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Probiotics and their effect on oral bacteria count in children: a pilot study

ABSTRACT

Aim *To assess the effect of the daily ingestion of a mixture of probiotics on the amount of Streptococcus mutans in the oral cavity of preschool-age patients with a high risk of caries.*

Materials and methods *Forty patients, aged between 4 and 6 years, with a high risk of dental caries were included in this pilot study. Patients were randomly assigned to two study groups: the Experimental Group (A) included patients who brushed their teeth and used fluoridated toothpaste in addition to consuming probiotics daily, and the Control Group (B) inclused patients who brushed their teeth and used fluoridated toothpaste but did not consume probiotics. Using the CariScreen®, the microorganism count was determined at different times: baseline, 7, 14, 21 and 30 days. To identify the differences between both groups, a Mann-Whitney U test was performed, with a significance level of 0.05.*

Results *It was observed that both groups showed similar microbial counts at the beginning of the trial ($p>0.05$), and a significant decrease in the count at the end of the study was found in the experimental group ($p<0.05$) 15 days after suspending ingestion.*

Conclusion *We found a significant reduction of RLU values in preschool children who ingested the tested probiotics in relation to the baseline values and 15 days after ceasing consumption.*

Keywords *Caries, Cariscreen, Prevention, Primary teeth, Probiora3®, Probiotics, S. Mutans.*

Introduction

The World Health Organization defines probiotics as living microorganisms that, when administered in proper amounts, provide beneficial effects to the host; thus, they are often used to treat or prevent different conditions and diseases in humans [Elavarasu et al., 2012; Tong et al., 2012]. The consumption of living microorganisms began in ancient times, and in the beginning of the twentieth century Metchnikoff reported that the inhabitants of Bulgaria lived longer than other populations, ascribing those effects to the consumption of dairy products that contained fermenting microorganisms, which he called *Lactobacillus bulgaricus* [Elavarasu et al., 2012; Cagetti et al., 2013]. Probiotics have diverse properties, including the inhibition of bacterial growth, immunomodulation, the production of bacteriocins and hydrogen peroxide, and competitive exclusion via the antagonistic activities of pathogens with roles like adhesion [Twetman, 2012].

Normally, diverse lactobacilli reside in the oral cavity, with *Lactobacillus plantarum* and *Lactobacillus rhamnosus* being the most common, found in 26 to 52% of the population [Stamatova and Meurman, 2009]. When probiotics first contact the oral tissues, different components of saliva, such as lysozymes, lactoferrin, hystatin, peroxidases and IgA, affect the viability and morphology of the probiotics. Previous studies have demonstrated that *Lactobacillus and Bifidobacterium* strains grow until after 24 hours of being in contact with saliva [Stamatova and Meurman, 2009]. Additionally, if the probiotic colonisation of the oral cavity happens at an early age, then the long-term effects will be better, especially when the probiotics are consumed daily; they can be detected up to 10 to 12 days after the last ingestion [Twetman and Stecksén-Blicks, 2008].

The main mechanism of action of probiotics in the oral cavity consists of the inhibition and control of the multiplication of the diverse regular resident microorganisms. *L. paracasei* and *L. rhamnosus* inhibit the action of *Streptococcus mutans* and *Porphyromonas gingivalis*, respectively [Snel et al., 2011]. Among the main beneficial properties of probiotics in the prevention of dental caries is their capacity to compete, antagonise and prevent the proliferation of cariogenic bacteria [Leão et al., 2011]. Güngör et al. [2013] evaluated the adhesive

