ORIGINAL ARTICLE

Assessment of the effect of probiotic curd consumption on salivary pH and *streptococcus mutans* counts

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ABSTRACT

Background: Antimicrobial methods of controlling dental caries that include probiotic agents can play a valuable role in establishing caries control in children at moderate to high risk for developing dental caries. Several studies have demonstrated the beneficial effects of use of various Probiotic products including curd. The objective of this study was to compare the effect of short-term consumption of probiotic curd containing Lactobacillus acidophilus and normal curd on salivary *Streptococcus Mutans* counts, as well as salivary pH. **Materials and Methods:** Forty, caries-free, 10-12 years old children were selected and randomly allocated to two groups. Test Group consisted of 20 children who consumed 200ml of probiotic curd daily for 30 days. Control Group consisted of 20 children who were given 200ml of regular curd for 30 days. Salivary pH and salivary *Streptococcus Mutans* counts were recorded at baseline and after 30 days and statistically compared using the Student's *t-test*. **Results:** Consumption of probiotic curd resulted in a statistically significant reduction in *S. Mutans* colony counts ($P<0.001$) as compared to regular curd. However, there was a slight reduction in pH ($P>0.05$) in both the groups. **Conclusion:** Short-term consumption of probiotic curds can reduce oral *S. Mutans* counts. However, this caused a slight reduction in salivary pH.

Key words: Dental caries, probiotic, curd, salivary pH, *streptococcus mutans*

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INTRODUCTION

Dental caries and periodontal diseases occur in nearly 95% of the population and are bacterially mediated processes.[1] In children at moderate to high risk for dental caries the predominantly surgical approach needs to be combined with an antimicrobial agent to ensure adequate control of the caries process. Several antibiotic or antimicrobial agents have been tested including Chlorhexidine, povidone-iodine, and fluoride mouthrinses.[2]

Another approach to bacterially- mediated diseases has been the use of Probiotics which has been defined as 'bacterial cultures or living micro-organisms which upon ingestion in certain numbers, exert health benefits beyond inherent general nutrition and support a good healthy intestinal bacterial flora.' The idea was that the harmless bacteria in the fermented products competed with pathogens injurious to health. A wide range of probiotic products containing different bacterial strains are commercially available such as lozenges, sucking tablets, chewing gums, dairy products such as milk, ice cream, cheese, yoghurt, etc.[1] The archetypical probiotic food is yoghurt (curd) and daily consumption of dairy products seems to be most natural way to ingest probiotic bacteria.[3] Dairy yoghurt is produced using a culture of *lactobacillus delbrueckii* subsp. *bulgaricus* and *streptococcus salivarius* subsp. *thermophilus* bacteria. In addition, *lactobacillus acidophilus*, *lactobacillus bifidus* and *lactobacillus casei* are also sometimes used in culturing yoghurt.

Recent studies have shown that certain gut bacteria including *lactobacilli* and *bifidobacterium* may exert beneficial effects in the oral cavity by inhibiting cariogenic streptococci and *Candida* species.[1]

In this study, the probiotic yogurt (curd) containing lactobacillus acidophilus has been used to investigate its effects on salivary pH and *streptococcus mutans* counts. These effects have been compared with those of normal curds.

Access this article online	
Quick Response Code:	Website: www.nigeriamedj.com
	DOI: 10.4103/0300-1652.104382

MATERIALS AND METHODS

This study was carried out in 40 healthy caries-free school children aged 10-12 years. A complete oral examination

was also carried out using the WHO format after obtaining an informed consent.

Using simple random sampling (lottery method) the samples were allocated to either Test or Control groups.

Inclusion criteria for selection of samples

1. Forty healthy children in the age group of 10-12 years
2. Caries free Children (d m f t = 0)

Exclusion criteria

1. Children with decayed teeth
2. Children under antibiotic treatment during the course of the study
3. Children on any other probiotic supplements during the course of the study
4. Children on use of any xylitol products for three weeks before and during the course of the study

Materials used

1. Basic examination kit consisting of disposable mouth mask and gloves, mouth explorer, tweezers and sterile cotton
2. Nestle dahi (curd) with probiotic
3. Nestle fresh 'n' natural dahi (curd)
4. Ependorff tubes
5. Digital pH meter
6. Mitis salivarius bacitracin (MSB) agar (hi-media)
7. Calibrated inoculating loop and bunsen burner
8. Micro slides
9. 1ml and 10 ml pipettes
10. Incubator
11. Autoclave

Procedure

Sampling of Saliva

Paraffin stimulated whole saliva was collected from all forty children in the morning on the Day 1 (baseline sample) and 30 days after the intervention period. The saliva was expectorated directly into ependorff tubes during a 5 min chewing period after a thorough rinse with water.

