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## Research Article

# COMPARATIVE EVALUATION OF A PROBIOTIC MOUTHWASH VERSUS A CHLORHEXIDINE MOUTHWASH ON PLAQUE INHIBITION AND GINGIVAL INFLAMMATION POST NON-SURGICAL PERIODONTAL THERAPY

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### ABSTRACT

**Introduction:** Antibiotic resistance is an increasingly important global problem. Chlorhexidine gluconate is considered the most effective antiplaque and antigingivitis agent. But Flotra *et al* have reported side effects like discoloration of teeth and tongue, taste disturbances, and paresthesia associated with its usage. Probiotic technology involves utilizing natural beneficial oral flora as a defense mechanism against detrimental bacteria. Objective-To assess and compare the effectiveness of a probiotic mouthwash with 0.2% chlorhexidine mouthwash on plaque inhibition and gingival inflammation.

**Methodology:** A randomized trial was designed for a period of 3 weeks on 40 subjects. The subjects were divided into two groups (20 subjects in each group) i.e. Group A- chlorhexidine mouthwash and Group B- probiotic mouthwash. All the subjects were instructed to rinse their mouth with 10 ml of their respective mouthrinse for 1 min twice daily for 21 days. Result- There was a statistically significant difference seen for the intra group comparison for both chlorhexidine group and probiotic group at 21<sup>st</sup> day with reduction in plaque index and gingival index for both probiotic group and chlorhexidine group. There was a statistically non-significant difference seen for the inter group comparison of the outcome variables with reduction in plaque index and gingival index for probiotic group as compared to chlorhexidine group.

**Conclusion:** The Probiotic mouth rinse tested was effective in reducing plaque accumulation and gingival inflammation. Therefore, the Probiotic mouth rinse has a potential therapeutic value and further long-term study is recommended to determine its efficacy.

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### INTRODUCTION

Plaque control measures employ a variety of mouthwashes to augment mechanical plaque removal by inhibiting or reducing plaque accumulation and gingival inflammation.<sup>1</sup> Tooth-brushing, when accomplished properly, results in effective plaque control. However, mechanical plaque control methods have certain inherent limitations as it does not have access to interdental areas and brush ends does not engage the gingival sulcus. Therefore, adjunctive chemical plaque control methods such as use of mouthwash have been suggested as additional therapeutic strategy to augment but definitely not to replace mechanical plaque control.<sup>2</sup>

Chlorhexidine gluconate is considered as the gold standard of antimicrobial rinses because of its broad spectrum antibacterial activity and substantivity of 8 to 12 hours.<sup>3</sup> Chlorhexidine is an antimicrobial agent. It prevents plaque accumulation, hence it is an antiplaque and antigingivitis agent.<sup>3</sup> However, unwanted

side effects, such as temporary loss of taste; staining of teeth, restorations, and mucosa; dryness and soreness of mucosa; bitter taste; and a slight increase in supragingival calculus formation are the limiting factors in extended use of this chemical plaque control agent.<sup>3</sup> With the threat of widespread antibiotic resistance rendering many antibiotics useless against important diseases, there is an increased necessity not only to minimize antibiotic use and develop novel nonantibiotic-based treatments, but also to raise the profile of disease prevention.<sup>4</sup>

Probiotics may be a promising area of research in periodontal therapy. Probiotics are defined as "live microorganisms that when administered in adequate amounts confer health benefits on the host." They repopulate the beneficial bacteria, which can help kill pathogenic bacteria and fight against infection. Probiotics administered orally may benefit oral health by preventing the growth of harmful microbiota or by modulating mucosal immunity in the oral cavity.<sup>4</sup> The rationale for using probiotics is to alter the microbial imbalance in caries and

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periodontal diseases by adding beneficial species. Probiotics have shown favourable properties in maintaining oral health by contributing to a healthier microbial equilibrium. To provide benefits in the oral cavity, probiotics should adhere to and colonize on dental tissues. Prebiotics have been proved to be an aid to complement probiotics in the treatment of oral diseases. These are non-digestible oligosaccharides that affect the proliferation of resident commensal bacteria, which may exert beneficial effects on the host. Their function is to enhance the growth and activity of beneficial organisms and simultaneously suppress the growth and activity of potentially deleterious bacteria. In this way prebiotics modify the balance of the intestinal micro-flora. Some of the commonly known prebiotics are lactose, inulin, fructo oligosaccharides, galacto oligosaccharides and xylo oligosaccharides. Inclusion of probiotic-enriched food promotes a healthy lifestyle by delaying and halting the pathophysiology of periodontal diseases.<sup>5</sup>

Hence, the present study was conducted to compare the efficacy of mouthrinsing with a probiotic mouthwash versus a chlorhexidine mouthwash with respect to plaque inhibition and gingival inflammation.

