Efficacy of Probiotic, Chlorhexidine, and Sodium Fluoride Mouthrinses on Mutans Streptococci in 8- to 12-Year-Old Children: A Crossover Randomized Trial

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Keywords

Bacteriotherapy · Children · Mouthrinse · Mutans streptococci · Prevention · Probiotics

Abstract

Introduction: The oral cavity is home to a diverse and distinct microbiome. While the role of oral bacteria in cariogenic and other dental diseases is irrefutable, their beneficial effects in the form of probiotics (PB) has been less studied, especially pertaining to oral diseases in children. This study compares the efficacy of a PB mouthrinse with 0.12% chlorhexidine (CHX) and 0.05% sodium fluoride (NaF) mouthrinse on the colony counts of mutans streptococci (MS) in children. **Methods:** A triple-blind crossover randomized trial between interventional groups was planned. Fifty-one children between 8 to 12 years of age were divided into three groups (I, II, and III) and were exposed to all three mouthrinses (A, B, and C) by randomized allocation for a period of two weeks with an inter-phase washout period of four weeks. Pre- and post-interventional MS counts (CFU/mL) were assessed, and the mean change was analysed using the *t* test (intragroup) and ANOVA (intergroup and crossover). **Results:** The mean changes in the colony counts obtained with the use of PB, CHX, and NaF mouthrinses were –1.0223

(–1.2201 to –0.8246), –0.9564 (–1.1503 to –0.7626), and –0.9511 (–1.1554 to –0.7467), respectively, which were statistically significant ($p < 0.0001$). However, the intergroup comparison for the mean change in colony counts revealed no statistically significant differences ($p > 0.05$). **Conclusion:** The study concluded that the PB mouthrinse was equally efficacious as compared to CHX and NaF mouthrinses against MS in 8- to 12-year-old children. However, further studies are recommended to strengthen the evidence.

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Introduction

Microbiome research has become central to disease pathogenesis and that of the oral cavity holds significance in the aetiology for many oral and systemic diseases. With over 700 bacterial taxa, the oral microbiome is extremely dynamic and maintains equilibrium despite its “continuum with the external environment” [1].

Dysbiotic states driven by ecological stress in the oral biofilm convert commensal bacteria to opportunistic, which lead to pathogenesis. Dental caries is the result of dysbiotic changes in the ecology of the oral biofilm [2]. Early cultivation studies and animal experiments isolated

Streptococcus mutans (*S. mutans*) from carious lesions [3, 4] and identified it as the principal bacteria responsible for dental caries in humans. *S. mutans* is highly acid-tolerant, creates impenetrable biofilms, and is genetically evolved to withstand oral environmental changes, which safeguards its cariogenic potential [2].

Recently, the prevalence of dental caries has increased in developing countries, due to high refined sugar consumption, inadequate fluoride exposure, high oral health service costs, etc. [5]. In India, 51.9% of 5-year-old, 53.8% of 12-year-old, and 63.1% of 15-year-old children are affected by dental caries [6].

Long-term prevention of dental caries can be implemented by interfering with deleterious factors that shift the microbial equilibrium [7], and regular tooth brushing is one means to achieve it. Regular tooth brushing prevents the accumulation of cariogenic biofilm; however, in children, factors such as lack of dexterity, individual motivation, and monitoring can limit its effectiveness [8]. Therefore, adjunctive use of chemotherapeutic agents in the form of mouthrinses is recommended [9, 10]. The chlorhexidine (CHX) mouthrinse, due to its proven efficacy, has been established as the “gold standard” or positive control for comparison with other agents [11]. Fluoride, since its inception through water fluoridation, is widely accepted as a caries-preventive regimen [12].

“Bacteriotherapy,” an alternative approach that works on the principle of harmonizing oral ecology and reducing the number of cariogenic bacteria, is of special interest [1]. Probiotics (PB) are being researched as a contender for bacteriotherapy. PB are defined as, “live microorganisms that, when administered in adequate amounts, confer a health benefit on the host” [13]. PB bacteria replenish the diversity of a dysbiotic microbiota [14]. Their mechanism of action on the oral biofilm is debatable; nonetheless, several theories postulate that PB bacteria secrete antimicrobial substances like bacteriocins, hydrogen peroxide, and organic acids, whilst against pathogenic microbes, they compete for nutrition, growth factors, and adhesion sites. Additionally, they can also stimulate non-specific immunity and improve the cellular and humoral response [15].