capability of 70 lactic acid bacteria, including *S. mutans*. They observed a great diversity in the ability to adhere to tooth surfaces among lactic acid bacteria and reported that all strains of lactic acid bacteria were capable of reducing the quantities of *S. mutans* on the surface of the saliva-covered tooth surface. It has been proven that *L. paracasei* possesses antibacterial capabilities, inhibiting the presence of different pathogens, such as *Streptococcus mutans, Streptococcus salivarius, Streptococcus sanguis, Staphylococcus aureus, Actynomyces viscosus, P gingivalis, Candida albicans, Candida tropicalis* and *Candida glabrata* [Chuag et al., 2011]. Additionally, it has been reported that the reduction of *S. mutans* associated with the consumption of probiotics reduces the incidence of dental caries [Näse et al., 2001; Ahola et al., 2002; Montalto et al., 2004; Meurman and Stamatova, 2007; Caglar et al., 2007; Campus et al., 2007]. However, a recent systematic revision suggests that the current scientific evidence is still insufficient, and highlights the need for new controlled clinical trials that support these preliminary observations [Cagetti et al., 2013].

In this context, the aim of this study was to evaluate the effects of the daily administration of a mixture of probiotic bacteria on the microorganism counts in the oral cavities of preschool age patients with a high risk of caries. It is assumed that this daily oral administration reduces the levels of microorganisms for several days after ingestion is interrupted.

Materials and methods

Study design

A comparative pilot study was conducted in accordance with the Declaration of Helsinki. The Ethics Committee of the Faculty of Dentistry approved this study, whose objective was explained to the subjects' parents or legal guardians and for which written informed consent was obtained. The participating children were recruited from two public preschool-level schools. After receiving the informed consent from parents or legal guardians, 40 healthy patients of both genders, aged between 4-6 years with a high risk of dental caries [≥ 1500 Relative Light Units (RLU)] were selected. Caries risk was determined using the CariScreen® and CariScreen Susceptibility Testing Swabs® (Oral Biotech, Albany, Ore) systems; patients under antibiotic therapy or who had gingival inflammation or psychomotor impairment were excluded.

The patients were assigned sequential numbers in the order of their enrollment and received their allocated treatment according to a previously designed randomisation schedule. The study groups were balanced using permuted blocks of 4 numbers. Even numbers corresponded to intervention A, or the experimental group, which ingested probiotics. Odd numbers corresponded to intervention B, or the placebo group, which did not consume probiotics.

The study was divided into two periods of two weeks each: an intervention period and a post-ingestion evaluation period. During the first period, patients of both groups were instructed to attend their activities at home, but without brushing their teeth, in order to perform it at school, under supervision and before the administration of the tablets. Patients used children's soft bristle toothbrushes with fluoridated toothpaste for brushing. Afterwards, each patient of the experimental group ingested Probiora3® oral tablets (Oragenics, Tampa, FL, USA), which contained *Streptococcus uberis* KJ2TM, *Streptococcus oralis* KJ3TM and *Streptococcus rattus* JH145TM. The tablets given to the control group had a similar presentation, but contained no probiotics or sugars. Both types of tablets were slowly dissolved in the mouth.

Sample collection

Baseline saliva samples were collected in the morning, before brushing and ingesting the tablets. The indirect microorganism count was determined at different times: baseline, 7, 14, 21 and 30 days. A luminescence assay was used; the CariScreen® and CariScreen Susceptibility Testing Swabs® (Oral Bio Tech, Albany, Ore) systems were used. The device was calibrated before taking the samples, according to the manufacturer's instructions. The collection device consisted of a swab and swab holder for the collection of oral samples and a reservoir containing luciferin. Each participant was instructed to swallow any excess saliva to collect a sample of the saliva film with a proper swab from the lingual surface of the four lower primary incisors, as well as from the buccal surface of the four upper first primary molars. Once the sample was collected, it was deposited in a reaction container, which contained a mix of luciferase and other components that, when mixed vigorously with the obtained sample for 10 seconds, allowed for a bioluminescence reaction in the CariScreen system. The results were recorded for each patient, obtaining values between 0 and 9.999. As described previously, the patients brushed their teeth, followed by the consumption of the specific tablet. This standardised protocol was performed daily for a period of 15 days, and then suspended. For 15 days following the interruption of the ingestion of probiotic or placebo tablets, the children were monitored and encouraged to continue their tooth brushing as instructed.

