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Infectious Disease

Antimicrobial efficacy of Xylitol, Probiotic and Chlorhexidine mouth rinses among children and elderly population at high risk for dental caries – A Randomized Controlled Trial

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Keywords

Probiotics • Xylitol • Streptococcus mutans • Dental caries

Summary

Introduction. *Chlorhexidine is considered the most potent chemotherapeutic agent against Streptococcus mutans. However, its side effects due to prolonged use, indicates need for alternatives. The study intended to assess and compare antimicrobial efficacies of probiotic, xylitol and chlorhexidine mouth rinses in children and elderly.*

Methods. *The study was a Double blind Randomized Controlled Trial conducted among residential school children aged 5-12 years and elderly greater than 60 years residing in old age homes. (ClinicalTrials.gov ID: NCT04399161). 30 participants each among children and elderly were chosen based on eligibility criterion (high risk for caries). They were further randomly divided into 3 groups with 10 participants in each group. Participants were asked to rinse with 15 ml of freshly prepared mouth rinses once daily for 2 minutes for 14 days. Antimicrobial efficacy was determined by assessing change in Streptococcus mutans levels in dental plaque.*

Results. *Significant reduction in Streptococcus mutans counts were observed in both children and elderly (Chlorhexidine: mean difference = 3.11 log10CFU/g, p = 0.022, Xylitol: mean difference = 0.93 log10CFU/g, p = 0.046, Probiotic: mean difference = 1.91 log10CFU/g, p = 0.023 in children); (Chlorhexidine: mean difference = 2.23 log10CFU/g, p = 0.004, Xylitol: mean difference = 1.39 log10CFU/g, p = 0.009, Probiotic: mean difference = 1.61 log10CFU/g, p = 0.018 in elderly). Intergroup comparison showed no significant difference.*

Conclusions. *Antimicrobial efficacy of xylitol and probiotic mouth rinses were comparable to that of chlorhexidine in both children and elderly. Probiotics could potentially be more efficacious than xylitol among children.*

Introduction

WHO defines dental caries as a localized, post eruptive pathological process of extreme origin involving softening of the hard tooth tissue and proceeding to the formation of cavity. The process involves bacterial interactions in plaque accumulated on the surface of the teeth. *Streptococcus mutans* in plaque is the most commonly isolated organism amidst all other cariogens. It ferments sucrose and the resulting acid causes demineralization of tooth enamel [1].

While mechanical methods of plaque control can maintain adequate oral hygiene, such methods are not being used appropriately. This necessitates use of adjuncts to mechanical plaque control methods [2]. Chlorhexidine mouth rinse has been considered the most effective agent in inhibiting *Streptococcus mutans* [3]. However, adverse effects due to prolonged use such as staining of teeth, xerostomia, altered taste sensation, mouth/ throat irritation, antimicrobial resistance, etc. has necessitated research on other alternatives.

Xylitol used as an artificial sweetener in foods, cannot be metabolized by oral bacteria thereby contributing to caries prevention. Adverse effects, as reported in studies, are due to consumption in large quantities and its magnitude, as compared to that of chlorhexidine, is indistinct. Although studies have assessed effects of xylitol chewing gum on caries, there is less literature on its efficacy as a mouth rinse [4].

Probiotics are live microorganisms that, when administered in adequate amounts, confer a health benefit on the host. Probiotic products seem to have an effect on the oral health of individuals by prompting beneficial bacteria to defend teeth and gums against harmful ones. There have been no issues of antibiotic resistance, as they contains only commensal flora and there has been no proof of intoxication or allergies on consumption [5]. Research on their use as mouth rinses are scanty.

Antimicrobial efficacies of probiotics and xylitol mouth rinses have not been compared till date. Also their effects on young and elderly population have not been compared. Hence the study intended to assess and compare antimicrobial efficacies of xylitol, probiotic and chlorhexidine mouth rinses in children and elderly.

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Methods

The research was a randomized, double blind, parallel, controlled trial (ClinicalTrials.gov ID: NCT04399161) conducted in full accordance with the World Medical Association Declaration of Helsinki. The study protocol was approved by the institution's ethics committee (No: JSSDCH/Ethical/05/2016-17). The study was conducted over a period of 10 months between February and December 2017.