Estimation of salivary pH

A digital pH meter analyzed the collected saliva**.**

Estimation of *s. mutans* count

A serial dilution of the collected salivary sample was done in saline. Using inoculating loop the diluted salivary sample was streaked on mitis salivarius bacitracin agar (MSB) selective media for *S. mutans*. The plates were incubated at 37°C for 36-48 h and the number of colonies was counted based on the colony characteristics [Figure 1] and confirmed by gram staining. [Figure 2]

The children were then randomly divided into two groups of 20 each. Operators were blinded, they were not aware of which yoghurt was given to them.

Test group: Consisted of children who were given probiotic curd.

Control group: Consisted of children who were given normal curd.

Both the groups were given curds (probiotic or normal) for 1 month. After 30 days of consumption of curds, salivary samples were collected and tested for salivary pH and salivary *S. mutans* count was estimated. The results were then tabulated and were statistically analyzed.

Statistical Analysis

Excel and SPSS (SPSS Inc, Chicago) software packages were used for data entry and analysis. The results were averaged (mean + standard deviation) for each parameter and are presented in Tables 1 & 2 and Figures 3 & 4.

RESULTS

In the control group, the mean pH values were higher at baseline compared to the mean pH at 30 days. A statistically significant reduction in the mean pH from baseline to 30 days was observed [Table 1].

In the test group, the mean pH values were higher at baseline compared to the mean pH at 30 days. A statistically significant reduction in the mean pH from baseline to 30 days was observed [Table 1].

At 30 days time interval, the mean pH value in test group was slightly higher compared to the Control Group but

Table 1: Comparison of the pH between test group and control group at baseline and at 30 days interval

	pH at baseline	pH at 30 Days	Mean difference	t	*P* value
Test group	7.13	6.72	0.434	6.607	<0.001*
Control group	7.06	6.63	0.410	5.208	<0.001*

*Denotes a significant difference; Mean±SD

Table 2: Comparison of pH between test group and control group at 30 days interval

	Mean	Std dev.	Mean difference	t	*P* value
Test group	6.72±0.32	0.32			
Control group	6.63±0.37	0.37	0.096	0.877	0.386

this difference was not statistically significant (P>0.05) [Table 2].

The mean colony count in Control Group at baseline was higher when compared with the mean colony count at 30-day time interval. But this reduction in mean colony count was not statistically significant (P>0.05) [Table 3].

The mean colony count in Test Group at baseline was higher when compared with the mean colony count at 30-day time interval. This reduction in mean colony count was found to be statistically significant (P<0.01) [Table 3].

At 30 days time interval, the mean number of colonies was found to be higher in Control Group compared to Test Group and this difference was found to be statistically significant (P<0.05) [Table 4].

