

Presence and count of *S. mutans* in children with dental caries: before, during and after a process of oral health education

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ABSTRACT

Dental caries is an infectious, multifactorial, localized, transmissible process that leads to the destruction of hard dental tissue. *Streptococcus mutans* is considered to be the main microorganism associated with its development. The aim of this study was to determine presence and count of *S. mutans* in saliva samples from children with dental caries before and after an educational process including interviews, lessons, lectures, educational workshops and recreational activities on the importance of oral care and hygiene. Twenty-three 3- to 6-year-old schoolchildren provided 3 unstimulated saliva samples: one before the educational process, one at 3 months and one at 6 months into the educational process. The samples were serially diluted and plated on Mitis Salivarius agar supplemented with

bacitracin and 20% sucrose, and incubated anaerobically for 2 days at 37°C. Presumptive *S. mutans* isolates were identified with biochemical tests. Before the beginning of the educational process, and at 3 and 6 months into the educational process, *S. mutans* was found, respectively, in 22 (95.6%), 15 (65.2%) and 10 (43.5%) of the 23 children. The *S. mutans* count was reduced by 64.8% and 86.6% at 3 and 6 months, respectively, compared to the levels found before the educational process. These results indicate that educational intervention produced a significant reduction in *S. mutans* levels in the saliva of children with dental caries at 3 and 6 months into the educational process.

Key words: Dental caries, children, *Streptococcus Mutans*, Health education.

Presencia y recuento de *S. mutans* en niños con caries dental: antes, durante y después de un proceso de educación en salud oral

RESUMEN

La caries dental es un proceso infeccioso multifactorial, localizado y transmisible que se caracteriza por la destrucción del tejido dental duro. *Streptococcus mutans* es considerado el principal microorganismo asociado al desarrollo de esta enfermedad. El objetivo de este estudio fue determinar la presencia y recuento de *S. mutans* en saliva de niños con caries dental antes y después de un proceso educativo. Con este fin se tomó saliva no estimulada de 23 niños con caries dental pertenecientes a un centro educativo con edades de 3 a 6 años. En todos los niños se tomaron 3 muestras de saliva: antes del proceso educativo y a los 3 y 6 meses de iniciado el proceso educativo. El proceso educativo consistió en entrevistas, enseñanzas, conferencias, talleres educativos y actividades lúdicas sobre la importancia del cuidado e higiene oral. Después de su recolección, las muestras de saliva fueron serialmente diluidas y sembradas en Agar Mitis Salivarius con bacitracina y

sacarosa al 20%. Los medios de cultivo sembrados se incubaron en anaerobiosis durante 2 días a 37°C y los aislamientos presuntivos de *S. mutans* se identificaron con pruebas bioquímicas. Antes del inicio del proceso educativo, a los 3 y 6 meses de iniciado el proceso educativo se encontró *S. mutans*, respectivamente, en 22 de los 23 niños (95.6%), en 15 de los 23 niños (65.2%) y en 10 de los 23 niños (43.5%). En cuanto al recuento de *S. mutans*, se encontró una reducción de 64.8% y 86.6% a los 3 y 6 meses, respectivamente, en comparación a los niveles encontrados antes del inicio del proceso educativo. En conclusión, los resultados indican que la intervención educativa realizada produjo una reducción significativa en los niveles de *S. mutans* en saliva de niños con caries dental después de 3 y 6 meses de iniciado el proceso educativo.

Palabras clave: Caries dental, niños, *Streptococcus mutans*, educación en salud.

INTRODUCTION

Dental caries is an infectious bacterial disease which is multifactorial, chronic, localized, post-eruptive and transmissible, and leads to destruction of hard dental tissue¹⁻³. Its development basically requires three factors sustained over time: a susceptible host, cariogenic microflora in the dental biofilm, and an adequate substrate. *Streptococcus mutans* (primarily serotype C), and to a lesser extent, *S. sobrinus* and *S. gordonii*, as well as *Lactobacillus* and *Actinomyces* species, in that order of frequency, are the primary microorganisms associated to the development and progression of dental caries^{4,5}.

Different studies have found a strong correlation between number of *S. mutans* colonies in the oral cavity and prevalence and incidence of dental caries^{1,4,5}. In addition, as a result of dental treatments, *S. mutans* can cause bacteremia, systemic infections and subacute endocarditis⁶.