The study was conducted among residential school children aged 5-12 years at high risk for caries chosen from a government residential school and elderly citizens (above 60 years) at high risk for caries residing in an old age home. The residential school and old age home were chosen by a convenience sampling method. Permission to conduct the study in the residential school and old age home was obtained from the concerned authorities. Details about the study were presented to the participants in the form of a study information sheet or communicated verbally in both English and in the local language. Only the eligible participants who provided written consent after they were briefed about the study procedure were included in the study. In case of children, written consent was obtained from the guardian. Eligibility criteria were as follows. Inclusion criteria: 1) Individuals with high risk for caries who were identified using a customized caries risk assessment tool. 2) Not under antimicrobial therapy or probiotic products for the past 1 month. Exclusion criteria: 1) Using mouth rinse routinely. 2) Undergoing any dental treatment during the study period. 3) Not able to brush their teeth and rinse on their own. Twelve participants were chosen per group. Sample size was based on previous literature with similar study objectives. With 3 products involved in each of the 2 population groups (children and elderly) the sample size was 36 child participants and 36 elderly participants.

The caries risk assessment tool comprised of information on socio-economic status, oral hygiene practices, fluoride exposure, caries experience amongst family members, symptoms of dry mouth, quantity and frequency of intake of sweetened food and caries experience in the past and present. Each item was scored as 0 or 1 and the total score was obtained by summing up the scores of all the items. Any participant with total score greater than 5 was considered to be at high risk for caries.

Antimicrobial efficacy of the mouth rinses was assessed by assessment of *Streptococcus mutans* levels in plaque. Participants were asked to refrain from brushing on the day of plaque collection. Plaque samples were collected from the buccal surface of a non-carious permanent maxillary first molar (adjacent tooth was considered if the index tooth was missing). Plaque collection was done using an autoclaved scaler under daylight. The collected plaque was then stored in a pre-weighed sterile eppendorf tube. The tubes were weighed again after plaque collection. The weight of the collected plaque (in grams) was determined by subtracting the weight of the empty eppendorf tube from the weight of the tube with the collected plaque. The samples were stored at -4°X and transported to a culture lab within an hour to avoid using transport media.

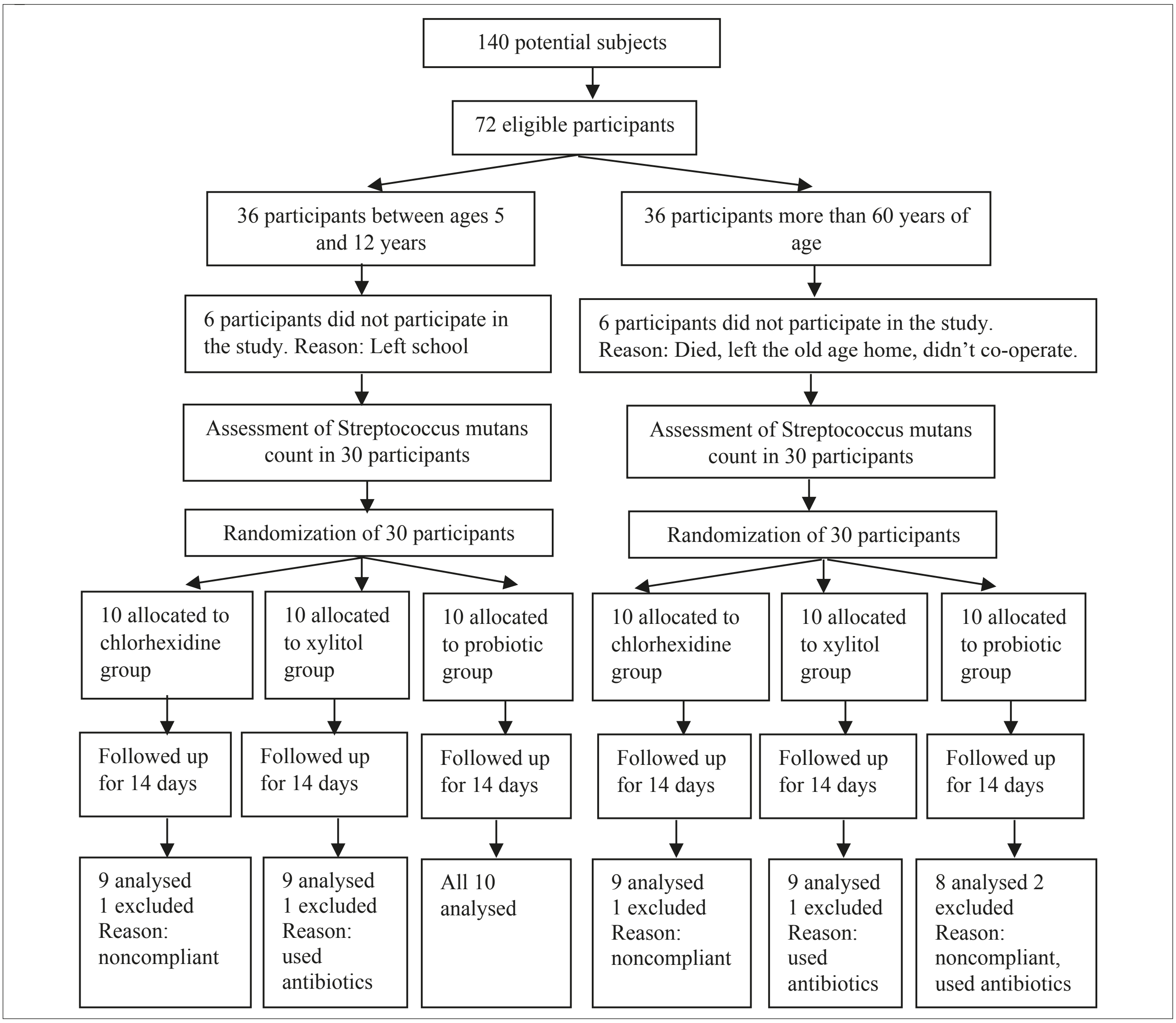
1 ml saline was added to the eppendorf tube and vortexed for even distribution of plaque. This mixture was used as stock solution for serial dilutions. From the stock solution, 100 µl was transferred to a sterile test tube containing 900 µl of saline and vortexed to arrive at 1:10 dilution. Similar dilutions were prepared to obtain 10^0, 10^{-1}, 10^{-2} and 10^{-3} dilutions. 50 µl from each of the dilutions was plated onto the selective medium (Mitis Salivarius Bacitracin Agar) by spread plate method. After plating, the MSB agar plates were placed in the anaerobic jar and incubated at 37°C for 72 hours.