Figure 1: MSB agar with *streptococcus mutans* growth

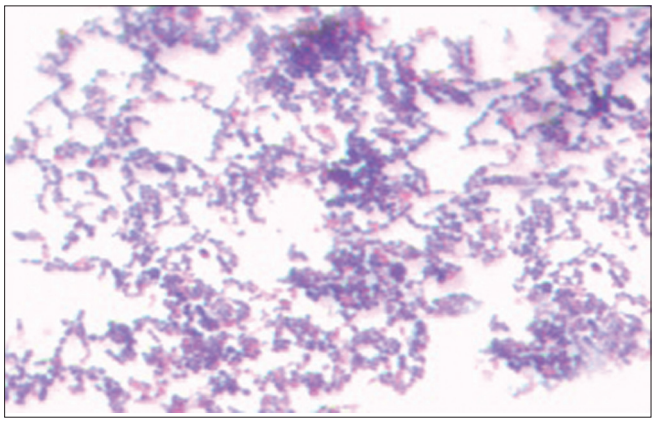

Figure 2: Gram stained *streptococcus mutans*

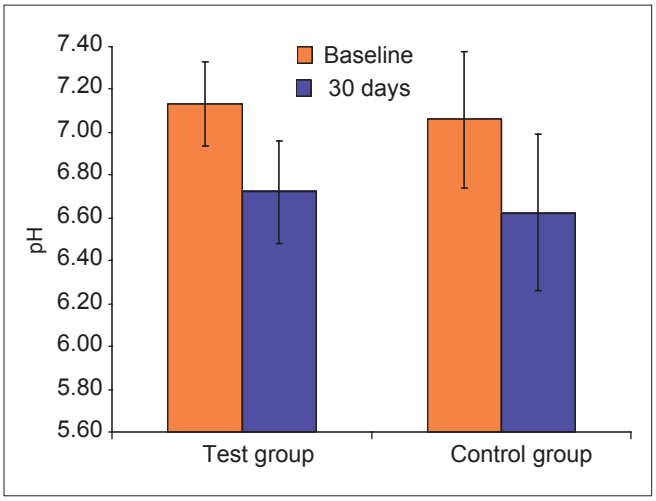

Figure 3: Mean pH values in the test group and control group at baseline and 30 days interval

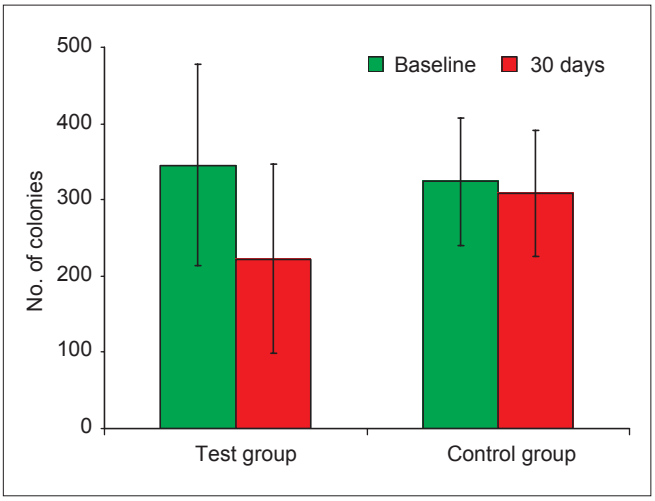

Figure 4: Mean number of colonies in test group and control group at baseline and 30 days interval

Table 3: Comparison of the number of colonies between test group and control group at baseline and at 30 days interval

	Colony count at baseline	Colony count at 30 days	Mean difference	t	*P* value
Test group	345.55±131.84	222.65±124.04	122.900	3.036	0.004*
Control group	323.85±83.15	309.15±82.61	14.700	0.561	0.578

* Denotes a significant difference; Mean±SD

Table 4: Comparison of the number of colonies between test group and control group at 30 days interval

	Mean	Std dev.	Mean difference	t	*P* value
Test group	222.65	124.04			
Control group	309.15	82.61	-86.500	-2.596	0.013

DISCUSSION

Dental caries being a multifactorial disease process often requires a multimodal approach to treatment and control. One of the antimicrobial approaches has been the use of probiotic-enriched products such as milk, ice cream, lozenges, cheese and yoghurt. Probiotic products contain living microorganisms such as *bifidobacterium* and *lactobacilli*. Several of these normal dietary constituents contain microorganisms, which can function as probiotic agents. However, these specially formulated probiotic products contain around 10^{10} to 10^{11} colony forming units of microorganisms per milliliter. The hypothesized mechanisms of action include (a) direct interactions in the dental plaque with interference of biofilm formation, plaque ecology, competing with oral microbes for the available substrate and production of antimicrobial substances and (b) Indirect actions including modulation of systemic immune function, local immunity, effect on nonimmunologic defense mechanisms, regulation of mucosal permeability and oral colonization by less pathogenic species.[4] The present study was conducted to investigate the inhibitory effect of short term consumption of probiotic curd containing lactobacillus acidophilus on salivary *streptococcus mutans* counts, its effect on salivary pH value and to compare this with normal curds in caries-free children.

