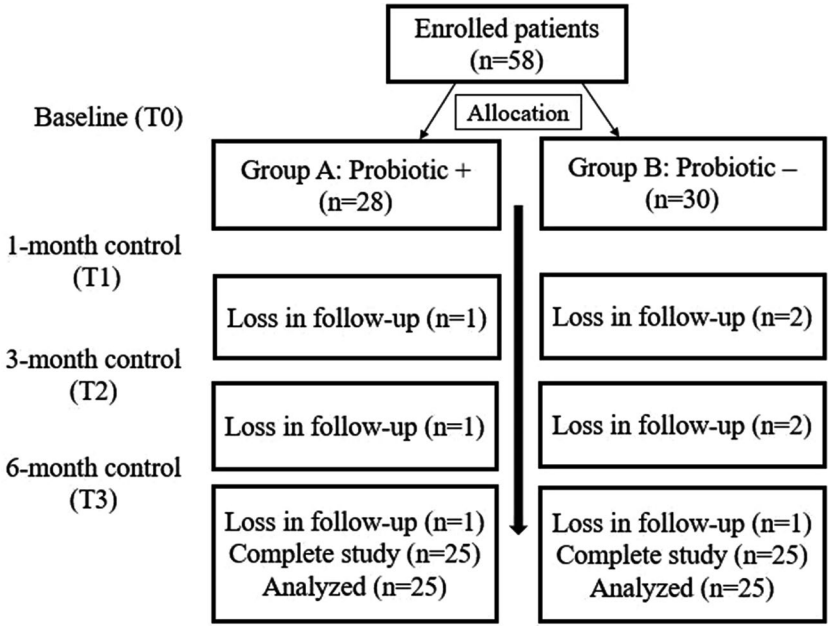

Fig. 1 Flow chart of the participating children in the study

missing, and filled permanent teeth (DMFT) and primary teeth (dmft) indexes were recorded by the same and experienced pediatric dentist at baseline assessment, which occurred concurrently with the collection of the first saliva sample. Following the completion of dental treatments under general anesthesia, all participating children were observed for a period of six months. Following the full mouth rehabilitation, each patient received comprehensive oral hygiene training, which encompassed the selection of an appropriate toothbrush and toothpaste, the correct brushing technique, and dietary recommendations.

The participating children were randomly assigned to one of two groups; Group A-Probiotics group: Patients who started using probiotic drops after general anesthesia procedure and Group B-Control group: Patients who did not use any probiotics after general anesthesia procedure. The patients in Group A were recommended to use a drop form probiotic (NBL Probiotic Drop, Nobel, Türkiye) which includes 5×10^8 active probiotics in every drop. These probiotics are *Bifidobacterium breve*, *Bifidobacterium infantis*, *Bifidobacterium longum*, *Bifidobacterium bifidum* in the content of each daily dose. In accordance with the instructions provided by the manufacturer, patients were instructed to administer six drops of the medication once per day, with each bottle of the medication providing sufficient dosage for a period of 30 days. During the control appointments, parents were asked to provide empty probiotic bottles as a means of ensuring the regular use of probiotics. During the six-month follow-up period, group A employed the use of probiotics for the first three months and then discontinued for the last three months.

Saliva samples were collected from each participating child on the day preceding the administration of general anesthesia (T0) and at one-month (T1), three-month (T2), and six-month (T3) control appointments by a pediatric dentist. The saliva samples were assigned a code based on the number of patients assigned after block randomization (i.e. 1) and the sample code (i.e. T0). Subsequently, the saliva samples were subjected to microbiological evaluation to ascertain the levels of *S. mutans* and *Lactobacillus* spp. at each follow-up interval, with a view to comparing Group A and Group B at each respective follow-up point. The microbiological analysis was performed by a microbiologist who was not aware of the patient group distributions included in the study.

Microbiological evaluation

Saliva samples were collected from each participating child into a sterile, plastic-capped tube and subsequently transferred to the microbiology laboratory for storage at -78°C. Prior to the real-time polymerase chain reaction (qPCR) experiment, genomic DNA was extracted from each saliva sample using a nucleic acid isolation kit (ZipPrime Biotechnology, Türkiye) in accordance with the manufacturer's instructions. Following the extraction of DNA, the samples were stored at -20 °C until qPCR analysis. Real-time polymerase chain reaction analysis was conducted to ascertain the number of target microorganisms, specifically *S. mutans* and *Lactobacillus* spp., in saliva samples. At this stage, primer sequences specifically designed for species and genus were employed.