PB, administered in different forms, have previously been studied to determine their effectiveness against cariogenic oral microbiota [15–18]. A crossover study in adults assessing Kefir PB drink against sodium fluoride (NaF) has reported significantly lower levels of salivary mutans streptococci (MS) [18]. Furthermore, a parallel group study comparing the efficacy of CHX, NaF, and PB powder as mouthrinses in adults found that the PB pow-

der reduced plaque *S. mutans* levels, without any untoward harmful effects [19]. As of yet, no crossover randomized trial has reportedly been performed in children with the three mouthrinses. Therefore, the purpose of this trial was to compare the efficacy of PB, CHX, and NaF mouthrinses on MS in children of 8–12 years of age. We also assessed the modifying effect of age, sex, and crossover; the patient’s acceptance to taste; and their compliance.

Methods

The CONSORT checklist was used in conducting and reporting this three-group, crossover randomized trial with an allocation ratio of 1:1:1.

Settings, Location, and Participants

The study was designed and carried out in the Department of Pediatric and Preventive Dentistry, YMT Dental College and Hospital, Navi Mumbai, India. One hundred and seventeen healthy children were screened for eligibility, of which, 51 children between 8 to 12 years of age with a total caries score (dmft + DMFT) (d/D: decayed, m/M: missing, and f/F: filled teeth) (primary + permanent dentition) between four and ten were selected. Children with recent H/o antibiotics, undergoing orthodontic treatment, with I/o soft tissue pathology, in whom saliva collection was not possible owing to disability or who could not rinse on their own were excluded.

Intervention

Demographic details and baseline total caries score (dmft + DMFT) of the participants were recorded by the primary investigator before commencement of the trial. The participants were randomly divided into three groups namely group I, group II, and group III using a computer-generated sequence. Participants in each group received all three mouthrinses, each coded as A, B, and C by randomized allocation, performed by an independent observer.

Each intervention phase spanned two weeks. Parents were instructed to measure 10 mL of CHX and NaF mouthrinses in a plastic calibrated measuring cup, and the participants were advised to rinse it for one minute, swish, and spit, twice a day, after tooth brushing under parental supervision. PB powder sachets were opened and freshly prepared by parents, by mixing the powder in 10 mL of water and using the solution as a mouthrinse. Participants were asked to refrain from eating, drinking, or brushing for at least one hour after rinsing. Before each new phase, along with the new mouthrinse, participants were provided with a customized pre-marked calendar that highlighted the number of times and days of use. They were instructed to fill it simultaneously while using the mouthrinse. The second and the third interventional phases were carried out in a similar manner with a washout period of four weeks. Throughout the trial, participants were to follow their regular diet and oral hygiene practices using a non-fluoridated toothpaste.

Saliva Collection and Bacterial Culturing

One millilitre of paraffin-stimulated mid-morning [20] saliva samples were collected by the primary investigator from all participants in duly labelled sterile Eppendorf tubes at baseline and at the end of each

phase. All the tubes were labelled by an independent investigator to ensure blinding of the primary investigator, immediately sealed, coded, and sent for culturing. Corresponding patient codes were also labelled onto the mitis salivarius-bacitracin (MSB) agar petri dishes for an accurate bacterial count record of respective participants.

MSB agar was prepared with addition of 1% potassium tellurite. The samples were streaked onto MSB agar plates and incubated in a 5% carbon dioxide incubator at 37°C for 48 h and examined for growth of MS. The colonies were identified by their morphology, i.e., raised, convex, undulate, opaque, pale-blue colonies with “frosted glass” or granular appearance on the agar surface. Colony counting was done with the help of a colony-counting meter as CFU/mL at the end of 48 h. The dilution factor used was 10⁵.

Outcomes

The primary outcome variable assessed was the colony counts of MS (CFU/mL) after the use of the mouthrinses. We also assessed the effect of crossover and the effect of modifier variables viz. age and sex.

