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A comparative evaluation of efficacy of probiotic and chlorhexidine mouthrinses on gingival health and plaque accumulation in 6-9 year old children

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Abstract
Introduction: The probiotics have the ability to modulate or influence the micro flora in a beneficial way leading to its increased use in various areas of healthcare. It provides a more natural and a non-invasive solution for management of commonly occurring oral diseases.
Aim & Objectives: To clinically evaluate and compare the efficacy of probiotic and chlorhexidine mouthrinses on plaque and gingival inflammation in children.
Materials and Method: The trial design was randomized parallel group study comprised of 60 healthy children of age group of 6-9 years. The subjects were assigned into three groups (A- control, B- chlorhexidine, C- probiotic). Plaque index and gingival index levels were recorded at baseline, 3rd day and 14th day.
Results: The Probiotic and chlorhexidine group had less plaque and gingival accumulation at the end of 14th day. There was significant difference in the plaque as well as gingival index between the chlorhexidine and probiotic group, probiotic group, being better than the chlorhexidine (mean value: 0.86±0.24 and 0.66±0.14) $p<0.05$ respectively.
Conclusion: Probiotic mouth rinse was found effective in reducing plaque accumulation as well as gingival inflammation. Hence, Probiotic mouth rinses have shown promising potential as an alternative effective and potentially safe anti-plaque agent in comparison to chlorhexidine.

Keywords: probiotic, chlorhexidine, gingival inflammation, children

Introduction
The oral biofilm is naturally colonized by a plethora of microorganism which exists in a exceedingly dynamic relationship with the host [1] The breakdown of this symbiotic relationship (Dysbiosis) or apparently the shift from a mutualistic relationship to a parasitic one is the principle behind the various endogenous oral infections [2, 3]. Dental caries and gingivitis are the commonly occurring plaque evoked dental disease which are mostly initiated in childhood and thus early prevention is of a great importance [4]. Efficient plaque control strategies should involve correct understanding of the structural and pathophysiological properties of the oral biofilm and identifythe factors responsible for the alteration of the existing microbial balance [5]. Also, the plaque biofilm should be maintained at levels compatible with oral health, focusing on maintaining helpful resident micro flora which can contribute to health, hence reducing the disease risk [1].
For decades the mechanical plaque control has been the conventional method of plaque control, which is mostly dependent on the cooperation, motivation, and patient compliance, hence certain chemical modalities such as use of antibiotics and antiplaque agents are used to augment its action to achieve desirable results [6] The chemical method of plaque control which interfere with the composition and metabolism of oral biofilm had certain drawbacks such as increasing antimicrobial resistance, disrupting the stability of beneficial micro flora hence increase in the number of exogenous microorganisms [7]. These limitations have encouraged researchers and clinicians to focus on alternate solutions of plaque management which are closer to nature.
Probiotic are a group of beneficial bacteria which have shown encouraging results in varied areas of healthcare. Primarily related to gut health, probiotics have also proved successful in modulation of adaptive immune response, management of urogenital and respiratory tract

infections, and atopic allergies in infants. The ability of probiotic bacteria to stimulate the immune system exposed prospects of its beneficial use in sites remote from the gut. Oral cavity which forms the primary part of gastrointestinal tract is thus, thought to be conceivable to the benefits of probiotics. Early colonization by helpful group bacteria within the developing oral microbiota in infants can facilitate to prevent caries and confer good mucosal immunity, although long term research is still needed to document the effects of probiotic in children [9].

Even though positive outcomes have been observed concerning the role of probiotics in preventing oral disease, there is scarcity of data about direct recommendations for their usage and longterm benefits on oral health of children. Therefore, the present study was done to evaluate clinically and compare the efficacy of probiotic and chlorhexidine on plaque accumulation and gingivitis in children with age group of 6-9 years old.

Materials and Methods

Patient selection criteria for the present study were 60 healthy children in the age group of 6-9 years visiting the outpatient department of Pedodontics and Preventive Dentistry in Inderprastha Dental College and Hospital, Ghaziabad were included in the study. Healthy children without any known systemic illness, with no recent history of use of antimicrobial agents or any other drugs were included in study whereas any history of oral prophylaxis within past six months, children using any other commercially available moth rinse were excluded in the present study. If the children met the above criteria informed consent from the parents of children was obtained before the study was initiated. Ethical clearance was obtained from the institutional review board of the institution.