The fact that *S. mutans* has been clearly recognized as the primary bacterial species involved in dental caries has led to the implementation of preventive and control measures tending to eliminate or reduce its presence in the oral cavity^{3,6-8}. Different strategies have been designed for such purpose, but because of lack of continuity, systematization, regulation and supervision, they have not been very effective⁸.

Dental caries is currently one of the most frequent diseases of the oral cavity, mainly affecting 5- to 12-year old children. Because it is a chronic disease, it progresses with age unless efforts are made to control it^{9,10}. The distribution and severity of dental caries varies among regions, and its onset is frequently associated to nutritional, sociocultural, economic and environmental factors^{11,12}.

Different comparative clinical studies on 2.5- to 7-year-old children with dental caries have shown reduction in oral levels of *S. mutans* and *Lactobacillus* species¹³⁻¹⁵ in dental biofilm and saliva, during and after conventional and non-conventional restorative treatments¹³⁻¹⁵. Although the results in these studies suggest that *S. mutans* and *Lactobacillus* species counts are lower after 6 months' treatment than at baseline, they also record a tendency for these microorganisms to become reestablished over time¹³⁻¹⁵. With regard to oral health educational strategies, very few studies to date present successful results of restorative dental

treatment in the reduction of microorganisms that are important in dental caries¹⁶⁻¹⁸.

In Colombia, no study has been published evaluating the impact of oral health educational intervention on presence and count of *S. mutans* in children with dental caries. The main aim of this study was therefore to determine *S. mutans* presence and count in saliva from children with dental caries before, during and after a 6-month oral health educational process.

MATERIALS AND METHODS

Study population

This was a longitudinal prospective study which met bioethical requirements for sampling and sample management. It included 23 3- to 7-year-old children with dental caries from the school Centro Educativo Fe y Alegría – José María Velaz- in the Suba locality of Bogota city, Colombia. Exclusion criteria were: use of topical or oral antimicrobial agents within 7 days prior to sampling; undergoing orthodontic treatment with fixed or removable appliances, and/or any kind of oral infectious process different from dental caries. Each child underwent an oral clinical examination to determine dental caries experience, performed by a single calibrated examiner using artificial light and a dentist's mirror. No X-ray was taken. Dental caries clinical stage was determined according to the International Caries Detection and Assessment System (ICDAS), which distinguishes early non-cavitated lesions from cavitated or dentinal lesions^{19,20}. Of the 23 children with dental caries included in the study, 12 were diagnosed as ICDAS score 3 and 11 as ICDAS score 6.

S. mutans isolation, identification and count

Three saliva samples were taken from each of the 23 children: one before starting the educational process (baseline), one 3 months into the educational process, and a final sample 6 months into the educational process. The samples were taken between 8:00 and 10:00 a.m., with prior commitment from the children not to eat anything and not to brush their teeth before sampling. Spontaneous saliva (0.2-1 ml) was sampled by gentle suction with a sterile plastic pipette²¹ and samples were immediately placed on ice to be transferred to the bacteriological laboratory. At the laboratory, the saliva samples were vortexed

for 30 seconds and serially diluted (1/10, 1/100 and 1/1000) with 0.05M phosphate buffer. For *S. mutans* selective isolation and count, 35 µl of the serial dilutions were inoculated in duplicate in Mitis Salivarius Bacitracin Agar (MSB; Difco Laboratories, Detroit, MI). MSB Agar contains digested pancreatic casein, proteose peptone No.3, proteose peptone, dextrose, 20% sucrose, dipotassium phosphate, trypan blue, crystal violet, agar, Chapman's tellurite and 0.2 U/ml bacitracin. The Petri dishes with MSB agar were incubated anaerobically (H₂:CO₂:N₂; 10:10:80) for 2 days at 37°C. After bacterial growth in the MSB agar, colonies with *S. mutans* morphological characteristics²² were counted and expressed as colony-forming units (CFU) per ml of unstimulated saliva. Then 5 to 10 colonies per sample with *S. mutans* characteristics were examined by Gram stain, catalase test and the following biochemical tests: fermentation of raffinose, mannitol, melibiose, trehalose and inulin; hydrolysis of esculin in presence and absence of bile; urease; hydrolysis of arginine; and resistance to 2 U of bacitracin. The biochemical profile for *S. mutans* is positive fermentation of raffinose, mannitol, melibiose, trehalose and inulin; negative hydrolysis of esculin in presence of bile and positive hydrolysis of esculin in absence of bile; negative urease; negative hydrolysis of arginine; and resistance to 2 U of bacitracin. The commercial system Api 20S (bioMérieux, Marcy-l'étoile, France) was also used in the identification of strains.