The secondary outcome variables assessed were the taste acceptance and the compliance to use of the three mouthrinses. The taste acceptance was recorded on a 5-point Likert rating scale (ranging from 1: very bad, 2: bad, 3: okay, 4: good, and 5: very good), by the primary investigator. As for the compliance, participants submitted the filled calendars to the primary investigator after each phase. Both outcomes were reviewed when participants reported for post-intervention saliva collection, immediately after each phase. Adverse effects, in the form of gastrointestinal dysfunction, taste disturbance, tooth staining, or any other symptoms, were also noted.

Sample Size Estimation

Considering that the proposed study was a three-group, crossover, randomized trial with α at 0.05 and β at <0.2 (power at 80%) with an assumed non-inferiority margin [19] at 0.6163, a sample size of 42 participants would be required. Assuming a dropout rate of 20%, a total of 51 participants were enrolled in the study.

Randomization and Blinding

Each participant was coded with numbers 01–51. Mouthrinses were coded as A, B, and C and dispensed to the participants according to the computerized sequence. An independent observer dispensed the pre-measured quantity of mouthrinses to the allocated participant, in white opaque bottles to mask the content before each phase began. The primary investigator collected the saliva samples at the start and end of each phase and hence was blinded and unaware of the type of the mouthrinse dispensed. The statistician was blinded to the coding of all the three interventions.

Statistical Analysis

The data were captured into a Microsoft Office Excel (version 2013) spreadsheet. Data analysis was done using Windows-based MedCalc statistical software version 13.3.1 (MedCalc Software bvba, Ostend, Belgium; <http://www.medcalc.org>; 2014).

The change in the colony counts of MS was expressed as the mean change and mean percentage change with standard deviation. The effect of crossover was expressed as the estimated marginal means and estimated marginal mean percentages. The patient acceptance to taste was expressed as ranked proportions. The patient compliance was recorded as a binary categorical variable and expressed as proportions.

Table 1. Distribution of study population (age and sex-wise)

Participants (n = 51)	8 years	9 years	10 years	11 years	12 years	Total
Boys (26), %	15.7	13.7	7.9	1.9	11.8	51
Girls (25), %	13.7	13.7	7.9	5.8	7.9	49
Total, %	29.4	27.4	15.8	7.7	19.7	100

An intent-to-treat (ITT) analysis was performed to account for the attrition in sample. A per protocol (PP) analysis was performed on participants who completed all three phases. Intragroup comparison was analysed using the paired-sample *t* test to assess the change in CFU/mL with the three interventions. Intergroup comparison and the effect of crossover on the mean change and mean percentage change in colony counts of MS were analysed using ANOVA. The χ^2 test was used to assess taste and compliance. Analysis of covariance (ANCOVA) was applied to assess the effect of age and sex in addition to crossover on the primary outcome. Spearman's coefficient of rank correlation was used to assess the correlation between acceptance to taste and compliance. $p < 0.05$ was considered significant.

Results

The study population comprised of 51 children of which 26 (50.98%) were boys and 25 (49.02%) were girls, the mean age being 9.6 ± 0.4 years (Table 1). Of the 51 participants, 19 completed all the three phases of the study (Fig. 1). The recruitment period for the trial extended from January 2017 to February 2017. The trial was completed by June 2017.

Primary Outcomes

A significant reduction in the colony count of MS was observed with use of the individual mouthrinses ($p < 0.0001$) (Table 2). Intergroup comparison using ITT and PP analysis revealed no significant difference in the reduction of colony counts of MS between the three mouthrinses ($p > 0.05$) (Table 3). Intergroup comparison with the crossover effect revealed no significant difference in the estimated marginal means and estimated marginal mean percentages of MS ($p > 0.05$) (Table 4).

Secondary Outcomes

Taste and Compliance

Most participants rated the taste as “good” ($\chi^2 = 9.189$, $p > 0.05$) with no significant difference between the three mouthrinses. The overall compliance, across the three

Fig. 1. Flow chart depicting participant allocation, randomization, washout period, follow-up phases, and subsequent attrition in the sample.