Table 1 Age, DMFT/dmft and gender distributions of each group and statistical analysis between them

Groups	Group A		Group B		*p*-value
	min-max	mean ± sd	min-max	mean ± sd	
Age	23–122	63.76 ± 22.25	26–141	70.36 ± 26.61	0.35
DMFT/dmft	8–16	11.68 ± 2.86	8–16	11.40 ± 2.61	0.72
Gender					
Girl	48%		36%		0.28
Boy	52%		64%		

min: Minimum; max: Maximum; sd: standard deviation; %: percentage; Age was evaluated as months; Age and DMFT/dmft was shown with statistical terms of minimum-maximum and mean ± standard deviation values. Independent Samples Test was employed for the analysis of age and DMFT/dmft between groups while, gender was analyzed with Pearson Chi-square Test, with a significance level of $p<0.05$

Real-time polymerase chain reaction analysis were optimized and standards were established using DNA obtained from bacterial suspensions at concentrations of 10^1 and 10^9, prepared with standard strains for quantitation. Subsequently, the DNA concentration in the saliva sample was quantified via NanoDrop 2000/2000 C (Thermo Fisher Scientific, Massachusetts, USA). The standard curve method was performed with standards of *Lactobacillus rhamnosus GG* ATCC 53103 and *S. mutans* ATCC35668 strains to detect levels of *Lactobacillus* spp. and *S. mutans*. The experiments were conducted using the QuantStudio 5 Real-time PCR System (Thermo Fisher Scientific, Massachusetts, USA) with PowerUp SYBR Green Master Mix (Thermo Fisher Scientific, Massachusetts, USA) and the following primer set:

Lactobacillus spp. F. 5'-3' GAATCTTCCACAATGGACG,

Lactobacillus spp. R. 5'-3' CGCTTTACGCCCAATAAATC,

Streptococcus mutans F. 5'-3' CCATGCGCAATCAACAGGT,

Streptococcus mutans R. 5'-3' CAACGCGAACATCTTGATCAG.

All experiments were conducted in triplicate, with a negative control group that did not DNA. A positive and negative control were included in each PCR set and for all sample processing. Subsequently, the data were analyzed using the QuantStudio 5 Real-Time PCR software.

Statistical analysis

The obtained data was evaluated with the IBM SPSS Statistics software, version 22. Descriptive statistics, including age, gender and DMFT/dmft index were provided for participating children in each group, with values for minimum, maximum, range, mean and standard deviation. The normality test conducted with Shapiro–Wilk revealed that all variables, except age and DMFT/dmft index, did not follow the normal distribution. Independent Samples Test was used to compare mean age and DMFT/dmft index between groups due to the normal distribution and Chi-square test was applied to compare gender between groups. Independent Samples Kruskal-Wallis Test and Mann–Whitney test was applied to compare between groups for variables that did not follow the normal distribution. Bonferroni adjusted Friedman test used for pairwise comparisons between different follow-up times. Statistical significance was accepted as $p<0.05$.

Table 2 *S. mutans* levels of each group with different follow-up times and statistical analysis between and within each group

Groups	T0	T1	T2	T3	*p*-value†
Group A	9.70×10^{14} ± 4.84×10^{15}	9.15×10^{12} ± 4.52×10^{13}	1.27×10^{14} ± 5.89×10^{14}	7.63×10^{11} ± 2.79×10^{12}	0.01* T0-T1 = 0.04 T0-T2 = 0.34 T0-T3 = 0.66 T1-T2 = 0.33 T1-T3 = 0.03 T2-T3 = 0.06
Group B	2.97×10^{16} ± 1.48×10^{17}	4.81×10^{13} ± 1.98×10^{13}	4.00×10^{12} ± 1.99×10^{13}	7.03×10^{13} ± 3.43×10^{14}	0.58
p-value‡	0.001*	0.04*	0.04*	0.03*	

S. mutans levels were given as mean ± standard deviation values; † refers statistical analysis with Friedman test with Bonferroni adjustment for intragroup comparison with 95% CI Difference and ‡ refers statistical analysis with Kruskal-Wallis Test for intergroup comparison with 95% CI Difference

Results

Fifty-eight children were enrolled in the study, with 50 children completing the study after a six-month follow-up period. Figure 1 shows the flow chart of the study. Table 1 shows the distributions of age, gender and DMFT/dmft index, as well as the results of the statistical analysis conducted on each group. The statistical analysis revealed no statistically significant difference between Group A and Group B with regard to age, gender and DMFT/dmft index of all participating children ($p\geq0.05$).