Statistical analysis

The RLU values were recorded in both groups as medians and graphically as box plots at different times. We performed a non-parametric Mann-Whitney U test, with a significance level set at 0.05, to identify possible significant differences between the results from both study groups.

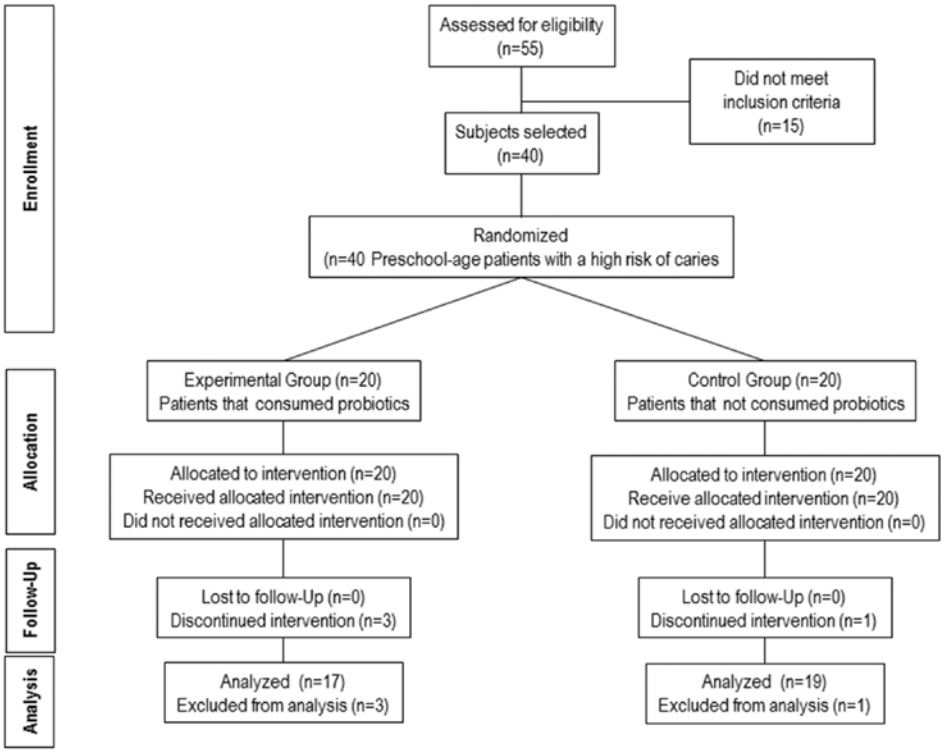

FIG. 1 Flowchart of the study, indicating the methodological stages (left) and characteristics of the study groups and their follow-up(right).

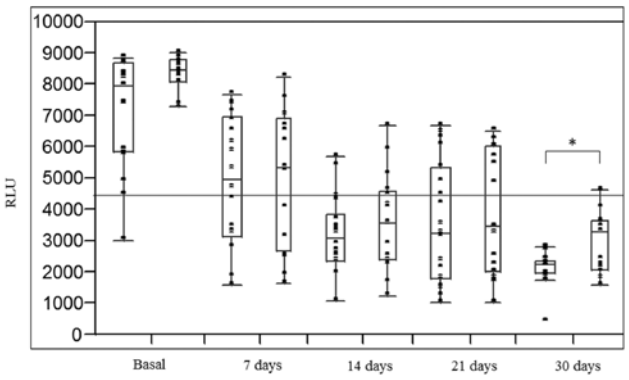

FIG. 2 This graph schematises the RLU values during the administration of Probiora3 tablets throughout the study. (*) Statistically significant (P < 0.05). Left box plot (Experimental group); Right box plot (Control group) in each period of time.

Results

A total of 19 participants in the experimental group and 17 in the control group completed the entire study period because four children did not attend any of the scheduled checkups (Fig. 1). When comparing the basal values of RLU, prior to the administration of probiotic or placebo tablets, it was determined that there were no statistically significant differences between both groups ($p>0.05$), indicating that at the beginning of the research, both groups showed similar values for their respective microorganism counts; we only included patients with a high risk of dental caries, with average initial values above 1500 RLU.