Table 2. Mean change in colony counts of MS in CFU/mL within PB, CHX, and NaF mouthrinse groups

Mouthrinse group	MS colony counts, mean $\pm$ SD*		Mean change in colony counts (95% CI)	<i>t</i>	<i>p</i> value [#]
	baseline	post-interventional			
PB (30)	2.625 $\pm$ 0.7725	1.6027 $\pm$ 0.5670	−1.0223 (−1.2201 to −0.8246)	−10.574	<0.0001
CHX (28)	2.4757 $\pm$ 0.8654	1.5193 $\pm$ 0.7376	−0.9564 (−1.1503 to −0.7626)	−10.122	<0.0001
NaF (28)	2.5021 $\pm$ 0.6589	1.5511 $\pm$ 0.4391	−0.9511 (−1.1554 to −0.7467)	−9.549	<0.0001

SD, standard deviation; CI, confidence interval. [#]*p* < 0.05, significant. * 10⁵ CFU/mL of the saliva.

Table 3. Mean reduction and mean percentage reduction in colony counts of MS between PB, CHX, and NaF mouthrinses considering ITT and PP analysis

Mouthrinse group	Mean $\pm$ SD*		Mean (%) $\pm$ SD	
	ITT (<i>n</i> = 86)	PP (<i>n</i> = 19)	ITT (<i>n</i> = 86)	PP (<i>n</i> = 19)
PB (30)	1.0223 $\pm$ 0.5290	1.0037 $\pm$ 0.6006	38 $\pm$ 15	36 $\pm$ 18
CHX (28)	0.9564 $\pm$ 0.5000	1.0637 $\pm$ 0.5195	37 $\pm$ 17	37 $\pm$ 18
NaF (28)	0.9511 $\pm$ 0.5270	0.9037 $\pm$ 0.4807	36 $\pm$ 16	35 $\pm$ 15
<i>F</i>	0.171	0.432	0.081	0.062
<i>p</i> value [#]	0.843	0.651	0.921	0.940

SD, standard deviation. [#]*p* > 0.05, non-significant. * 10⁵ CFU/mL of the saliva.

groups, was 81%, the individual compliances being 85% for PB, 80% for CHX and 77.7% for NaF, with no statistically significant difference between the three mouthrinses ($\chi^2 = 0.343$, *p* > 0.05). Furthermore, we also assessed if any correlation existed between acceptance to taste and compliance to usage; however, the results found no significant association between the two outcomes {Spearman's coefficient of rank correlation (ρ) = −0.0724 [95% confidence interval (−0.290 to 0.153)]; *p* > 0.05}.

Effect of Confounding Variables

An analysis of the confounder variables using ANCOVA suggested that age (*p* = 0.246), sex (*p* = 0.425) and crossover (*p* = 0.147), individually as well as collectively (*p* = 0.890), had no significant effect on the efficacy of the three mouthrinses.

Discussion

In the last two decades, considerable scientific evidence has been gathered to assert that PB is beneficial for oral health as a preventive therapy. This form of bacteriotherapy has also been applied for dental caries, as a measure to re-establish a non-cariogenic flora in susceptible

individuals. Though the evidence is plenty, it is weak [21]. This study assessed the efficacy of a PB powder in the form of a mouthrinse in children with a high risk of caries. One gram of freeze-dried PB powder is consumed by mixing in 10 mL of water, as prescribed by general medical practitioners and paediatricians for gut health, and the same has previously been assessed as a mouthrinse in children [17].

Mouthrinses are an ideal vehicle to incorporate chemicals and are appreciated for their ease of use, plaque reduction, and breath-freshening effect [22]. Previous reports have shown that MS is highly susceptible to CHX [23, 24]. NaF (0.05%) is the most commonly prescribed mouthrinse in children, and its regular use has shown a reduction in caries increment in permanent teeth [12]. However, CHX and NaF mouthrinses have exhibited undesirable adverse effects, which include staining of teeth by CHX, questionable fluorosis by NaF, and allergic reactions. Additionally, the emerging resistance of bacteria to antimicrobial agents cannot be ignored [25]. Synthetic antimicrobial agents kill an array of bacteria, and this can alter the ecology of the oral microbiome. “Bacteriotherapy” in the form of PB can maintain a healthy microbiota and prevent pathogenesis at the same time [1]; hence, a continuous supply of PB microbes, with an acceptable de-