Table 2 shows the *S. mutans* levels of both groups due to the follow-up periods and, their comparisons for each time interval. The levels of *S. mutans* at T0 exhibited a decline over time for both groups, though this trend was not statistically significant for Group B ($p=0.58$; $p\geq0.05$). In contrast, the decline was statistically significant for Group A ($p=0.01$; $p<0.05$). Repeated comparisons of Group A revealed statistically significant difference between T0 and T1 ($p=0.04$; $p<0.05$) and T1 and T2 ($p=0.03$; $p<0.05$). Additionally, a statistically significant difference was observed between Group A and Group B for T0 ($p=0.001$; $p<0.05$), T1 ($p=0.04$; $p<0.05$), T2 ($p=0.04$; $p<0.05$) and T3 ($p=0.03$; $p<0.05$). In summary, the levels of *S. mutans* exhibited a decline in both groups during the follow-up period, even after the probiotic was discontinued. However, this decline was not statistically significant. A statistically significant decrease was observed in the probiotics group during the initial month of follow-up.

Table 3 shows the *Lactobacillus* levels of each group with different follow-up times and statistical analysis between and within each group. The statistical analysis of *Lactobacillus* levels revealed no statistically significant difference between Group A ($p=0.54$; $p\geq0.05$) and Group B ($p=0.96$; $p\geq0.05$) with time. Additionally, no statistically significant difference was observed between groups at T0 ($p=0.09$; $p\geq0.05$), T1 ($p=0.14$; $p\geq0.05$) and T2 ($p=0.08$; $p\geq0.05$). However, a statistically significant difference was noted as T3 ($p=0.03$; $p<0.05$). The levels of *Lactobacillus* spp. exhibited an increase that did not reach statistical significance during the follow-up period in both groups.

Discussion

According to the American Association of Pediatric Dentistry [12], full mouth oral rehabilitation of all dental caries is a necessary, but insufficient measure to reduce the level and colonization of cariogenic bacteria, including *S. mutans* and *Lactobacillus* spp., which are responsible for dental caries. Consequently, following the completion of dental treatments, the application of preventive or protective agents is necessary to maintain low levels. The most efficacious method of maintaining a minimum level of cariogenic bacteria is to prevent dental plaque formation through the application of correct brushing techniques with the utilization of optimal remineralization agents. It is further recommended that patients attend regular controls to facilitate the outcomes [13–15].

Lin et al. [14] reported a study about clinical and microbiological evaluation of children after full mouth rehabilitation under general anesthesia. According to the results of the study, there was statistically significant difference between before and after the general anesthesia. Therefore, the present study was established on patients whose dental treatments were completed under general anesthesia. As all dental treatments for participating patients were completed prior to the administration of probiotics, it was ensured that both groups, those using and those not using probiotics, were at comparable levels of cariogenic bacteria. Furthermore, the presence of *S. mutans* and *Lactobacillus* spp. has an active role in the formation of mature dental biofilms and the development of caries. Consequently, the included children were selected from the patients who had completed and accepted an oral rehabilitation program, were willing to attend regular control appointments, and had received instructions in oral hygiene methods designed to reduce bias. Therefore, the patients whose dental treatments were completed under general anesthesia in one session started to follow up with zero (0) tooth caries to provide standardization of the presence of tooth caries. The results of the present study indicate that there was no statistically significant difference between the groups regarding DMFT/dmft, which represents the number of decayed-missed-filled teeth number for permanent and primary dentition. These results are corroborated by the comparable baseline levels of *S. mutans* and *Lactobacillus* spp. in both groups, which play the most crucial role during caries formation.

Table 3 *Lactobacillus* spp. levels of each group with different follow-up times and statistical analysis between and within each group

Groups	T0	T1	T2	T3	*p*-value†
Group A	9.68×10^{10} ± 4.73×10^{11}	1.66×10^{12} ± 7.76×10^{12}	4.42×10^{12} ± 2.21×10^{13}	1.41×10^{12} ± 6.22×10^{12}	0.54
Group B	1.55×10^{9} ± 6.45×10^{9}	2.63×10^{11} ± 9.99×10^{11}	3.32×10^{10} ± 1.65×10^{11}	2.25×10^{10} ± 1.09×10^{10}	0.96
p-value‡	0.09	0.14	0.08	0.03*	