Total aerobic microbial count

Total aerobes were counted in all saliva samples (before and after the beginning of the educational process). To do so, 35 µl of all the serial dilutions of saliva with phosphate buffer (as described above) were inoculated in blood agar (BHI agar supplemented with 5% lamb's blood). After inoculation, the dishes of blood agar were incubated aerobically at 37°C for 48 hours. Total aerobic microbe CFU/ml were counted.

Oral health educational process

The educational process began with a survey to determine parents' and/or guardians' knowledge, attitudes and practices regarding oral health, acquired previously in family and school settings. After the survey, the following activities were

conducted with parent/guardian-child pairs: 1. Talks and workshops for parents/guardians and children on the importance of good oral health; 2. Educational workshops on tooth brushing, evidence of bacterial plaque or dental biofilm, and the importance of good nutrition; 3. Workshops and feedback with parents/guardians and children over the 6 month educational period, on the importance of good oral hygiene; and 4. Recreational-educational workshops for the children on dental care and the practice of good oral health. Throughout the 6-month educational process, oral hygiene kits consisting of 22 ml dentifrice and a child toothbrush were delivered individually to each child every 15 days. In addition, throughout the 6-month study, the four oral hygiene strategies described above were reinforced every 15 days.

Statistical analysis

Descriptive statistics (frequency, mean, standard deviation and maximum and minimum values) and paired Student's t-test were used to establish differences between *S. mutans* counts in the groups with caries (ICDAS 3 and 6) before (baseline) and after beginning the educational process (at 3 and 6 months). Student's t-test was performed with the program IBM SPSS Statistics version 22.0 and the level of statistical significance was set at $p < 0.05$.

RESULTS

Table 1 shows caries experience and demographics for the 23 children with dental caries included in the study. The 11 children (5 female and 6 male) diagnosed with ICDAS score 3 were within an age range of 3.5-4.5 years, average age 4.2 ± 0.24 years and dmft 3.7 ± 3.1 . The 12 children (5 female and 7 male) diagnosed with ICDAS score 6 were within an age range of 5.7-6.7 years, average age 6.5 ± 0.3 years and dmft 5.3 ± 3.9 .

Table 2 shows the frequency of *S. mutans* in baseline, 3- and 6-month samples in the 23 children with caries (ICDAS groups 3 and 6). The 11 children with ICDAS score 3 had *S. mutans* frequencies of 91% (10/11), 27% (3/11) and 9% (1/11), at baseline, 3 and 6 months, respectively. The 12 children with ICDAS score 6 had *S. mutans* frequencies of 100% (12/12), 100% (12/12) and 75% (9/12) at baseline, 3 and 6 months, respectively. Aggregate results for both groups showed *S. mutans* frequencies of 95.7% (22/23),

65.2% (15/23) and 43.5% (10/23) at baseline, 3 and 6 months, respectively (Table 2).

Fig. 1 shows average S. mutans values in saliva from children at the two clinical stages of dental caries for the three sampling times. For clinical stage ICDAS

3, there was a significant reduction in S. mutans from baseline to month 3 (p=0.0221) and from baseline to month 6 (p= 0.0182). For reduction of S. mutans at clinical stage ICDAS 6, baseline to month 3 had p= 0.0526 and baseline to month 6 had p=0.0607.

Table 1: Baseline: Caries experience and demographics of children included in the study.

