

## Evaluating the effect of probiotics on salivary microflora: A clinical controlled trail

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### Abstract

**Objective:** Probiotics are dietary supplements of live microorganisms thought to be healthy for the host organism. The aim of the study was to investigate the effect of probiotic administered in the form of multi-strain suspension on the salivary levels of *Streptococcus mutans* and *Lactobacilli* in children of mixed dentition period.

**Materials and Methods:** A randomized, double-blind, placebo study was done. Saliva samples were collected at baseline and after 15 days of the study, microbial cultures were done to count the number of colony forming unit. This data was tabulated and analyzed.

**Result:** There was a significant reduction in *Streptococcus mutans* count in the study group after 15 days as compared to its initial count and placebo group. But no significant difference was observed in *Lactobacilli* count between the two groups. Probiotics reduced the salivary levels of *Streptococcus mutans* in children of mixed dentition period.

**Conclusion:** The application of probiotics may in the near future, provide the end of new cavities.

**Keywords:** Probiotics, Randomized, Salivary, *Streptococcus mutans* and *Lactobacilli*.

### Introduction

Probiotics, literally meaning “for life” are microorganisms proven to exert health promoting influences in human.<sup>1</sup> The term “probiotic” was first used in 1965, by Lilly and Stillwell, to describe substances secreted by one organism which stimulate the growth of another.<sup>2</sup> Probiotics are live microorganisms (in most cases, bacteria) that are similar to beneficial microorganisms found in the human gut. They are also called - “Friendly bacteria” or “Good bacteria”.

The use of antibiotics, immunosuppressive therapy and irradiation, amongst other means of treatment, may cause alterations in the composition and have an effect on the Gastro intestinal flora.<sup>3</sup> Therefore, the introduction of beneficial bacterial species to GI tract may be a very attractive option to re-establish the microbial equilibrium and prevent disease.<sup>4</sup> *Lactobacillus rhamnosus* strain GG has proven beneficial effects on immunity. It increases the number of IgA and other immunoglobulin secreting cells in the intestinal mucosa and even increases salivary IgA levels.<sup>5</sup> It also stimulates local release of interferons.<sup>6</sup>

Probiotics has made its way in treatment of number of conditions ranging from infantile diarrhoea, necrotizing enterocolitis, inflammatory bowel disease to cancer, female uro-genital infection and surgical infections.<sup>7</sup>

Probiotics are available to consumers even in the form of non-dairy products example- tablets, straws, suspension etc. They can be used as complementary and alternative medicine (CAM). The WHO deemed probiotics to be the next most important immune defense system when commonly prescribed antibiotics are rendered useless by antibiotic resistance.<sup>8</sup>

The most commonly used strains belong to the genera *Lactobacillus* and *Bifidobacterium* that are commonly found in the oral cavity, including carious lesions. Numerous studies have been performed with different probiotic bacteria in the intestine, as the same *Lactobacillus* species are found on both rectal and oral mucosa and most of the

probiotic products are consumed orally, it is feasible that the consumed probiotic bacteria also attach to oral surfaces.<sup>9-11</sup>

Many studies on probiotics have been done earlier, but only a few has been done on children, however no study has been done in children of mixed dentition age. Therefore the aim of this study was to investigate the effect of probiotic administered in the form of multi-strain suspension containing *Lactobacillus rhamnosus*, *Bifidobacterium longum*, and *Saccharomyces cerevisiae*<sup>12</sup> on the salivary levels of *Streptococcus mutans* and *Lactobacilli* in children of mixed dentition period.

### Materials and Methods

The study was a randomized, double-blind, placebo design over a time period of 15 days. Twenty out of twenty five healthy children were randomly selected, who were residing in the orphanage and having high salivary *Streptococcus mutans* count. The age range of all the children was 6 to 12 years. All the children were living in same environmental conditions (orphanage), with caries score (DMFT) of at least one and having no history of any antibiotic or any fluoride intake for past 2 weeks. The ethical committee approval and the written consent from the officials of the orphanage were obtained prior to the onset of the study.