Lactobacillus spp. levels were given as mean ± standard deviation values; † refers statistical analysis with Friedman test for intragroup comparison with 95% CI Difference and ‡ refers statistical analysis with Kruskal-Wallis Test for intergroup comparison with 95% CI Difference

In recent years, probiotics have been the preferred treatment for diarrhea, Crohn's disease, some cardiovascular diseases, some types of cancer, urogenital infections, oropharyngeal infections and as a supportive treatment in cases where antibiotic use is necessary [16–18]. Probiotics have also been employed to diminish the number and colonization of pathogens that cause dental caries and gingival diseases, thereby improving oral health. Accordingly, the action mechanism of probiotics varies depending on the specific combination of live bacteria contained within each strain [1, 5, 8, 19]. As evidenced by prior research [7, 20, 21], the probiotic bacteria most commonly utilized in clinical studies targeting oral health are *Lactobacillus* spp. and *Bifidobacterium* spp. These are administered in a variety of vehicles, including milk, yogurt, cheese, drops, gum, ice cream, lozenges and tablets. Probiotic *Lactobacilli* and *Bifidobacteria* are considered to be both acidogenic and aciduric. The acid production of probiotic bacteria is believed to be a crucial factor in their ability to affect other bacteria. However, this is also the direct causative factor for the demineralization of teeth [22, 23]. Consequently, the use of probiotics may results in an elevated acidogenicity within the dental plaque [1]. So, some studies [24–26] even claim probiotics to not have beneficial, but potentially harmful effects. The purpose of this study was to evaluate the efficacy of *Bifidobacteria* probiotics in maintaining optimal levels of *S. mutans* and *Lactobacillus* spp. without the use of a vehicle.

S. mutans is one of the most thoroughly studied oral symbionts and a significant contributor to dental caries. Its colonization of the dentition can serve as an early indicator of caries development [3, 4, 19, 27]. The presence of oral *Lactobacillus* spp. is associated with an elevated

prevalence and augmented severity of dental caries [19, 27]. In contrast, *Lactobacillus* spp. is rarely detected in the saliva of individuals who have not experienced dental caries. However, the considerable diversity of species and genotypes that colonize the oral cavity indicate that the natural sources of *Lactobacillus* include exogenous and opportunistic colonizers that reside outside of the human oral cavity, likely originating from food products or other fermented materials [20, 21, 24, 28]. According to the results of this study, the initial levels of *S. mutans* and *Lactobacillus* spp. was observed statistically different. Because there are many species of *Lactobacillus* that are natural members of the oral microbiota and *Lactobacillus* is a large genus that has species with probiotic and cariogenic properties. However, there should be an acidogenic environment with such a reason as cariogenic diet consumption that provide retention of *S. mutans* on the enamel surface of teeth. Therefore, it would be stated that the significant difference between baseline levels was a proof of the dominant feature of *Lactobacillus* spp. rather than *S. mutans* for a child patient with early childhood caries.

The evidence indicates that probiotic bacteria do not establish a long-term colonization of the oral cavity, whether in early life interventions or in subjects with a mature microbiota. The strains under investigation have been observed to be transiently present in saliva during and shortly after an intervention [16]. Therefore, this information corroborates the results of the present study regarding alterations in *S. mutans* and *Lactobacillus* spp. levels subsequent to cessation of probiotics use. The results of the *S. mutans* level analysis revealed a statistically significant difference between probiotics and control groups for each control period. This result supported the beneficial effect of probiotics for oral rehabilitation, as evidenced by a reduction in the level of *S. mutans.* Additionally, intragroup comparisons of follow-up times revealed a decline in the *S. mutans* level for probiotics group, particularly at the one-month control. A statistically significant difference was observed between the baseline and one-month, as well as between one-month and six-month. However, no statistically significant difference was identified for Group B over time.

However, this beneficial effect of probiotics was not observed among *Lactobacillus* strains. There was not any statistically significant decrease in the level of *Lactobacillus* spp. at baseline, one-month and three-month control period. This might be attributed to the specific probiotics strain utilized in recent studies, namely *Bifidobacterium.* The action mechanism of probiotics might diverge from the features of probiotic strains, potentially influencing the colonization capacity and extent within oral tissues. Consequently, the underlying cause might be the insufficient colonization of *Bifidobacterium* spp. as opposed to *Lactobacillus* spp.