## MATERIALS AND METHODS

The study was conducted by selecting the subjects from the Out Patient Department, Department of Periodontology of Y.M.T. Dental College, Kharghar, Navi Mumbai. This study was conducted on subjects who gave signed written informed consent in the language that was best understood by them and after obtaining prior ethical clearance from the institutional review board (IRB). Subjects between the ages of 20-30 years having gingivitis were included in the study. They were selected on the basis of the following criteria:

### Inclusion criteria

1. Subjects within the age group of 20-30 years of either sex.
2. Subjects with minimum of 20 teeth present in the dentition.
3. Systemically healthy and cooperative subjects.
4. Mean plaque score > 1 according to plaque index (PI) (Silness and Loe, 1964)
5. Subjects with gingivitis having probing pocket depth (PPD) of ≤ 3mm (with mean Gingival index scores >1; according to Loe and Silness, 1963)

### Exclusion Criteria

1. Subjects who had undergone any periodontal treatment in the past 3 months.
2. Subjects with a history of antibiotic intake within the past 3 months.
3. Subjects with a history of oral prophylaxis within the past 6 months.
4. Pregnant or lactating women.

The clinical parameters- plaque index (PI), gingival index (GI) were recorded and the case histories of 40 subjects were taken. The plaque score and gingival score was measured using a UNC-15 graduated periodontal probe. Thorough scaling and polishing were performed and the subjects were randomly

assigned by simple randomized sampling (lottery method) into two groups:

Group A (20 subjects) – 0.2% Chlorhexidine mouthwash (Hexidine®) group

Group B (20 subjects) - Probiotic mouthwash group

For the probiotic group, Darolac® Sachets (Aristo pharmaceuticals Pvt Ltd, India) containing 1 gm powder of 1.25 billion freeze dried combination containing *Lactobacillus acidophilus*, *Lactobacillus rhamnosus*, *Bifidobacterium longum*, and *Saccharomyces boulardii* and 10 ml ampules of distilled water were given. The patients were demonstrated and instructed to prepare the experimental probiotic mouthwash by mixing together the contents of the sachet and 10 ml of distilled water. Emphasis was made to explain to the patient that the solution had to be stirred thoroughly until all the contents were completely dissolved in the distilled water. The formulation had to be prepared and rinsed immediately once prepared. A randomized controlled trial was designed for a period of 2 weeks on 40 systemically healthy subjects between 20 and 30 years. They underwent complete scaling at baseline. The subjects were randomly divided into two groups i.e. Group A (20 subjects) - chlorhexidine mouthwash group and Group B (20 subjects) - probiotic mouthwash group. All the subjects were instructed to rinse their mouth with 10 ml of their respective mouthrinse for 1 min twice daily for 21 days, 30 min after brushing. The study design is summarized in the following fig 1.

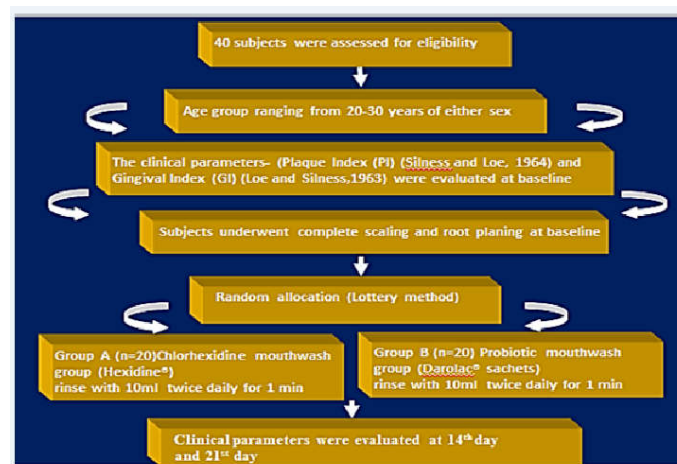


Fig 1 Study design

### Statistical Analysis

Data obtained was compiled on a MS Office Excel Sheet (v 2010). Data was subjected to statistical analysis using statistical package for social sciences (SPSS v 21.0, IBM). Inter group comparison of scoring was done using t test. Intra group comparison in both the groups was done using ANOVA test (Analysis of variance). p values of <0.05 was considered to be statistically significant.