The subjects who fulfilled the required inclusion and exclusion criteria were randomly assigned into three groups as group A – control, group B- chlorhexidine and group C- probiotic with 20 children in each group.

Baseline scores of Plaque Index (PI) (Loe and Silness) [10] and Gingival Index (GI) (Loe and Silness, 1963) [11] were taken from all the participants followed by thorough oral prophylaxis. Each of the recipient groups were made to rinse with the designated mouth rinse. Subjects were instructed to brush twice daily with the given toothbrush and toothpaste (non-fluoridated). Group A individuals were given distilled water, group B was given chlorhexidine mouth wash 10 ml of 0.02% (Hexidine) for 60 sec followed by expectoration of the residual solution. Group C was instructed to use the probiotic mouth rinse. Probiotic mouth rinse was prepared using commercially available probiotic product Darolac (Aristo pharmaceuticals, India). As per the manufacturer details composition of Darolac powder used in the present study contained freezed dried bacterial combination comprised of Lactobacillus acidophilus, Lactobacillus rhamnosus, Bifidobacterium longum, and Saccharomyces boulardii (1.25 billion). The sachet contents were dissolved in 10 ml of distilled water and solution was stirred thoroughly which was used as a mouth rinse [12]. It was emphasized that the solution be freshly prepared and immediately used. Each mouth rinse was used by the children after 30 min of brushing (twice or once) under the strict supervision of the parents. On third day plaque index and gingival index were again taken. The oral health was assessed again at 14th day by taking the plaque and gingival score. The data was entered into Microsoft excel spread sheet and was analyzed by SPSS (21.0 version) (Chicago, IL U.S.A).The summarized data was presented using tables and graphs. The data was normally distributed as tested using the Shaperio-Wilk W test (p-value was more than 0.05) and analyses were performed using the parametric test i.e. the one-way analysis of variance (ANOVA). Level of statistical significance was set at p-value less than 0.05.

Results

Descriptive analysis was carried out among 60 subjects. The mean plaque index and gingival index level in control group at baseline, 3rd day and 14th day were found to be similar i.e. 2.44±0.34 whereas the mean values of plaque index and gingival index level in chlorhexidine group and probiotic group were found to be different at baseline, 3rd day and 14th day. (Table 1 and Figure 1, Table 2 and Figure 2) Comparison of plaque index and gingival index level among three groups at baseline, 3rd day and 14th day was carried out. The results were found to be significant at 3rd day and 14th day among the groups ($p<0.05$) At 14th day, the mean plaque index and gingival index levels were recorded maximum in control group i.e. 2.44±0.34, 2.44±0.35, followed by chlorhexidine group i.e.0.81±0.22, 0.86±0.24 and least in probiotic group i.e. 0.72±0.21, 0.66±0.14 respectively. (Table3 and Figure3) Post hoc analysis for intergroup comparison showed significant differences between control group and chlorhexidine group as well as control group and probiotic group. At 14th day, the mean plaque levels and gingival levels were recorded maximum in control group, followed by chlorhexidine and least in probiotic group (p>0.05) Hence significant differences were found between control group and probiotic group only. (Table 4)

Table 1: Descriptive analysis of mean and standard deviation (SD) of Plaque Index among 3 groups at baseline, 3rd day and 14thday

		Control		**Chlorhexidine**		**Probiotic**	
		MEAN	SD	MEAN	SD	MEAN	SD
1	Baseline	2.44	0.34	2.14	0.27	2.30	0.45
2	3rd day	2.44	0.34	1.49	0.26	1.57	0.42
3	14thday	2.44	0.34	0.81	0.22	0.72	0.21