Curds (Nestle fresh 'n' natural dahi) was selected as it is a common dietary constituent of the Indian population, is readily available, inexpensive and can be used in several different combinations that are acceptable to the paediatric age group. Unlike milk and milk products, curd being semisolid may be retained in the oral cavity for a longer period of time with an extended beneficial effect. Further, this study aims to compare the relative effects of regular curd with a specially formulated probiotic curd (Nestle dahi with probiotic) that contains 10^{10} colony forming units per milliliter of lactobacillus acidophilus. Several studies have shown that *lactobacilli* may inhibit the growth of other bacteria including *streptococci*[5] and *lactobacilli*[6] by producing low molecular weight bacteriocins.[7]

There have been no documented studies correlating the effect of probiotic containing curd on salivary pH. In the present study, salivary samples were used to determine any change in pH and *S. mutans* counts on the day before onset (baseline) and 30 days after the intervention period (consumption of probiotic curds). The assessment was therefore of the impact of probiotic, as well as normal curd on the general ecology of the oral cavity rather than a specific plaque evaluation.

On comparison of mean salivary pH in the Control Group, as well as the Test Group, a statistically significant lowering of mean pH value was seen at 30 days interval as compared to baseline [Table 1].

At 30 days interval, the mean pH value in Test Group was slightly higher as compared to the Control Group but this difference was not statistically significant [Table 2].

This decrease in salivary pH on consumption of both probiotic containing curd and normal curd was primarily due to the acidic nature of the curds. However, the pH levels were still above the critical pH (5.2-5.5), which otherwise could have been considered as harmful for the causation or progression of dental caries. This further proves that curd though acidic in nature does not pose any risk for caries active children. Conversely it may prove beneficial due to its calcium, phosphorous, protein and vitamin content.

Streptococcus mutans has been implicated as one of the major and most virulent of the caries-producing organisms. Therefore, the effect of probiotic curd containing lactobacillus acidophilus on *S. mutans* count was looked into.

In this study, the *S. mutans* colony counts in the Test Group at 30-day time interval was significantly lower than at baseline. In the Control Group a slight but statistically insignificant reduction was observed [Table 4]. The results are in accordance to other studies which support the use of probiotics against salivary *streptococcus mutans*, oral malodour and other oral infections like *candida albicans*.[8-16] This effect against *streptococcus mutans* has been attributed to the general antimicrobial activity of curd rather than a specific competitive activity of curd microorganisms.6[7] Due to the paucity of studies using curd/yoghurt a definite conclusion on the possible mechanism of action in reducing cariogenic bacteria could not be drawn from the present study. Further studies need to be conducted to investigate the effect of probiotic agents on oral ecology.

Lactobacilli have been implicated in the progression of dental caries especially in dentin. However, the use of *lactobacillus acidophilus* based milk product has been justified due to the good buffering capacity of milk, presence of tooth protective minerals including calcium.[17] However, the use of these products has been contraindicated in children with open carious lesions.[3] Also in patients on long term use of probiotics monitoring of oral health becomes important along with lactobacilli counts.[18] Most antimicrobial strategies are effective only as long as the patient is on the treatment. Following cessation of the intervention the oral bacterial counts rapidly return to the original counts. In this regard probiotics may prove to be beneficial especially when combined with a modification in dietary patterns along with effective restorative treatments.

Further studies need to be done in the use of these probiotic products in children with varying degrees of

caries risk over a longer period of time. Monitoring of plaque microbial counts may provide additional indicators of the ecological changes that may occur with use of these products.

CONCLUSION

The following conclusions can be drawn from the present study:

- Probiotic curds containing lactobacillus acidophilus was found to have an inhibitory effect on the salivary *streptococcus mutans* counts
- The consumption of normal curd and probiotic curd caused a small decrease in salivary pH that is still above the critical pH level
- Probiotic curds containing lactobacillus acidophilus may be used as adjuncts, for prevention of dental caries especially as a part of dietary modification in children at varying risk for dental caries.

ACKNOWLEDGMENT

The Authors would like to acknowledge the help rendered by Mr. Thejasvi Venkateshmurthy from Statintelligence network.

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How to cite this article: Sudhir R, Praveen P, Anantharaj A, Venkataraghavan K. Assessment of the effect of probiotic curd consumption on salivary pH and *streptococcus* mutans counts. Niger Med J 2012;53:135-9.

Source of Support: Nil, **Conflict of Interest:** None declared.