## RESULT

The intragroup comparison of plaque index and gingival index was assessed for group A and group B (Table 1 and 2 and Graph 1 and 2). There was a statistically significant difference

seen for the intra group comparison for both chlorhexidine group and probiotic group at 14<sup>th</sup> day and 21<sup>st</sup> day with reduction in plaque index and gingival index for both probiotic group and chlorhexidine group. The intergroup comparison of plaque index and gingival index was assessed for group A and group B (Table 3 and 4 and Graph 3 and Graph 4). There was a statistically non-significant difference seen for the inter group comparison of the outcome variables with reduction in plaque index and gingival index for probiotic group as compared to chlorhexidine group.

**Table 1** Intra group comparison of Plaque Index using repeated measures of ANOVA

Group (n=20)	Baseline	14 days	21 days	p-value
Group A (CHX)	1.71±0.29	0.35±0.34*	0.40±0.42*	0.000*
Group B (Probiotics)	1.70±0.28	0.35±0.33*	0.26±0.34*	0.000*

\*Values highly significant (p < 0.001) as compared to the baseline levels using repeated measure ANOVA

**Table 2** Intra group comparison of Gingival Index using repeated measures of ANOVA

Group (n=20)	Baseline	14 days	21 days	p-value
Group A (CHX)	1.59±0.53	0.20±0.35*	0.24±0.28*	0.000*
Group B (Probiotics)	1.75±0.56	0.18±0.27*	0.18±0.27*	0.000*

\*Values highly significant (p < 0.001) as compared to the baseline levels using repeated measure ANOVA

**Table 3** Inter group comparison of Plaque Index using student t-test

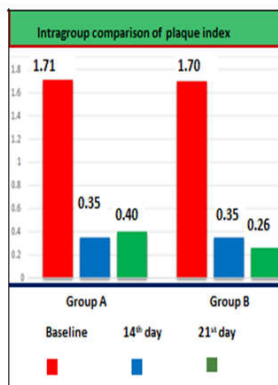
Group (n=20)	Group A (CHX)	Group B (Probiotics)	p-value
Baseline	1.71 ±0.29	1.70±0.28	0.825 <sup>#</sup>
14 days	0.35 ±0.34	0.35±0.33	1.000 <sup>#</sup>
21 days	0.40±0.42	0.26±0.34	1.09 <sup>#</sup>

#(p>0.05) Values non-significant using student t-test

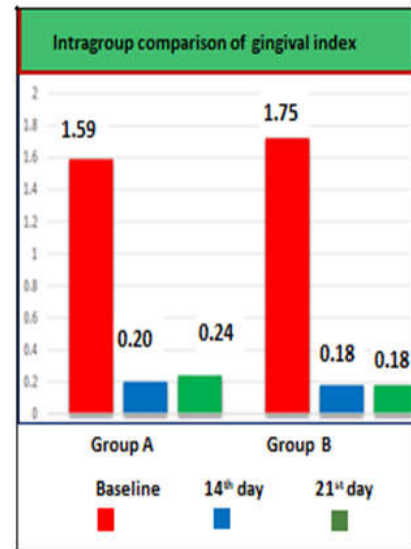
**Table 4** Inter group comparison of Gingival Index using student t-test

Group (n=20)	Group A (CHX)	Group B (Probiotics)	p-value
Baseline	1.59 ±0.53	1.75±0.56	0.352 <sup>#</sup>
14 days	0.20 ±0.35	0.18±0.27	0.842 <sup>#</sup>
21 days	0.24±0.28	0.18±0.27	0.423 <sup>#</sup>

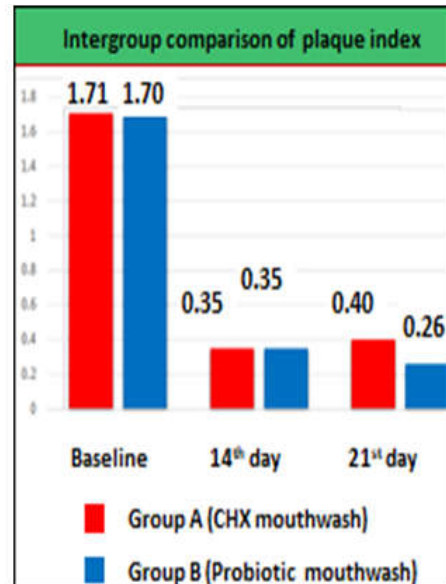
#Values non-significant (p>0.05) using student t-test