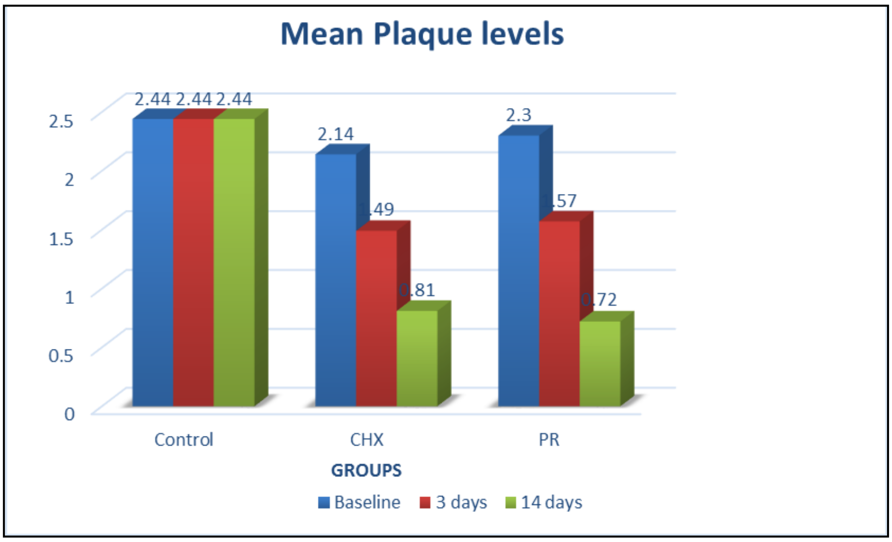


Fig 1: Mean Plaque Index among three groups at baseline, 3rd day and 14th day

Table 2: Descriptive of mean and standard deviation (SD) of Gingival Index among 3 groups at baseline, 3rd day and 14th day

		Control		**Chlorhexidine**		**Probiotic**	
		Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**
1	Baseline	2.44	0.35	1.98	0.31	2.08	0.46
2	3rd day	2.44	0.35	1.38	0.26	1.41	0.31
3	14th day	2.44	0.35	0.86	0.24	0.66	0.14

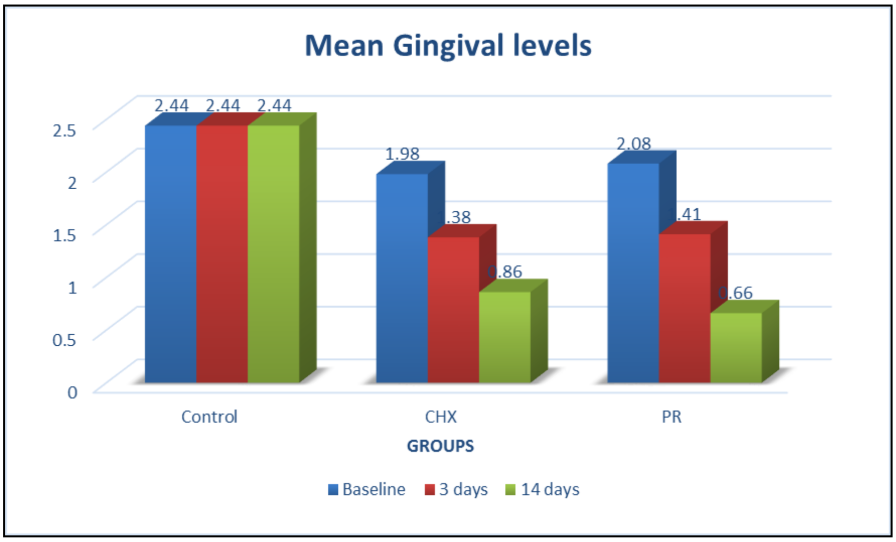
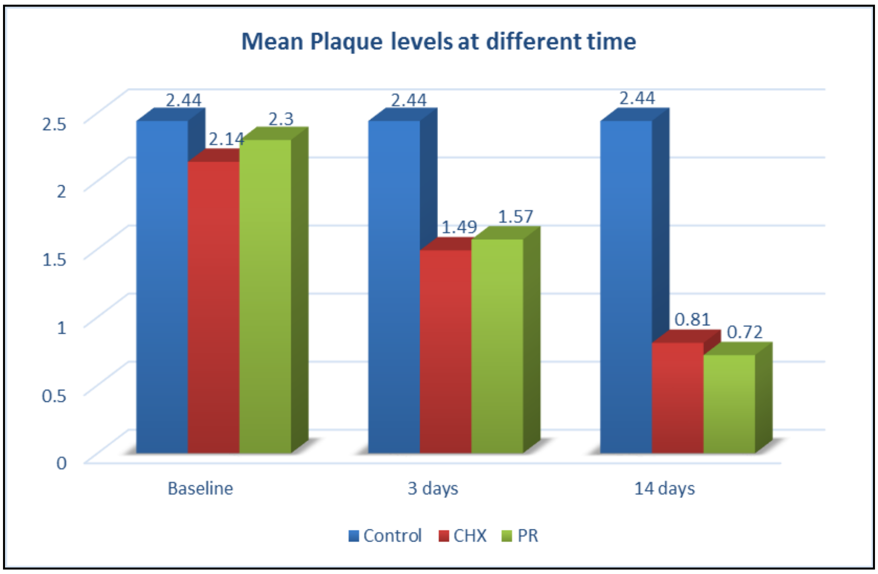