Before starting the study, the caries and plaque scores of all the children were recorded by an independent trained examiner. Following the screening examination, their tooth brushing habit was evaluated and modified during one week of preparatory period to establish healthy conditions. They were refrained from the use of any fluoridated products and any other oral hygiene aids except brushing.

The children were then randomly divided into two groups i.e. ten in the placebo group and ten in the study group. The children under study group were asked to place the probiotic suspension containing 1gm powder of 1.25 billion freeze dried bacterial combination of *Lactobacillus rhamnosus*, *Bifidobacterium longum*, and *Saccharomyces*

*cereviasae* mixed in 20ml of water in the oral cavity for 1 min and then swallow.<sup>12</sup> The children under placebo group were asked to keep water in oral cavity for 1 min and then swallow. This procedure was repeated twice a day for 15 days. No brushing was allowed for at least 1 hour after swallowing. The caries index (DMFT index) and Plaque index sillness and loe, 1964<sup>13</sup> scores were recorded again at the end of 15<sup>th</sup> day.

### Saliva sampling

Samplings were obtained using paraffin stimulated whole saliva during morning hours, before and after 15 days trial. The saliva was collected directly in sterile containers of Hi-media and transferred directly to culture plates.

The laboratory procedure was as follows: Using an inoculation loop (4 mm inner diameter) 10 µl of the vortexed 1:5 dilution sample was streaked in duplicate on Mitis salivarius bacitracin agar (MSB) selective for *Streptococcus mutans* and on Rogosa SL agar for *Lactobacilli*. The MSB agar plates were incubated anaerobically for 48 hours at 37°C. The Rogosa SL agar plates were incubated anaerobically for 96 hours at 37°C. Following incubation, counts were made of colonies with morphological characteristic for *Streptococcus mutans* on the MSB agar and of colonies exhibiting the typical morphology of *Lactobacilli* on Rogosa SL agar.<sup>14,15</sup> Identification for *Streptococcus mutans* was confirmed by biochemical tests like mannitol and sorbitol fermentation and catalase test. Gram staining was also performed. Catalase test and Gram staining confirmed the identity of *Lactobacilli*. The colony count of each petri dish was recorded and the mean colony forming units per milliliter was determined after multiplying the colony count of each plate with its respective dilution factor.

### Statistical analysis

The data collected were statistically analyzed & tabulated using SPSS 12. ANOVA and paired-t test were performed to evaluate the intergroup comparison and P-value of less than 0.05 was considered statistically significant.

### Results

Twenty out of twenty five healthy children were randomly selected, having high salivary *Streptococcus mutant* count (>10<sup>5</sup>) of 6 to 12 years of age. Children had high mutans count and mean DMFT score was 3.7 and 3.5 in study and placebo group respectively at baseline. The null hypothesis was that the probiotics in the form of multi-strain suspension would not affect the level of salivary *Streptococcus mutans* and *Lactobacilli*.

The study showed a significant reduction in *Streptococcus mutans* (s) final count (f) in the study group (S) after 15 days as compared to its initial count (i) (p=0.000). [Table 1] Whereas in placebo group (C) it remained almost same or there was a slight increase in mutans count (p=.859) which was statistically non-significant. After 15 days post treatment with probiotic

supplement there was significant reduction in *Streptococcus mutans* count in study group as compared to placebo group.

Salivary *Lactobacilli* (l) showed a slight increase in its count in study group after 15 days although levels remained almost same in placebo group.

The mean plaque score significantly reduced in study group after 15 days post treatment, whereas it remained almost same in placebo group.

The trend was different in study group as compared to placebo group which shows the effect of probiotics on oral microflora after 15 days period.

