Official Title of the study: Antimicrobial Efficacy of Xylitol, Probiotic and Chlorhexidine Mouth Rinses Among Children and Elderly Population at High Risk for Dental Caries - A Double Blind Randomized Controlled Trial.

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<center>**Study Protocol**</center>

Need for the study:

World Health Organization 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.

While mechanical methods of plaque control can maintain adequate oral hygiene, such methods are not being utilized appropriately by the population. This necessitates use of adjuncts to mechanical plaque control methods in the form of antiplaque mouth rinses. Chlorhexidine mouth rinse has been considered the most effective agent in inhibiting Streptococcus mutans. Although considered the gold standard, its adverse effects due to prolonged use such as staining of teeth, xerostomia, altered taste sensation, mouth/ throat irritation, antimicrobial resistance, etc. indicates the need for alternatives which have been extensively studied of late in literature.

Xylitol used as an artificial sweetener in foods, is a non-cariogenic sugar substitute. This polyalcohol cannot be metabolized by oral bacteria thereby preventing caries. The magnitude of adverse effects caused by xylitol as compared to that of Chlorhexidine is indistinct. Although studies have assessed effects of xylitol chewing gum on caries, very few have studied its efficacy as a mouth rinse.

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.

The advantages of probiotics as compared to that of chlorhexidine mouth rinse are that there are no issues of antibiotic resistance as it contains only commensal flora and there has been no proof of intoxication or allergies on consumption.

Antimicrobial efficacies of probiotics and xylitol mouth rinses have not been compared till date. Also their effects on the young and elderly population have not been compared. Hence the purpose of the present study is to compare the antimicrobial efficacies of probiotic and xylitol mouth rinses with the gold standard in children and elderly population.

Aim of the study:

To compare the antimicrobial efficacy of probiotic and xylitol mouth rinses with chlorhexidine (gold standard) among children and elderly population at high risk for dental caries.

Objectives of the study:

1. To assess Streptococcus mutans levels at baseline and 14 days after intervention in children and elderly population.

2. To compare efficacies of the 3 mouth rinses in reducing Streptococcus mutans levels in children and elderly.

Materials and methods:

Source of data: Residential school children aged 5-12 years at high risk for caries. Elderly population above 60 years residing in old age homes at high risk for caries.

Eligibility Criteria:

Inclusion Criteria:

1. Children aged 5-12 years at high risk for caries.

2. Elderly citizens (above 60 years) at high risk for caries.

3. Those willing to participate in the study and consented.

4. Not under antimicrobial therapy or used probiotic products during 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.

Study Design: Double Blind Randomized Controlled Trial.

Sample Size: Twelve participants will be chosen per group. With 3 products involved in each of the 2 population groups (children and elderly) the sample size will be 36 child participants and 36 elderly participants.

Consent from participants: Permission to conduct the study in the residential school and old age home will be obtained from the concerned authorities. Details about the study will be 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 provide written

consent will be included in the study. In case of children, written consent will be obtained from the guardians.

Caries risk assessment: Individuals with high caries risk will be identified using a customized caries risk assessment tool. The caries risk assessment tool will comprise 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 will be scored as 0 or 1 and the total score will be obtained by summing up the scores of all the items. Any participant with total score greater than 5 will be considered to be at high risk for caries.

Method:

1. Preparation of mouth rinses
2. Baseline data collection and Microbial Analysis
3. Randomization and Group Allocation
4. Intervention
5. Post intervention data collection and Microbial Analysis

1. Preparation of mouth rinses:

Xylitol mouth rinse at 10 percentage concentration will be used. Probiotic mouth rinse will be prepared by using a commercially available probiotic product (Sporolac Plus powder- 1gm sachet containing not less than 1.5 billion cells of Lactobacillus acidophilus, Lactobacillus rhamnosus, Bifidobacterium longum, Bacillus coagulans and Saccharomyces boulardii). Each

sachet will be dissolved in 15 ml of water in a measuring cup and will be used as a mouth rinse. A commercially available Chlorhexidine mouth rinse (Hexidine- 0.2 percentage Chlorhexidine gluconate) containing 0.2 percentage chlorhexidine gluconate per 10 ml will be used.

2. Baseline data collection and microbial analysis:

Plaque samples will be collected from the buccal surface of a non-carious permanent maxillary first molar. Plaque collection will be done using an autoclaved scaler under daylight. The collected plaque will be stored in a pre-weighed sterile eppendorf tube. Tubes will be weighed again after plaque collection. The weight of the collected plaque (in grams) will be determined by subtracting the weight of the empty eppendorf tube from the weight of the tube with the collected plaque. The samples will be stored at - 4 degree Celsius and transported to a culture lab within an hour to avoid using transport media.

1 ml saline will be added to the eppendorf tube and vortexed for even distribution of plaque. This mixture will be used as stock solution for serial dilutions. From the stock solution, 100μl will be transferred to a sterile test tube containing 900μl of saline and vortexed to arrive at 1:10 dilution. Similar dilutions will be prepared to obtain 1/10, 1/100 and 1/1000 dilutions. 50μl from each of the dilutions will be plated onto the selective medium MSB (Mitis Salivarius Bacitracin Agar) by spread plate method. After plating, the MSB (Mitis Salivarius Bacitracin Agar) agar plates will be placed in the anaerobic jar and incubated at 37 degree Celsius for 72 hours.

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

3. Randomization and Group allocation: After data collection at baseline, the study participants will be allocated to three groups, Group A, B and C, by simple random sampling (lottery method). Group A will be given Chlorhexidine mouth rinse; Group B will be given Xylitol mouth rinse and Group C Probiotic mouth rinse. The participants and the investigator will be blinded from the allocation sequence.

4. Intervention: The participants will be asked to rinse their mouth once daily (at night) for 2 minutes, using 15 ml of mouth rinse. The intervention will be carried out for a period of 14 days. Mouth rinsing will be supervised during the study period by an assistant. A record will be maintained to document regular usage of the mouth rinse and also to record any adverse effects occurring during the intervention period.

5. Post intervention data collection: After 14 days of using the mouth rinses, the same procedures will be repeated and compared with baseline.

Statistical Analysis Plan: Data will be analysed using SPSS (Statistical Package for the Social Sciences Version 22.0; SPSS Incorporated, Chicago, Illinois). Comparisons before and after interventions will be done using Paired t Test. Comparisons between Chlorhexidine, probiotic and xylitol groups will be done using ANOVA (Analysis of Variance) and Tukey's Post Hoc Tests. Comparison of antimicrobial efficacy of mouth rinses between children and elderly will be done using Independent sample t Test. Statistical significance will be fixed at $p \leq 0.05$.