Caries Status	Children (n=)	Age range in years	Age* (years)	Sex	dmft*
ICDAS 3	11	3.5 – 4.5	4.2 ± 0.24	Female: 21.7% (n= 5) Male: 26.1% (n= 6)	3.7 ± 3.1
ICDAS 6	12	5.7 – 6.7	6.5 ± 0.3	Female: 21.7% (n= 5) Male: 30.4% (n= 7)	5.3 ± 3.9
Total	23	3.5 – 6.7	5.4 ± 1.2	Female: 43.5% (n= 10) Male: 56.5% (n= 13)	4.2 ± 1.1

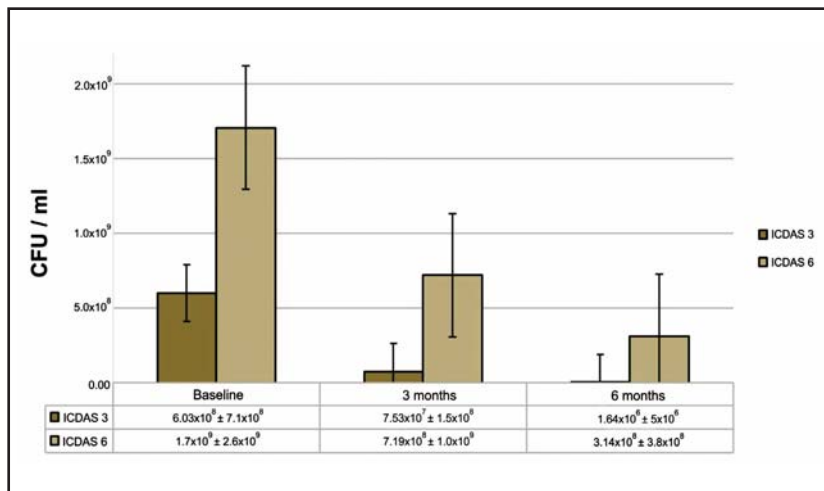
* Values expressed as means (± standard deviation).

Table 2: Frequency of S. mutans in saliva samples at baseline and 3 and 6 months into the educational process.

Time	Presence of S. mutans	No. of children with:		Total
		ICDAS 3	ICDAS 6	
Baseline	Positive	10	12	22
	Negative	1	0	1
	Frequency	10/11 (91%)	12/12 (100%)	22/23(95.7%)
3 months	Positive	3	12	15
	Negative	8	0	8
	Frequency	3/11 (27%)	12/12 (100%)	15/23(65.2%)
6 months	Positive	1	9	10
	Negative	10	3	13
	Frequency	1/11 (9%)	9/12 (75%)	10/23(43.5%)

Fig. 1: S. mutans count (CFU/ml)* in saliva of children classified by ICDAS into two clinical stages of dental caries.

*CFU/ml (mean ± SD) are expressed on logarithmic scale. Paired Student t-test was used to analyze the differences between baseline and month 3, and between baseline and month 6. There was a significant reduction (p = 0.0221) for ICDAS 3 between baseline and month 3. There was also a significant reduction for ICDAS 3 between baseline and month 6 (p = 0.0182). No significant difference was found for ICDAS 6 between baseline and month 3 (p = 0.0526), between baseline and month 6 (p = 0.0607) or between month 3 and month 6 (p=0.0569).



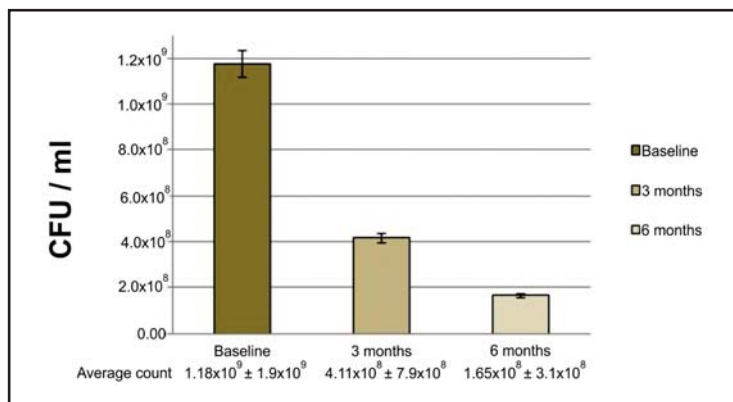


Fig. 2: *S. mutans* count (CFU/ml)* found in saliva from children with dental caries (ICDAS 3 + ICDAS 6). * CFU/ml (mean \pm SD) expressed on logarithmic scale. Paired student t-test was used to analyze the differences. *S. mutans* was reduced significantly between baseline and month 3 ($p = 0.007$) and between baseline and month 6 ($p = 0.002$).