Colonies of *Streptococcus mutans* were identified based on the following morphologic characteristics, a) 0.5 mm raised convex undulated colonies b) light blue color with rough margins c) granular frosted glass appearance [6]. The colonies were confirmed by a catalase test (negative catalase reaction) and gram staining (gram positive cocci). Bacterial colonies were counted manually. The standard formula used for determining Colony Forming Units (CFU) was CFU/g = [Number of colonies * Dilution factor] / [Volume plated (in ml) * Amount of plaque (in g)].

After data collection at baseline, the subjects were allocated into three groups, Group A, B and C, by simple random sampling (lottery method). Group A was given Chlorhexidine mouth rinse; Group B was given Xylitol mouth rinse and Group C Probiotic mouth rinse. The participants and the investigator were blinded from the allocation sequence. After 14 days of using the mouth rinses, the same procedures were repeated and comparisons were made with baseline values.

Xylitol mouth rinse at 10% concentration was used. Studies have observed that xylitol at concentrations as low as 1% inhibited *S. mutans* growth [7]. Hence a concentration of 10% was expected to inhibit S. mutans growth. The mouth rinse was prepared by dissolving 1.5 g of xylitol powder (Loba Chemie, code 06512) in 15 ml of water. Probiotic mouth rinse was prepared by using a commercially available probiotic product (Sporolac Plus powder-1 g sachet containing not less than 1.5 billion cells of *Lactobacillus acidophilus*-R 0052, *Lactobacillus rhamnosus*-R 0011, *Bifidobacterium longum*-R 00175, *Bacillus coagulans*-SNZ 1969, *Saccharomyces boulardii*). Each sachet was dissolved in 15 ml of water in a measuring cup and was used as a mouth rinse. A commercially available chlorhexidine mouth rinse (Hexidine-0.2% Chlorhexidine gluconate) containing 0.2% chlorhexidine gluconate per 10 ml was used. 7.5 ml of the concentrate was diluted with equal amounts of water to make 15 ml that was used for rinsing. The subjects were asked to rinse their mouth once daily (at night) for 2 minutes, using 15 ml of mouth rinse. The intervention was carried out for a period of 14 days. Mouth rinsing was supervised during the study period by an assistant. A record was maintained to document regular usage of the mouth rinse and also to record any adverse effects occurring during the intervention period.

Fig. 1. Flow of participants throughout the study.



Data was analyzed using SPSS (Version 22.0; SPSS Inc., Chicago, IL, USA). Comparisons before and after interventions were done using Paired t Test. Comparisons between Chlorhexidine, probiotic and xylitol groups were done using ANOVA. Statistical significance was fixed at $p \leq 0.05$. Effect size estimates were obtained for pre and post intervention comparisons and also for comparison between groups.

Results

A total of 72 participants (36 children and 36 elderly) were recruited for the study of which 12 (6 children and 6 elderly) were excluded. The remaining (30 children and 30 elderly) were randomly divided into 3 groups with 10 participants per group. Amongst the participants chosen for the study 2 children and 4 elderly were lost to follow-up (Fig. 1).