Fig 2: Mean Gingival Index among 3 groups at baseline, 3rd day and 14th day

Table 3: Comparison of mean and standard deviation (SD) of Plaque Index and Gingival Index among 3 groups at baseline, 3rd day and 14th day (Intra Group Comparison).

		Control		**Chlorhexidine**		**Probiotic**		**P value**
		Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	
Plaque Index	Baseline	2.44	0.34	2.14	0.27	2.30	0.45	0.093
	3rd day	2.44	0.34	1.49	0.26	1.57	0.42	0.00
	14th day	2.44	0.34	0.81	0.22	0.72	0.21	0.00*
Gingival Index		Control		Chlorhexidine		Probiotic		P value
		Mean	SD	Mean	SD	Mean	SD	
	Baseline	2.44	0.35	1.98	0.31	2.08	0.46	0.005*
	3rd day	2.44	0.35	1.38	0.24	1.41	0.31	0.00*
	14th day	2.44	0.35	0.86	0.24	0.66	0.14	0.00*

One way ANOVA, *Significance of relationship at $p < 0.05$



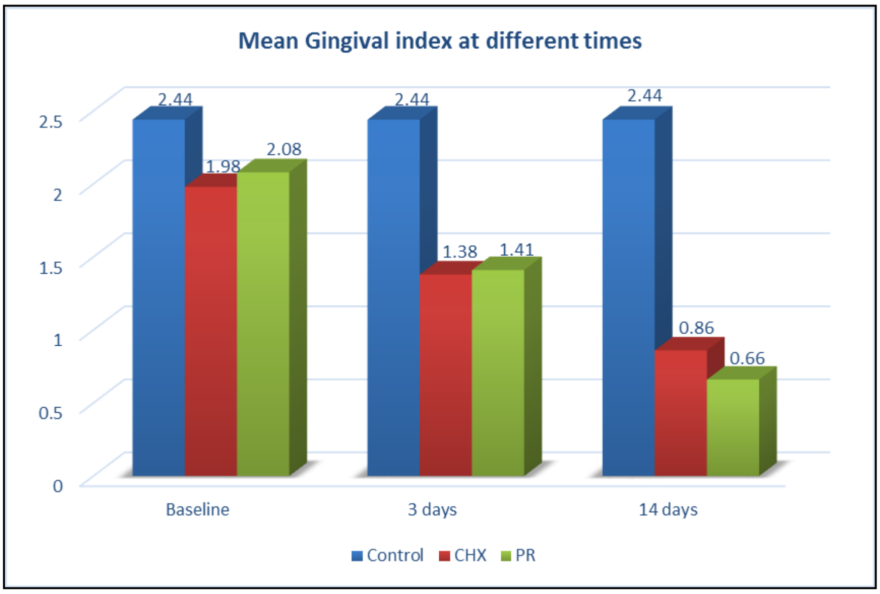


Fig 3: Mean and standard deviation (SD) of Plaque Index and Gingival Index among 3 groups at baseline, 3rd day and 14th day (Inter Group Comparison).