Table 4. Mean change and mean percentage reduction in colony counts of MS in CFU/mL with PB, CHX, and NaF mouthrinses with cycle (sequence of drug administration) as the variable considering ITT and PP analysis

Mouthrinse group	ITT (n = 86) (95% CI)		PP (n = 19) (95% CI)	
	estimated marginal mean*	estimated marginal mean percentage	estimated marginal mean*	estimated marginal mean percentage
PB	1.0794 (0.8346–1.3243)	40.3682 (32.9118–47.8606)	1.2200 (0.8329–1.6071)	40.6267 (28.6254–52.6281)
CHX	0.9636 (0.6504–1.2768)	37.3831 (27.8218–46.9443)	1.0120 (0.5224–1.5016)	31.8651 (16.6844–47.0457)
NaF	1.1562 (0.8681–1.4442)	43.0557 (34.2606–51.8508)	1.1500 (0.7031–1.5969)	43.1682 (29.3102–57.0262)
F	0.685	1.132	0.471	1.188
p value [#]	0.605	0.348	0.756	0.328

CI, confidence interval. [#] $p > 0.05$, non-significant. * 10^5 CFU/mL of the saliva.

livery route, is necessary for optimal effectiveness [26, 27]. Darolac™ PB powder (M/s Aristo Pharmaceuticals Pvt. Ltd., Mumbai, India) is a 1 g freeze-dried powder with not <1.25 billion live cells of *Lactobacillus rhamnosus*, *Lactobacillus acidophilus*, *Bifidobacterium longum*, and *Saccharomyces boulardii*. This PB mouthrinse is a blend of *Lactobacilli* and *Bifidobacterium*, which are commensal bacteria and also most recommended for human use due to its adhesion capacity, ability to co-aggregate and colonize on the oral surface [28]. At least 10^6 – 10^7 live PB per gram or mL of product are necessary, at the time of its consumption to benefit human health [29].

The three mouthrinses evaluated in this study have unique anti-cariogenic actions against MS. The 0.12% CHX gluconate mouthrinse contains hydrosoluble active ingredients that bind to the negatively charged bacterial cell wall, especially to that of Gram-positive MS [30]. NaF, in addition to remineralization of tooth structure, has biological effects against bacteria through inhibition of cellular enzymes by increased permeability of hydrogen fluoride protons that interrupt with the intracellular glycolysis of *S. mutans* to reduce its acid tolerance and acid production [31]. PB mouthrinses act by means of “defence by diversity” [14] and are known to influence levels of MS in saliva [32].

Our results suggest that the PB mouthrinse proved to be equally efficacious as compared to CHX and NaF mouthrinses against MS in 8- to 12-year-old children. Hence, the study fails to reject non-inferiority. To the best of our knowledge, this is the first triple-blinded, three-phased, crossover randomized controlled trial reporting on the efficacy of PB, CHX, and NaF mouthrinses in 8- to 12-year-old children. We chose to perform triple-blinding to reduce assessment bias, increase accuracy, and ob-

jectivity of results [33]. A crossover design was chosen to reduce subject variability at baseline so that they act as their own control and a “within subject” comparison of treatments promotes internal validity of results [34]. We chose the age group of 8 to 12 years as children of a younger age group might inadvertently swallow liquids, leading to adverse outcomes [35]. Healthy children with a caries score between four and ten were included as the AAPD recommends the use of mouthrinses for children with a high caries risk [36]. Children with an ongoing or recent history of antibiotics were excluded as it influences oral microbial diversity which could have altered clinical outcomes [37]. To re-establish the oral microbiota at baseline before each phase, a washout period of four weeks was provided [18]. Despite stringency in follow-up and planning, there was considerable attrition for which ITT and PP analyses were considered.