Among children, significant reduction in *Streptococcus mutans* counts were observed in all three groups (chlorhexidine: mean difference = 3.11 log_{10}CFU/g; SD = 2.32; t = 3.28; p = 0.022); (xylitol: mean difference = 0.93 log_{10}CFU/g; SD = 0.86; t = 2.64; p = 0.046); (probiotic: mean difference = 1.91 log_{10}CFU/g; SD = 1.67; t = 3.02; p = 0.023). Likewise significant reduction in *Streptococcus mutans* counts were observed among elderly (chlorhexidine: mean difference = 2.23 log_{10}CFU/g; SD = 1.08; t = 5.07; p = 0.004); (xylitol: mean difference = 1.39 log_{10}CFU/g; SD = 0.81; t = 4.19; p = 0.009); (probiotic: mean difference = 1.61 log_{10}CFU/g; SD = 0.92; t = 3.90; p = 0.018). The before and after comparisons also showed large effect sizes in all three groups in both children and elderly (Tabs. I, II).

There was no statistically significant difference in *Streptococcus mutans* count on comparing the 3 mouth rinses in children (F = 2.39; p = 0.123) and elderly (F = 1.26; p = 0.314). Medium effect size (ω^2 = 0.13) was observed on comparing *Streptococcus mutans*

Tab. I. Comparison of Streptococcus mutans counts in children before and after using mouth rinses.

Mouth rinse used	Streptococcus mutans count in children $log_{10}CFU/g$ (SD)			t	p-value	Hedges' g
	Before using mouth rinse	After using mouth rinse	Mean difference			
Chlorhexidine	6.43 (1.28)	3.33 (2.71)	3.11 (2.32)	3.28	0.022*	1.4
Xylitol	6.60 (0.95)	5.67 (1.12)	0.93 (0.86)	2.64	0.046*	0.8
Probiotic	6.88 (0.76)	4.97 (2.36)	1.91 (1.67)	3.02	0.023*	1.0

* Significant; SD: standard deviation; CFU/g: Colony Forming Units/ gram.

Tab. II. Comparison of Streptococcus mutans counts in elderly before and after using mouth rinses.

Mouth rinse used	Streptococcus mutans count in elderly $log_{10}CFU/g$ (SD)			t	p-value	Hedges' g
	Before using mouth rinse	After using mouth rinse	Mean difference			
Chlorhexidine	7.13 (1.15)	4.91 (0.73)	2.23 (1.08)	5.07	0.004*	2.1
Xylitol	6.42 (1.10)	5.03 (0.47)	1.39 (0.81)	4.19	0.009*	1.5
Probiotic	7.16 (0.80)	5.55 (0.43)	1.61 (0.92)	3.90	0.018*	2.2

* Significant; SD: standard deviation; CFU/g: Colony Forming Units/ gram.

Tab. III. Comparison of mean reduction in Streptococcus mutans counts between three mouth rinses in children and elderly.

Study groups	Mouth rinse used		Mean difference in *Streptococcus mutans* reduction (I-J) $log_{10}CFU/g$	F	p-value	ω^2
	(I)	(J)				
Children	Chlorhexidine	Xylitol	2.17	2.39	0.123	0.13
	Chlorhexidine	Probiotic	1.19			
	Xylitol	Probiotic	-0.98			
Elderly	Chlorhexidine	Xylitol	0.84	1.26	0.314	0.03
	Chlorhexidine	Probiotic	0.62			
	Xylitol	Probiotic	-0.22			

CFU/g: Colony Forming Units/ gram.

counts between the three mouth rinses among children while the effect size was small ($\omega^2 = 0.03$) on making similar comparisons among elderly (Tab. III).

Percentage reductions in *Streptococcus mutans* counts were greater with probiotic (28%) than xylitol (14%) among children while they were similar (probiotic: 23%, xylitol: 22%) among elderly (Fig. 2).