The values of the microbial counts in both groups from the beginning of the period of probiotic ingestion up to 15th day exhibited a statistically significant continuous decrease; however, this value was higher in the probiotic group ($p<0.05$). This tendency continued for the next 15 days after suspending probiotic ingestion. Data corresponding to the microbial count was significantly lower ($p<0.05$) in those patients who performed daily toothbrushing with fluoridated toothpaste in conjunction with probiotic ingestion (Fig. 2).

Discussion

Despite its reported decline in prevalence in some countries, dental caries is still one of the most important global public health problems that affect children [Marja-Leena et al., 2008]. The occurrence of this disorder is associated with several biological, dietetic, hygienic and social factors. Over time, different preventive approaches have been implemented, from chemoprophylactic agents such as chlorhexidine, triclosan and plant-derived compounds, eventually leading to the development of vaccines and the introduction of living microorganisms called probiotics [Chen and Wang, 2010]. During recent years, there has been a dramatic rise in the consumption of industrialised food products among the world's population. These foods contain a large amount of cariogenic carbohydrates, and some of these products

have been improved by the addition of probiotic bacteria due to their potential benefits [Twetman and Stecksén-Blicks, 2008].

The results of the present study suggest that the oral administration of probiotics may help maintain, and even decrease, low counts of cariogenic pathogens. Tong et al. [2012] evaluated the *in vitro* growth of strands of *S. mutans* and *L. lactis*, either individually or combined, and showed that the addition of *L. lactis* to an environment populated by a cariogenic microbial strand can affect its development, as well as that of the biofilms on the surface of the enamel, thus lowering the caries risk. In a randomised controlled clinical trial, Caglar et al. [2009] compared the effect of *L. reuteri* contained in tablets to the administration of a placebo and concluded that gradually dissolving the tablets allows for closer contact between the probiotic and the habitual oral environment, causing the reduction of *S. mutans* levels in saliva after two weeks of consumption. However, Twetman Stecksén-Blicks [2008] noted that regardless of the route of administration, whether oral or systemic, there was a decrease in the count of cariogenic bacteria when liquid and encapsulated forms were used; thus, they could evaluate the direct contact of probiotics with the oral tissues, proving that the route of administration is associated with increases in the count of *lactobacilli* in saliva, and thus maintaining lower *S. mutans* levels.

Various studies have evaluated different probiotics, particularly *Lactobacillus*, and have measured the effect of their ingestion, obtaining diverse results [Twetman and Stecksén-Blicks, 2008; Tanzer et al., 2010]. The present work showed that the administration of Probiora3® oral tablets, which contain a mixture of *Streptococcus uberis* KJ2™, *Streptococcus oralis* KJ3™ and *Streptococcus rattus* JH145™, produces encouraging effects by decreasing cariogenic bacteria in preschool patients. Chuan et al. [2011] established the effect of the consumption of *L. paracasei* GMNL-33 for 15 days on the count of *S. mutans* and the salivary pH in adults aged from 20 to 40 years. They did not observe a significant decrease in the counts of *S. mutans* nor in the pH values throughout the probiotic ingestion period and up to fifteen days after the cessation of intake. Campus et al. [2013] reported that the oral administration of L. brevis CD2 caused a significant decrease in the acidogenicity of dental plaque, in the counts of *S. mutans*, and in the gingival bleeding on probing. Their study examined 191 children, aged 6 to 8 years, who presented 2 to 3 carious lesions, and salivary counts of *S. mutans* $\geq 10^5$ UFC/ml; they delivered placebo tablets or *L. brevis* CD2 twice a day for six weeks.