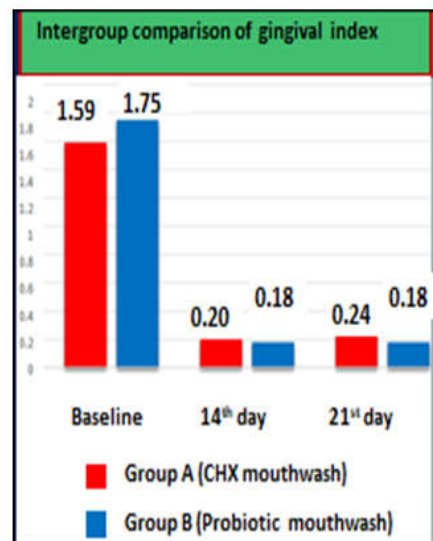
**Graph 1**



**Graph 2**



**Graph 3**



**Graph 4**

## DISCUSSION

The present study compared the efficacy of probiotic and CHX mouthwashes on oral health using two variables, viz. PI and GI. The results obtained showed that there was a significant improvement in gingival bleeding and plaque accumulation after 21 days in both the groups. But at 21st day, probiotic mouthwash was found to be more effective than chlorhexidine mouthwash in terms of plaque index and gingival index which is in accordance with the studies obtaining results similar to the present study i.e. Haukoja *et al* (2009), Shimauchi *et al.* (2008), Krasse *et al.* (2006), Ishikawa *et al.* (2003), Harini and Anegundi (2010), and the results were contradictory to the present study conducted by Shah RK *et al* (2014), Mishra R *et al* (2014).

The combination of probiotic strain was similar to those used by Haukoja *et al* (2009). The author reported the clinical treatment of periodontitis and gingivitis seems to be a potential target for probiotic bifidobacteria. A basic prerequisite to be an oral probiotic is the ability to bond and inhabit over the oral mucosal surfaces.<sup>7</sup>

Shimauchi *et al.* (2008) did a study in patients with gingivitis who were given freeze-dried *Lactobacillus salivarius* tablets for 8 weeks. The intake of tablets containing *L. salivarius* resulted in benefits in terms of pocket probing depth and PI. Krasse *et al.* (2006) did a study in patients with moderate gingivitis who were given one of the two different *L. reuteri* formulations (LR-1 or LR-2). LR-1 was efficacious in reducing both gingival index and plaque index.<sup>8</sup>

Ishikawa *et al.* (2003) observed inhibition of *P. gingivalis*, *P. intermedia*, and *Prevotella nigrescens* by daily ingestion of *L. salivarius* in tablet form. The administration of probiotics neutralized the pH of the saliva thus decreasing the acid production of plaque flora.<sup>10</sup>

Harini and Anegundi (2010) found significant reduction in the GI score, with the probiotic group (mean GI score = 0.2300) being better than the chlorhexidine group (mean GI score = 0.6805). The authors attributed the antiplaque effect of probiotics to its ability in reducing bacterial adhesion resulting in diminished bacterial growth and proliferation.<sup>6</sup>

Mishra R *et al.* (2014) found maximum reduction in PI and *Streptococcus viridans* counts with chlorhexidine rinse, followed by herbal mouthwash and minimum in probiotic mouthwash at the end of one week in children between 6 to 14 years of age.<sup>14</sup> Shah RK *et al.* (2014) demonstrated no significant difference in the gingival inflammation between *L. reuteri* probiotic and CHX mouthrinses at the end of study duration.<sup>13</sup>

The mere spelling of the word microorganism often gives a threat of health hazard. But, friendly microorganisms called probiotics have changed this concept and have given a new dimension for both general and oral health. The basic rationale behind the use of probiotics was that the human body lives in a heavily contaminated environment associated with millions of bacteria. Probiotics can be utilized by replacing pathogenic microorganisms with healthy ones. This concept of using beneficial bacteria has gained much popularity in the field of medical research in recent years where antibiotic resistance is