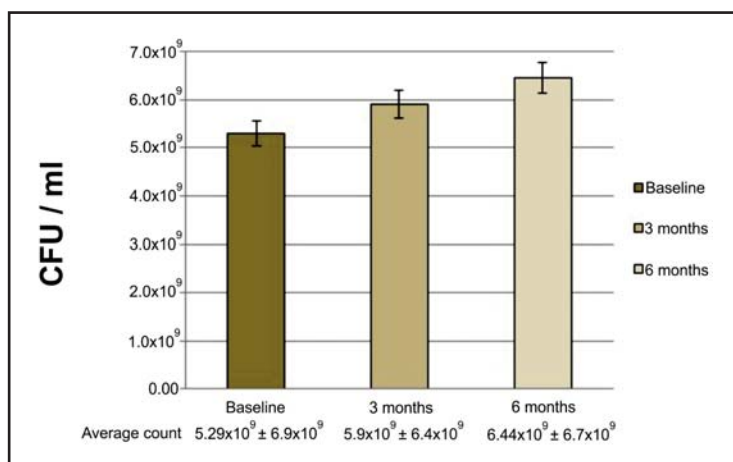


Fig. 3: Total aerobic microorganism count* in saliva from children with dental caries (ICDAS 3 + ICDAS 6). * CFU/ml (mean \pm SD) expressed on logarithmic scale. Paired student t-test was used to analyze the differences. No difference in aerobic microorganisms was found between baseline and month 3 ($p = 0.63417$), between baseline and month 6 ($p = 0.5711$) or between month 3 and month 6 ($p = 0.6124$).

Fig. 2 shows aggregate average values for *S. mutans* count in saliva from all 23 children with dental caries (ICDAS 3 + ICDAS 6) at baseline, month 3 and month 6. According to Student's t-test, average values for total count of *S. mutans* present in saliva decreased with statistically significant differences, comparing counts at baseline, month 3 and month 6 ($p < 0.05$). Bacterial counts diminished significantly between baseline and month 3 (64.8%, $p = 0.007$), and between baseline and month 6 (86%, $p = 0.02$). Fig. 3 shows total aerobe count, expressed as CFU/ml, for saliva samples from the 23 children with dental caries at baseline, 3 months and 6 months. Total average value at baseline was 5.29×10^9 CFU/ml, increasing at 3 and 6 months by 11.6% (5.9×10^9) and 21.8% (6.44×10^9) respectively, without statistically significant differences (Student t-test $p > 0.05$, Fig. 3).

DISCUSSION

Dental caries is considered to be a multifactorial disease as a result of an imbalance in the oral

ecosystem leading to the predominance of flora previously considered normal in the oral cavity, which then becomes pathogenic. Microbial imbalance, as well as the presence of different external factors, including nutritional, sociocultural, economic, environmental and individual behavioral, impact the development of dental caries. For a long time, *S. mutans* has been considered the primary etiological agent involved in the development of dental caries, playing a very important part in initial stages of dental enamel deterioration^{23,24}. In children, *S. mutans* is associated to a specific period, known as "window of infectivity", which coincides with the eruption of teeth and occurs between 6 and 30 months of age, with higher risk from 18 to 30 months of age²⁴⁻²⁶. Clear recognition of *S. mutans* as the primary bacterial species involved in dental caries has led to the search for, and development and implementation of prevention and control measures aimed at eliminating or reducing *S. mutans* in the oral cavity²⁵. Previous clinical studies on children with dental caries have shown the positive effect of restorative

treatment on the reduction of *S. mutans* levels¹³⁻¹⁵. On the other hand, with regard to educational strategies, very few studies conducted to date present similar results to those of restorative dental treatment for reduction of microorganisms which are important in dental caries¹⁶⁻¹⁸.

In view of the fact that dental caries is basically a controllable disease and considering the failure of many preventive and therapeutic actions, greater efforts and resources need to be allocated to preventive and educational measures to reduce the presence and quantity of cariogenic microorganisms in the oral cavity, thereby reducing dental caries^{27, 28}. Proper oral health education should be a strategy leading to a change in attitude in children and adults to achieve better individual and collective health and wellbeing indicators^{27, 28}. In the current study, the 23 children complied with all the educational strategies proposed over the 6-month educational process. They received relevant oral health and hygiene education in accordance with the plans outlined: 1. Survey on knowledge of oral health; conferences and workshops for parents/guardians and children on the importance of good oral health and hygiene; 2. Personalized educational workshops on tooth brushing, identifying bacterial plaque, and the importance of healthy eating; 3. Recreational-educational workshops for the children on dental care and practicing good oral health. The oral health educational strategies were reinforced periodically, according to the proposed schedule, and also by leaving in classrooms leaflets, guides, notice boards and documents relevant to oral health and hygiene education. It should be highlighted that all the activities required constant, disciplined commitment of the dentists, preschool teachers and children's parents and/or guardians who participated in this project.