### Discussion

Dental caries is a complex disease. From the 1950s to 60s, the landmark studies of the 'Keyes and Fitzgerald revolution'<sup>16</sup> proved the strong causal relationship of certain specific microorganisms such as streptococci, lactobacilli, and actinomyces present in the dental plaque with the incidence of caries. *Streptococcus mutans* and *Lactobacilli* are considered crucial for the initiation and progression of dental caries.

A study investigated the effect of probiotics on levels of salivary *Streptococcus mutans* and *Lactobacillus* in young adult by two non-dairy delivery systems that is tablets and straws and found a significant reduction in *Streptococcus mutans* count (p=.000), which was similar to our study. Thus showed that a direct contact with oral tissue may not be a pre-requisite for beneficial effects.<sup>9</sup> A similar but conflicting finding was reported by Montatlo M in a study where oral or systemic administration of probiotics could change cariogenic microflora and found that both could enhance *Lactobacillus* proliferation (p = 0.005 and p = 0.02, respectively).<sup>17</sup>

A randomized, double-blind, placebo-controlled study involving 20 healthy young women was done by Calgar E. In which the study subjects sucked the *Lactobacilli* –derived probiotics delivered via a medical device once daily for 10 days, while the control subjects received placebo medical devices without bacteria. Salivary *S. mutans* levels in the probiotic test group were significantly reduced, with statistical significance of reduction (P < 0.05).<sup>18</sup>

The results of the present study indicate the significant reduction in mutans count (p=.000) which was similar to findings of the study designed to examine whether milk containing *Lactobacillus rhamnosus* has an effect on caries when compared with normal milk. The children received the milk with meals from coded containers 5 days a week in the day-care centres for 7 months. The result showed beneficial effect of long term consumption of probiotics that is milk containing *Lactobacillus rhamnosus* on dental caries in 3-4 year old day care children.<sup>19</sup> However, these findings were in contrast to study where statistically insignificant difference was found between the groups in *Streptococcus mutans* counts after the intervention, by short term consumption of probiotics containing cheese on cariogenic microflora. However, *Streptococcus mutans* counts decreased in 20% (P=0.01) (OR=0.37, 95% CI 0.08-1.75, P=0.21).<sup>19</sup>

**Table 1:** Distribution of Streptococcus mutans count and Lactobacilli count at baseline and after 15 days trail. (in CFU/ML)

Initial count(i) (CFU/ML)	Streptococcus mutans (s)		P-value	Lactobacilli count (l)		P-value
	Study group N=10	Placebo group N=10		Study group N=10	Placebo group N=10	
10 <sup>5</sup>	0	0	.016	1	0	.487
10 <sup>6</sup>	8	9		9	10	
10 <sup>7</sup>	2	1		0	0	
Final Count(f) (CFU/ML)	Study group N=10	Placebo group N=10	Final Count	Study Group N=10	Placebo group N=10	P-value
10 <sup>5</sup>	6	0		0	0	
10 <sup>6</sup>	4	9	7	10		
10 <sup>7</sup>	0	1	3	0		
P-value	0.000	0.859		0.000	0.768	

The present study showed that probiotic intervention to be beneficial especially to those with high mutans count. The *Streptococcus mutans* count was found to be statistically reduced (p=.000) in study group as compared to placebo group (p=.859) during the post treatment period.

### Conclusion

Probiotic administered in the form of multi-strain suspension reduced the salivary levels of *Streptococcus mutans* in children of mixed dentition period. Given appropriate release mechanisms, some of these technologies may be parenterally administered to treat life-threatening infections and emerging drug-resistant organisms.<sup>21</sup>

Probiotic strategies are part of the continuing evolution of the treatment of oral infection that produces the clinical manifestations of dental caries. Bacteriotherapy in the form of probiotics seems to be a natural way to maintain health and protect oral tissues from disease. Data suggest that potential benefits increases as early childhood start.<sup>22,23</sup> The application of probiotics may in the near future, provide the end of new cavities.

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None.

### Conflict of interest

None.

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