Discussion

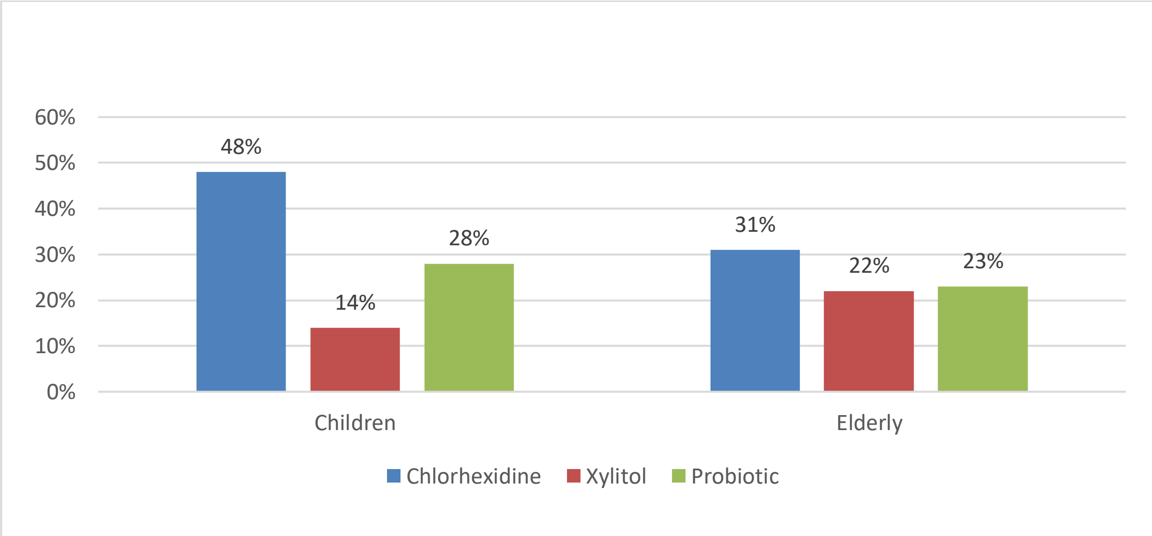
The bisbiguanide chlorhexidine, which has been studied extensively for over 20 years, is currently the most potent chemotherapeutic agent against *Streptococcus mutans* and has been often used as a positive control for assessment of the anticariogenic potential of other agents [8]. Side effects reported with use of chlorhexidine provides opportunities to study the potency of other products in inhibiting *Streptococcus mutans* activity [4, 9-11]. In this background the study attempted to assess and compare the antimicrobial efficacies of chlorhexidine, xylitol and probiotic mouth rinses by assessment of *Streptococcus mutans* levels in plaque.

The methodology involved assessment of Streptococcus mutans levels in plaque among children and elderly who were at high risk for dental caries. Concentration of mouth rinses used were based on inhibitory concentrations as determined in previous studies [7, 12]. Plaque was used as an alternative to saliva (due to variable secretion rates, duration of contact with the biofilm, etc.) as it has been proven to be a better indicator of microbial load in the oral cavity [13, 14]. The dropout rate in the study was around 7%. The main reason for dropout was systemic illnesses and antibiotic use.

We observed significant reduction in Streptococcus mutans counts after using the 3 mouth rinses for 14 days. The results were supported by studies conducted by Yousuf et al. [9], Jindal et al. [15], Priyadharshini et al. [10] and Laleman et al. [16] in case of probiotics and by El Salhy et al. [4] and Arunakul et al. [11] in case of xylitol. Large effect sizes were obtained in all the groups. Hence it may be inferred that use of any of the mouth rinses significantly reduces microbial load in plaque as compared to non-use.

Comparison of the mean reduction in *Streptococcus mutans* counts between groups gave insignificant results. Also the effect sizes observed between the different mouth rinses were trivial. Hence it may be inferred that the antimicrobial efficacy of three mouth rinses are comparable. Similar results were reported in another study [17] conducted among adults. Better compliance was however observed with probiotic and xylitol mouth rinses as compared to that of chlorhexidine mouth rinse (few complained of burning sensation on using chlorhexidine mouth rinse). Percentage reduction in *Streptococcus mutans* counts show that probiotics could be potentially more efficacious than xylitol among

Fig. 2. Percentage reduction in Streptococcus mutans counts in children and elderly after using the three mouth rinses.



children while both had similar effects among elderly. There is scanty documenting of the effect of mouth rinses on compromised individuals (high caries risk) which may be considered a strength in our study. Antimicrobial efficacies of probiotics and xylitol mouth rinses especially among elderly population have not been compared till date. A major limitation was the small sample size. Also, the effect of the mouth rinses was tested on *Streptococcus mutans* alone. The fact that more than one microbe is involved in the caries process provides scope for further research.

Conclusion

Antimicrobial efficacy of xylitol and probiotic mouth rinses were comparable to that of chlorhexidine in both children and elderly. Probiotics could potentially be more efficacious than xylitol among children.

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Conflict of interest statement

The authors declare no conflict of interest.

Authors' contributions

Concepts, design, definition of intellectual content: KNC, THM; Literature search, data acquisition, data analysis, manuscript preparation: KNC; Manuscript editing, manuscript review: THM, CBR.

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