Table 4: Intergroup comparison of plaque index and gingival index among 3 groups at baseline, 3rdday and 14th day

			Control	**Chlorhexidine**	**Probiotic**
Plaque Index	**Baseline**	Control	-	-	-
		Chlorhexidine	-	-	-
		Probiotic	-	-	-
	3rd day	Control	-	0.00*	0.00*
		Chlorhexidine	-	-	0.808*
		Probiotic	-	-	
	10th day	Control	-	0.00*	0.00*
		Chlorhexidine	-	-	0.601*
		Probiotic	-	-	
Gingival Index	Baseline	Control	-	-	-
		Chlorhexidine	-	-	-
		Probiotic	-	-	-
	3rd day	Control	-	0.00*	0.00*
		Chlorhexidine	-	-	0.970*
		Probiotic	-	-	-
	10th day	Control	-	0.00*	0.00*
		Chlorhexidine	-	-	0.103*
		Probiotic	-	-	-

One way ANOVA, *Significance of relationship at $p < 0.05$

Discussion

Gingival and periodontal diseases are amongst the most commonly occurring diseases in children and adolescents [13] Virtually the foundation of periodontal diseases seen in adults is generally laid down in childhood, hence early diagnosis and prevention is imperative [14].

Dental plaque plays a vital role in dental diseases such as dental caries, gingivitis and periodontitis [6] A clinicians understanding of the ecology of the dental plaque, the complex microbial relationships, role of host immune reaction and the entire change process behind the occurrence of the oral disease is necessary to implement the successful management strategies. Mechanical plaque control in combination with topical antimicrobial chemotherapeutic has long been used to reduce plaque and gingivitis [15]. Antimicrobial mouth washes are a good home oral care adjunct which provides additional benefits to the daily brushing and flossing, however, increasing potential of rising antibiotic resistance with the long term and indiscriminate use of antibiotic /antimicrobial agent has been observed.[7] Chlorhexidine, a bisbiguanide, having a broad spectrum of action and good substantivity is considered a "gold standard" antimicrobial agent for plaque control. This most extensively studied, highly effective antiplaque and anti-gingivitis chemical plaque control agent is not devoid of its bound side effects. Long term use of chlorhexidine mouthwash can lead to brown discolorations of teeth and tongue, oral mucosa erosion and taste perturbations, rarely unilateral / bilateral parotid swellings are also seen. Oral discomfort in patients with chemotherapy-induced mucositis, xerostomia or ulcerative oral mucosal conditionshas also been observed [16, 17] With these inherent limitations, a great inclination towards use of natural remedies such as probiotics has been observed in previous years. In contrast to chemical agents, probiotics restore a balance between the oral flora hence, these group of beneficial bacteria contend with the harmful bacteria to attain a biological plaque control by eliminating the pathogenic micro-organism and have minimal or no adverse side effects when compared to chlorhexidine, also the immune system is beneficially modulated to achieve anti-inflammatory properties [18, 19]

Due to the promising results in various strata's of healthcare and wide range of application, the interest in probiotic application for oral health and disease management is steadily expanding and gaining interest [20]

The term probiotics was coined by Lilly and Stillwell in 1965 who described them as 'substances secreted by one organism which stimulate the growth of another'. [21] A consensus definition of probiotics was given by United Nations Food and Agriculture Organization and the World Health Organization in 2001 which defines probiotics as "live micro-organisms which, when administered in adequate amounts, confer a health benefit on the host." [22] These groups of health promoting bacteria exert their positive influence on the oral health through the following potential mode of action - normalization of the balance between the pathogenic and benefiting resident microflora, modulative the immune response, and metabolic pathways. Contending for the binding sites these microorganisms aim at blocking the epithelial adherence of the disease causing species. Further the growth and aggregation of the harmful bacterial species is prevented by release of antimicrobial factors such as bacteriocin, microcins, hydrogen peroxide, defensins and short chain fatty acids. etc. Inhibiting the pro-inflammatory molecules and increasing the mucosal immunity is contributed to the immune modulatory action. The interaction between the pathogenic bacteria is obstructed by blocking the "quorum sensing signals." [23]

The beneficial actions of probiotics are however strain and dose specific, the desired health benefit should determine and verify the selection for the individual probiotic. Every strain ought to be analyzed individually to assess their health edges [24].

Most commonly used probiotics are Lactobacillus and Bifidobacterium species [25]. These bacteria are found in fermented dairy products and colonize gastro-intestinal tract soon after birth. Probiotics have been made commonly available in various formulations such as milk and its products like yogurt, cheese, as dietary supplements (non-dairy products) such as powder, capsule, gelatin tablets, as culture concentrates added to a beverage or food (such as a fruit juice) or inoculated into probiotic fibers [26]. In the present study a commercially available probiotic preparation Darolac (Aristo Pharmaceuticals, India) containing 1 g powder of 1.25 billion freeze-dried bacterial combination (a mixture of, Lactobacillus acidophilus, Lactobacillus rhamnosus, Bifidobacterium longum, and Saccharomyces cereviasae was used to prepare probiotic mouth rinse.