an increasing global problem.<sup>7</sup>

The present study employed Darolac sachets dissolved in water. Each 1 gm sachet of Darolac contains probiotics not less than 1.25 billion cells of *L. acidophilus*, *L. rhamnosus*, *B. longum* and *S. boulardii*. Lactobacilli produce low molecular weight bacteriocins with an inhibitory effect against a wide range of bacterial species related to oral diseases. *L. rhamnosus* demonstrates both high antimicrobial activity and high tolerance of environmental stress. Assistance of Bifidobacterium species includes metabolism of lactose, generation of lactic ions from lactic acid and vitamin synthesis. *Saccharomyces boulardii* has anti-microbial action. It is probably the synthesis of compounds like bacteriocin and the ability of probiotic bacteria to inhibit, cell association, colonization and invasion by pathogenic bacteria that are responsible for the anti-plaque action of probiotics like Darolac. Hence, in the present study, Darolac improved gingival health due to above mentioned facts.<sup>2</sup>

The reduction in the mean gingival index could be due to the bacteriocins secreted by probiotic bacteria such as *Lactobacillus* spp. They also activate immunocompetent cells to secrete both inflammatory and anti-inflammatory cytokines, which in turn modulates the mucosal immune system. Probiotics may also exert their beneficial effect in the oral cavity by directly interacting with microorganisms in dental plaque and indirectly by modulation of the innate immune systems. Aggregation alteration is another important mechanism of action of probiotics for inhibition of *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis* and *Prevotella intermedia*.<sup>5</sup> Probiotics may achieve the antiplaque activity by inhibiting the growth of microorganisms, reducing the adhesion of bacteria to the tooth surface, inhibiting the formation of the intercellular plaque matrix, reducing the formation of cytotoxic products by modifying plaque biochemistry and ecology to a less pathogenic flora.<sup>5</sup>

In our study we used probiotic mouthrinse containing combination of *Lactobacillus* strains and strain of bifidobacterium and *Saccharomyces* that contains 1.25 billion freeze dried bacterial combination. It is possible, in the complex environment of the human mouth, that probiotic “cocktails” of multiple strains would be more effective than any single probiotic agent.

In our study, the reduction in plaque accumulation and gingivitis could also be due to a confounding factor known as the Hawthorne effect or the attention bias. The subjects participation involved repeated dental examinations which may have stimulated the participants to improve their mechanical tooth cleaning measures. The participants usually improve their oral hygiene although they were unaware of the regimen administered to them.<sup>5</sup> It is pertinent to highlight some limitations and future prospects of this study in order to subsidize future clinical trials in this field. A cross-over design period could have been a more valid study design as it eliminates the bias of variable host response. The cross-over design would help to assess the patient’s acceptance and preference after exposure to both the plaque control measures. However, we would like to state that the major limitation of our probiotic preparation is that it needs to be used immediately once prepared and cannot be stored. Thus, we would

recommend that a proper vehicle is needed for delivering probiotics so that patient compliance can be improved.

Apart from the unacceptable taste, no other adverse effects on the oral mucosa such as ulcerations were noted with probiotic mouthrinse. Likewise, it would be interesting to evaluate the additional gastrointestinal benefits of probiotics in studies, where the patient is instructed to ingest rather than expectorate the probiotic mouthrinse. This was not possible to evaluate in this study, since it was a comparative clinical trial assessing the efficacy of two mouthrinses. A longer trial involving probiotics and further microbiological evaluation will also essential to assess the effect of probiotics.

Further randomized controlled trials are required to prove the long-term effects of probiotics on oral health. It is recommended for the manufacturers to improve the strain performance and activity by conducting further research to determine the exact dosage, improve consumer acceptance, stability and efficacy of probiotic-containing products by incorporating flavoring agents and making the products more palatable and more pleasing for use. 5

## CONCLUSION

The Probiotic mouth rinse tested was effective in reducing plaque accumulation and gingival inflammation. Therefore, the Probiotic mouth rinse has a potential therapeutic value and further long-term study is recommended to determine its efficacy.

It is perhaps surprising that chemical anti-plaque agents of superior or atleast equivalent efficacy, as an alternative to CHX, to overcome its undesirable side effects, safety and better acceptability have largely not been found and CHX remains the so-called gold standard of plaque control agents. With the public being increasingly cautious about the use of synthetic drugs owing to their adverse effects, "run of the masses" towards natural remedies is on an uptrend and oralhealth is no exception to this. Probiotics are proved to have dual health benefits, both locally on oral health as well as systemically on general health. Thus, probiotic mouthwashes can be advocated as suitable alternatives to CHX if their use and prescription is supported by strong scientific evidence.

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