PB (labelled as A), 0.12% CHX (labelled as B) (PerioGard™, M/s Colgate-Palmolive Pvt. Ltd., Mumbai, India), and 0.05% NaF (labelled as C) (Fluoritop™, M/s ICPA Health Products Ltd, Mumbai, India) were dispensed by an independent observer. Before each phase, participants reported for collection of respective mouthrinses and were instructed about its preparation, direction of use, and on their objective role as a participant. Mid-morning paraffin-stimulated salivary samples were collected to minimize effects of the circadian rhythm [20]. Dental treatments of participants were performed in the washout period only as discontinuation for the sake of the trial would have been unethical. Treatments, in addition to crossover, could influence the regrowth of oral microbiota. However, our results suggest that despite cyclic administration of interventions, the baseline counts were re-established in the washout period and had no effect on

the overall resultant change in colony counts. We additionally considered the difference in mean percentage change instead of assessing absolute change in colony counts to compensate for this possible alteration.

The results of this trial are in accordance with a previous study in adults using similar interventions, with no significant difference in the extent of their effectiveness against MS [19]. Equivalent reductions have also been reported by Ghasempour et al. [18] and Patil et al. [38]. Jindal et al. [17] compared two PB powders as a mouthrinse with a placebo mouthrinse, in 7–14-year-old children and found significant reduction in the salivary MS counts. However, in the study by Kulkarni and Damle [39], CHX was more effective against MS compared to NaF, which is in contradiction to our findings. Aminabadi et al. [30] have shown that CHX used prophylactically before PB can strengthen the survival of PB bacteria *Lactobacillus rhamnosus* GG in the oral cavity. PB introduced through different vehicles of administration have been tested in a similar age group. One-week consumption of PB in the form of curd and ice cream showed reduced MS levels at 1 h [40] and on the 7th day [40, 41]. Reduction has been reported to last up to a month [41, 42], while at 6 months, normal levels of MS were re-established [41]. Hence, long-term effectiveness of PB is questionable.

Numerous health-beneficial bacteria are being tested to reduce virulence of cariogenic bacteria. *Bacillus coagulans* Unique IS2 (2 billion CFU) through chewable tablets reduced levels of MS and *Lactobacilli* in saliva and plaque after two weeks, in 5–15-year-old children [43]. PB ice cream containing *Bifidobacterium lactis* Bb12 and *Lactobacillus acidophilus* La5 reduced MS, but levels of oral *Lactobacilli* remained unaffected [44]. *Lactobacilli* counts have also remained unaffected after long-term use of a fluoride mouthrinse in contrast to reduced MS levels in children [45, 46]. *Lactobacilli* have been known to inhibit the growth and development of *S. mutans* and overall biofilm formation, which might explain results of aforementioned studies [30].

The taste perception of a paediatric formulation is an important factor to determine patient compliance [47]. To analyse the taste acceptance, we used a Likert rating scale as it is a pre-validated, psychometric scale, commonly used for examining self-reported perceptions and attitudes. It has also been used for assessment of children's feelings, beliefs, and attitudes [48]. The results for taste showed that the PB mouthrinse was “most accepted” for its taste, while all three interventions were ranked “good.” Flavouring agents added to mask the original taste of the active ingredient can influence the ranking

[49]. However, individual taste preferences need to be considered while interpreting the results. Compliance was assessed through self-reported calendar markings. Most participants of the study were well-compliant to the three mouthrinses.

Age and sex did not influence the change in colony counts. *S. mutans* have not been known to have any specific predilection for age or sex in children [50]. No adverse effects were reported by the participants in this trial, possibly due to usage for only two weeks. Parents were advised to supervise the participants while mouthrinsing to prevent inadvertent swallowing. Contact numbers of both investigators were provided to the parents to report emergencies. Even as participants dropped out, no reasons related to side effects were reported when contacted.

Darolac™ is one of the most commonly prescribed PB by general practitioners for improvement of gut health [51]. It is easily available and cost-effective (1 sachet is ~10–11 INR), therefore economical to purchase. Assessment of an inexpensive product with two-way benefits against commercially prominent and costlier products adds to the cost-benefit ratio of the mouthrinses chosen in this trial, and this will specifically benefit population of low socio-economic status. Developing countries like India fail to put strenuous efforts in the implementation of community-level prevention programmes for oral health, and consequently, treatment-based approaches gain focus [17]. The best cost-effective community-level prevention for children would be a school-based intervention in high-risk areas, with PB powder suspended in milk or water, distributed and monitored by school staff [14], or similar to fluoride recommendation for children, annual exposure to calibrated doses of PB in acceptable vehicles can be administered to children based on their caries risk. This might frequently replenish the oral microbiota with consistency and reduce the caries susceptibility. Researching cost-effective and easily available products for oral health can aid in large-scale prevention programmes and in future, formulate preventive guidelines for public health [17].