Regarding microbiological results, *S. mutans* count was found to be directly proportional to caries clinical stage, i.e., at higher levels of dental caries there were greater numbers of *S. mutans* colonies. The health and oral hygiene educational strategies enabled a reduction in *S. mutans* levels at 3 and 6 months, by 64.8% and 86% respectively, in children with dental caries (ICDAS 3 + ICDAS 6, Fig. 2). The educational strategy had greater impact on the reduction of *S. mutans* at 6 months in the group of children with ICDAS score 3, where *S. mutans* was only detected in 1 of the 11 children. The impact

was weaker in the children with ICDAS score 6, since at the end of the 6 months *S. mutans* persisted in 9 of the 12 children, at concentrations higher than 500.000 CFU/ml.

The high counts at the end of the 6-month educational process in children with ICDAS score 6 may indicate the high risk for originating carious stages and that educational strategies alone were insufficient. It is highly likely that future educational activities will need to cover longer times, and/or strategies to achieve absence and/or reduction of *S. mutans* levels will need to be strengthened, as proposed in other studies²⁷⁻²⁹, even considering joint use of educational strategies and dentifrices with greater bactericidal power²⁹. A study by Kumar et al.²⁹ analyzed the effect of oral health education and the use of fluoridated and non-fluoridated dentifrices on oral health status, using *S. mutans* and *Lactobacillus* counts before beginning the educational strategy, and 3, 6 and 12 months post-treatment. They found post-treatment reductions in the counts of both microorganisms, both in children who used fluoridated dentifrices and in those who used non-fluoridated dentifrices. However, the reduction was significantly greater at 3, 6 and 12 months post-treatment in children who used fluoridated dentifrices, and the authors suggest that this may have been due to the bactericidal action of fluoride. Another study on children found that the long-term use of fluoride mouthrinse led to lower levels of *S. mutans*³⁰. However, long-term studies have been proposed to clarify the effect of chemotherapeutic agents on the reduction of *S. mutans* and their impact on caries incidence³¹.

It is important to clarify that although the current study focuses the microbiological risk factor, other studies have shown that long-term caries prevention needs to act simultaneously on all risk factors involved in this disease^{1-4, 19, 25}.

Different studies have shown the direct association between dental caries incidence and *S. mutans* presence and quantity in the oral cavity³²⁻³⁵. Similarly, the current study found high frequency (95.7%, or 22 out of 23 children) and high *S. mutans* colonization rate at baseline in both groups of children with dental caries (ICDAS 3 and ICDAS 6). The current study also determined levels of aerobic microorganisms before and after the oral health educational strategies. In general, the counts remained stable, with slight, non-statistically signi-

ficant increase over time. These levels of aerobic microorganisms may also be indicative of stable oral health, in which the place left by the reduction of *S. mutans* colonies achieved through educational strategies was taken over by aerobic microorganisms, which are compatible with the healthy microflora that is part of the oral microbial ecology⁸.

This study was intentionally sought children with dental caries. There was predominance of male children, with 56.5% (13/23), and overall average age was 5.4 ± 1.2 years. Oral examination detected that 47.8% (11/23) of the children had ICDAS score 3 and 52.2% (12/23) had ICDAS score 6. Overall average dmft index was 4.2 ± 1.1 for the 23 children. Other studies conducted in Colombia, which also used ICDAS and dmft diagnostic criteria, have

reported similar results³⁶⁻³⁸. These results indicate a high rate of dental caries in the child population studied, which may serve as a sentinel and be an indicator of the results of the public health strategies being implemented³⁹. On the other hand, microbiological analyses of saliva and monitoring children under 6 years old with dental caries may be useful for identifying individual and community risk of caries, and for improving treatment and delaying the process of *S. mutans* colonization and multiplication in the oral cavity⁴⁰.

To conclude, the results of this study indicate that the oral health educational process conducted led to a significant reduction in *S. mutans* level in the saliva of children with dental caries, 3 and 6 months into the educational process.

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