A statistically significant difference was observed in the levels of *Lactobacillus* spp. between probiotics and control groups during the sixth month control period. The probiotics group exhibited a higher level of *Lactobacillus* spp. than the control group. This result might be attributed to the influence of standardized oral hygiene methods and the diminished efficacy of probiotics following cessation during the wash-out period of probiotics. However, the *Lactobacillus* level in the group without probiotics reduced in the 6-month follow-up, and this was not statistically significant. This observed result may be associated with the oral hygiene training provided during regular control appointments and determination of the total *Lactobacillus* spp. which may possess probiotic or cariogenic properties, without the knowledge of the dominant species.

Although the positive effects on general health and oral health of the probiotics are known, possible side effects and complications are still being investigated. The safety of probiotics is tied to their intended use, which includes consideration of the potential vulnerability of the patient, dose and duration of consumption [19]. Unique to probiotics is that they are alive when administered, and unlike other food or drug ingredients, possess the potential for infectivity or in situ toxin production. Additionally, the presence of transferable antibiotic resistance genes, which comprises a theoretical risk of transfer to other members of the flora is one of the important points that creates questions in the mind and should be considered [29]. There are a lot of questions about the risk levels of probiotics using as main or adjunct treatment agents. However, negative effects of probiotics and the mechanisms of probiotic interaction with the host and colonizing microbes should be better understood to determine the risk level of probiotics.

The administration type of the probiotics used in the present study, in the form of drops, was a key strength. This method of administration was straightforward for all participating children. However, the study was limited by the use of probiotics without a vehicle and the lack of clarity regarding the six drops of daily use. It should be noted that the amount of food consumed by each individual may vary, as may the remineralizing effects of the vehicles used, such as beneficial nutrients, including milk, yoghurt and cheese. Since dental caries is a multifactorial disease, its development is influenced by a number of factors beyond bacterial balance including diet, fluoride use and oral hygiene, which is a common limitation for this type of clinical studies.

After all dental treatments were completed under general anesthesia, there was a notable decrease in the levels of *S. mutans* and *Lactobacillus* spp. This suggests that full

mouth oral rehabilitation might facilitate the remineralization ability of oral hygiene methods with remineralization agents. The use of probiotics might prove an effective method of preventing the colonization of the oral cavity by *S. mutans* and *Lactobacillus.* This is based on the results of this study, which indicate a reduction in the number of these cariogenic bacteria. It may therefore be anticipated that the administration of probiotics, which are commonly used to support general health, will have a beneficial effect on the reduction of these microorganisms and on maintaining a stable microbial population. It is recommended that treatment protocols for patients with early childhood caries be modified to include the use of probiotics as part of their daily routine. The most crucial aspect within this context might be summarized as the administration of an appropriate probiotic strain at an optimal dosage, coupled with an awareness of the significance of oral hygiene practices. Therefore, future studies should be conducted to show the role of probiotics, prebiotics, synbiotics, and postbiotics on oral health with long-term studies. In particular, the assessment of the most effective and convenient ways to deliver probiotics and the isolation of the probiotic strain from the bacterial components of the patient's oral microbiota i.e. *S. mutans* and *Lactobacillus* spp. should be investigated.

Conclusions

Probiotics, which are widely used for the treatment of various health conditions, have recently been employed as a means of reducing oral pathogens. The administration of *Bifidobacterium* spp. probiotics was observed to result in a notable decline in *S. mutans* levels, particularly at the one-month follow-up. However, no such reduction was evident in *Lactobacillus* spp. levels. The combination of probiotics and proper oral hygiene may prove advantageous in preventing the colonization of cariogenic bacteria, potentially leading to alterations in treatment protocols for early childhood caries. Selecting the most appropriate probiotic strain and dosage, in addition to maintaining satisfactory oral hygiene, is essential for achieving the optimal outcome.

Author contributions

D.S.U. and B.M.Ö. conceived the idea; D.S.U., E.Ç., A.Ü.G., B.M.Ö., A.A.K. and A.C.B. attended the project design; D.S.U. collected the data; D.S.U., E.Ç. and A.Ü.G. analyzed the data; and D.S.U. led the writing; A.Ü.G. revised the manuscript. All authors have read and approved the final version of the manuscript.

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Data availability

The datasets analyzed during the current study are not publicly available due to the ethical limitation of being private information of the participated children but are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

Procedures followed during the study were in accordance with the Helsinki Declaration and the study was approved by Baskent University Institutional Review Board and Ethics Committee (Project no: D-KA19/41). In addition, written informed consent was obtained from the parents of each child participating in the study and from the children who knew how to write before the first saliva sample was taken and before the general anesthesia procedure.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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