The results of this trial may be generalizable to children of a similar age group in similar settings. Future trials with different study designs, settings, and ethnic population groups may yield interesting results, depending upon the strain-specific action of PB [29].

Limitations

Complete blinding (of the participants) could not be achieved as PB was available in powder form and hence was dispensed as sachets. Additionally, CHX and NaF

mouthrinses also have unique colours, blue and orange, respectively, which are visible after one begins usage. Individual caries activity of the participants was not assessed. Both streaking of saliva samples and colony counting were performed by the primary investigator. Though this standardized the process of culturing, the effects of intra-examiner variability on the colony counting procedure cannot be overlooked. Participants in our trial were instructed to spit the PB mouthrinse, so any effect on the gut microbiota could not be understood.

The Indian market for PB has improved in the past decade; however, ready-to-use preparations are not easily available. Most products are available in a capsule form; syrup preparations have the disadvantage of having added sugar which can contribute to decay. Till date, the products follow an old nomenclature to label PB preparations [52]. One study has found that the most commonly available drug is Darolac [51] and can reflect the state of availability of different preparations in most urban metro cities in India.

The present trial suffered more than the estimated attrition as only 19 of the 51 participants completed the study. We observed that maximum participants were lost after the first phase and first washout period (39.22% loss), while the consecutive phases and washout period had lesser dropouts (Fig. 1). The attrition in this trial was highly influenced by the study period. This trial was planned between the months of March to June, which in India, coincides with school final examination, followed by a long vacation period of approximately two months. This time of the year, residents widely leave for a visit to native places, and a very large number of participants were lost due to this reason. We failed to foresee that this could crucially affect the trial. Additionally, reasons for non-compliance specifically in the first phase could have been related to participation by transiently motivated parents and children, who withdrew when no visible effects were seen after first usage. For this, individual motivation, counselling and oral health awareness may play a key role in compliance. Individuals with a high risk for disease are known to indulge in high-risk behaviours, non-compliance, and laxity in follow-ups [53]. This factor was beyond the control of the investigators. The attrition rate shows that non-adherence to a trial conducted with children can be influenced by factors such as academic schedules, seasonal diseases, and quality of life [54]. Crossover studies are generally prone to attrition as it demands high compliance from participants, wherein a 5–10% dropout rate can extend up to 25% [55]. Future crossover studies

must take into consideration the challenges developed in this trial and factors that may influence dropouts, especially when recruiting children.

There exists a statistically significant association between reduction in the counts of MS and use of a gut-beneficial PB bacterial therapeutic agent. Within limitations and based on the results of this trial, we may conclude that PB is as effective as CHX and NaF mouthrinses; however, further studies are needed to confirm this claim. PB may be considered as an alternative bacteriotherapeutic regimen effective as a preventive measure in children with moderate to high risk of caries. Future trials can take into consideration any dual beneficial or adverse effects of PB on the gut microbiome and calibrate the dosage that can benefit both oral and gut health. Cost-to-benefit analysis of PB to other commonly marketed antimicrobial drugs may highlight its effect on economic expenditure needed for prevention to recommend at a community level.

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Statement of Ethics

The trial was conducted in accordance with the Declaration of Helsinki and was approved by the Institutional Ethics Committee at YMT Dental College and Hospital (YMTDC&H/IEC/2015/112). Information sheets were provided to the parents, and a written consent was obtained from the parents before commencement of trial following which an assent was taken from children. This trial is registered with the UMIN-CTR Trial Registry (UMIN ID: UMIN000042958).

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

A.N.K, K.Y.G: research proposal, theoretical formulations, data analysis, interpretation, manuscript draft. A.N.K: blinding, supervision. K.Y.G: participant recruitment, data collection, literature review. A.N.K, K.Y.G: final manuscript and critical evaluation for intellectual content. Authors are accountable for all aspects of the research conducted.

Data Availability Statement

All data generated through this trial has been included in this paper. Any further enquiries are to be directed to the corresponding author.

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