The Present study was conducted with the aim to evaluate and compare the efficacy of probiotic and chlorhehexidine mouth rinse in reducing plaque accumulation and gingival inflammation in children. A 14 day randomized placebo controlled trial conducted in 60 healthy children in the age group of 6- 9 years were randomly allocated in three groups the placebo, chlorhexidine and probiotic group respectively. Oral health assessment was done at 3rd and 14th day of the study. The mean value of plaque index and gingival index was 1.05 ± 0.54, 0.17 ±0.47 in control group, 1.31±0.57 and 0.61±0.51 in chlorhexidine group whereas 1.64±0.55, 1.34±0.58 in probiotic group. Therefore, statistically significant reduction of plaque levels and gingival inflammation was observed more in the probiotic group when compared to chlorhexidine and control group mouth rinses at the end of 14th day.

Efficacy of probiotic mouthrinses in reducing plaque accumulation and gingivitis in children has been explored in various comparative studies. A study done by Purunaik *et al* among 90 school children aged 15–16 years to evaluate efficacy of probiotic mouthrinses (1 g powder of 1.25 billion freeze dried combination, a mixture of L. acidophilus, L. rhamnosus, B. longum and S. boulardii and chlorhexidine mouth rinses on plaque and gingival scores demonstrated that probiotic had comparable efficacy to chlorhexidine mouthrinses and caused significant reduction in plaque and gingival score [27]. In a similar study done by Shah RK on healthy children in the age group of 6- 10 years using probiotic, chlorhexidine, fluoride mouthwashes and comparing the gingival and plaque status after 28 days, found that probiotic group to be equally effective in the prevention of plaque formation and reducing gingivitis and thus proposed probiotic to be good alternative therapeutic agent to other antibacterial mouthrinses [28] Probiotic group of mouthrinses were observed to be better over chlorhexidine group in reducing plaque formation and incidence of gingivitis in another study done by Harini PM and Anegundi RT in 2010 in pediatric population of the age group 6-8 years where a significant difference in the Gingival Index between the Probiotic and the Chlorhexidine groups was observed.[29] However in our study significant reduction in both gingival and plaque index was observed.

Thakkar PK in (2013) conducted a study in children and allocated them into three groups-the placebo, chlorhexidine, and probiotic groups it was found that Probiotic mouth rinse was more effective against plaque accumulation both at 14 days of study and 3 weeks after discontinuation of probiotics [12]. The result of the current study reveal that probiotic group of mouthrinses had a significant reduction on plaque accumulation and gingival inflammation when compared with chlorhexidine and placebo group of mouthrinses. Probiotics in the form of mouth rinse had been tested among adults in another study by Noordin K in 2007 and concluded that rinsing with probiotic mouth rinse resulted in a significant reduction of plaque accumulation and gingival inflammation which is similar to our study [30].

Probiotics are thought to play an antagonist action against S. mutans and other caries causing acidogenic/acidouric bacteria. Different strains of probiotics in various other forms such as milk, lozenges, ice cream, yogurt etc have been studied. A significant dental caries reduction was found in 3- to 4-year-old children after 7 months of daily consumption of probiotic milk which contain probiotic bacterium, Lactobacillus rhamnosus G in a study conducted by Nase L *et al* in 2001. [31] Taipale T, *et al* in his study on infant s concluded that permanent colonization of the probiotic in the oral cavity is not definitive even when it's early administered in infancy [32]. The fact that probiotic action is strain specific and may differ individually and also the short term or temporary colonization of the host by the probiotic bacteria necessitates the need for a lot of research in this area. Follow up studies are needed to ensure its long term effects, the specific probiotic strain for the specific disease, age according dosage and frequency of supplementation.

Conclusion

Recent years have witnessed a lot of research to prove the efficacy of probiotics in oral health although there is scarcity of studies in children. Extensive research is recommended to determine the strain of probiotics, the appropriate vehicle and amount of probiotic administration so, that it can be effectively used and recommended by clinicians as an effective tool in plaque control and maintaining oral health.

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