Similarly, the present work was designed to be carried out in preschool patients, as the effect of probiotics is better and longer lasting at early ages, as reported by Vestman et al. [2013], who evaluated oral colonisation levels from 133 infants aged 0-2 months who were distributed in three groups, according to the type of feeding: 1) simple milk formula, 2) supplemented milk formula, and 3) breast milk. Children from the last group exhibited at least 37% *Lactobacillus* in the oral cavity, while the other two groups had levels below 10%, which suggests that early colonisation by probiotics derived from breast milk helps colonization by probiotics. However, we considered that the presence of primary teeth plays a fundamental role in the modification of the caries-associated oral microbiota because their colonisation begins on the dental surface after the eruption of these teeth in the presence of adequate substrates. Additionally, unlike other studies, we considered that either the probiotics' effect in decreasing caries risk must be accompanied by other preventive measures, like effective and frequent oral hygiene; it was also shown that the oral administration of ProBiora3® significantly reduces the cariogenic flora, even up to 15 days after the last intake.

The evaluation of caries risk via the CariScreen system uses ATP bioluminescence technology to detect the level of acid production by cariogenic bacteria residing in the dental plaque. This is an indirect quantification method that has already been previously validated, offers high sensitivity in detection, and the advantage of easy application in relation to conventional methods [Fazilat et al., 2010].

According to our results and to previous findings, probiotics showed anticariogenic and cariostatic potential, and they likely promote enamel remineralisation by reducing the production of acids that affect the tooth structure. In our clinical trial, we evaluated a probiotic mixture of three *Streptococcus* species. Paradoxically, two of these species, *S. oralis (mitis* group) and *S. rattus (mutans* group) have been identified as *streptococci* isolated from caries-associated dental plaque; however, both species are secondarily related, in low concentrations, to high adhesion to the enamel and to acidophilic properties, which grants them a competitive advantage over other bacteria such as *Lactobacilli. Lactobacilli* are closely associated with dentin caries, with an acidogenicity that is much lower than that of *Streptococcus mutans* and other primary cariogenic species, which, when administered in high concentrations, breaks the established ecological balance in the dental plaque. This phenomenon distorts their progress and maturation, and very likely stimulates a host adaptive immunologic reaction against these bacteria. Additionally, it is known that some Lactobacillus species have beneficial effects on periodontal health, as reported by Riccia et al. [2007], who observed anti-inflammatory effects with L. brevis tablets; after consuming this probiotic for 4 days, plaque levels, gingival inflammation and bleeding decreased, along with levels of prostaglandin E2 and metalloproteinases. Therefore, less nitric acid was produced. Keller et al. [2012] studied 25 subjects with diurnal oral halitosis who were enrolled in a double blind study with an intervention period of

14 days; they were instructed to chew a gum containing L. reuteri DMS 17938 and L. reuteri ATCC PTA 5289 or a placebo as control. Odor was detected by means of a halimeter, together with cysteine levels. They reported a significant reduction in halitosis in those subjects who ingested probiotics compared to the placebo group; in this regard, in the current study, we did not evaluate the effects of Streptococcus probiotics over these two variables. Additionally, it must be noted that probiotics have been shown to be nontoxic; Hillman et al. [2009] and Zaharadnik et al. [2009] evaluated the toxic effects of a Probiora3® probiotic mixture on rats and humans and reported the absence of toxicity or adverse effects at the systemic level, as well as a high susceptibility to common antibiotics such as erythromycin and penicillin. Therefore, it is important to note that, aside from the results observed in this research, in the reduction of the levels of cariogenic bacteria, it is essential to obtain more evidence to explain the mechanism of action of streptococcal probiotics on the prevention of dental caries in preschool children.

Conclusion

In conclusion, when comparing the results from both study groups, we found a significant reduction of RLU values in preschool children who ingested the tested probiotics (Streptococcus uberis KJ2TM, Streptococcus oralis KJ3TM and Streptococcus rattus JH145TM), in relation to the baseline values and fifteen days after ceasing consumption. Furthermore, the reduction in cariogenic microbial counts was significantly higher in children from the experimental group.

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