Original Article

Oral Microbial Shift Following 1-Month Supplementation of Probiotic Chewable Tablets Containing *Lactobacillus reuteri* UBLRu-87 as an Adjunct to Phase 1 Periodontal Therapy in Chronic Periodontitis Patients: A Randomized Controlled Clinical Trial

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Abstract

Context: Although *Lactobacilli* as a probiotic was established as a treatment for a wide range of systemic infections, its role in periodontitis and oral microbiota is still under investigation. **Aims:** The present randomized clinical trial was aimed to evaluate the effects of probiotic chewable tablets containing *Lactobacillus reuteri UBLRu-87* along with initial periodontal therapy on clinical parameters and oral microbiota of chronic periodontitis (CP) patients. **Settings and Design:** The randomized controlled clinical trial. **Subjects and Methods:** Thirty CP patients were selected who received scaling and root planing (SRP) and were randomly allocated into two treatment groups; Groups A and B. Group A received *L. reuteri*-containing chewable probiotic tablets. The clinical parameters (plaque index, gingival index, probing pocket depth, clinical attachment level), and microbiological parameters (*Porphyromonas gingivalis* and *L. reuteri* levels using real-time polymerase chain reaction) were evaluated at baseline, following treatment at 1 month and 3 months in both groups. **Statistical Analysis:** Paired *t*-test and unpaired *t*-test were used for the statistical analysis. **Results:** On intergroup analysis, statistically significant improvement in clinical as well as microbiological parameters was observed in Group A (SRP + PROBIOTIC) compared to Group B (SRP ALONE) at all evaluation time points. **Conclusion:** Probiotic chewable tablets containing *L. reuteri* may be a useful adjunct along with initial periodontal therapy to slow recolonization of periopathogens along with improvement in clinical outcomes of CP. Further long-term trials are necessary to establish the optimal dosage of probiotics.

Keywords: *Chronic periodontitis, Lactobacillus reuteri, probiotics, root planing*

Introduction

The World Health Organization defined probiotics as live microorganisms, which, when administered in adequate amounts, confers health benefits to the host.[1] Probiotics evolved since the past ten decades since Elie Metchnikoff popularized it in his work "Prolongation of life."[2,3] Probiotics had proven effective against a wide range of systemic infection. In the field of periodontics, it possesses a high potential in terms of modification of plaque biofilm, management of halitosis, reviving anaerobic bacterial colonization, Probing pocket depth (PPD) reduction and Clinical attachment level (CAL) gain through several mechanisms including specific pathogen inhibition, host immune response modulation production of antimicrobial substances, competitive exclusion, etc.[4,5]

One probiotic strain that has been widely researched is *Lactobacillus reuteri* due to its potential to form antimicrobial substances. *L. reuteri* is an obligate hetero-fermentative organism and a true autochthonous Lactobacillus species.[5]

Even though the effects of the probiotics on periodontal conditions were evaluated in the literature,[6-10] their results have been controversial. Some of these studies have shown that probiotic application along with scaling and root planing (SRP) resulted in improvement in clinical and microbiological parameters,[6,7] whereas some literature did not prove any supplementary benefits of probiotics on periodontal diseases.[11,12] And only limited studies have evaluated

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the recolonization pattern of *L. reuteri* in the subgingival environment after the administration of probiotics.[10] Therefore the present study is designed to evaluate the efficacy of chewable tablets containing probiotic *L. reuteri*, clinically as well as microbiologically as an adjunct to conventional periodontal therapy in mild-to-moderate chronic periodontitis (CP) patients and to examine whether, in CP patients, *L. reuteri* can colonize the periodontal pockets.

Subjects and Methods

Study population and study design

The study population was those patients visiting the outpatient Department of Periodontology of the institution where the study was conducted. Altogether, 35 patients were examined for their eligibility to take part in the present study, of which five patients were excluded based on the inclusion criteria. The protocol was followed for the study analysis. Systemically healthy 30 patients with CP were selected between 20 and 60 years of age, which included 24 males and 6 females. This parallel-arm, double-blinded, randomized controlled clinical trial was conducted according to 1975 Helsinki declaration guidelines as revised in 2013. Informed written consent was acquired from all participants, after a full explanation of the study aspects. The study protocol was performed between March 2019 and September 2019.

The inclusion criteria for the study were: patients with Stage II/Stage III and Grade A/Grade B periodontitis according to the American Academy of Periodontology 2018 classification[13] with CAL 3–6 mm in at least 2 quadrants, presence of at least 16 remaining teeth with a minimum of four teeth in each quadrant, presence of at least single tooth with PPD 5–7 mm in minimum 2 quadrants.

The exclusion criteria for the study were as follows: periodontal therapy or antibiotic treatment in the preceding 6 months before the initial examination, patients undergoing active orthodontic therapy, uncontrolled systemic conditions, known drug allergies or infectious diseases, smoking/alcohol consumption, pregnant and/or lactating females.

Treatment protocol

The sample size for the present study was calculated using n-Master software. Minimum 12 patients were required in each group to provide 80% power to the study with an $\alpha = 0.05$. Rounding the sample size to 15, altogether 30 patients were enlisted in the trial once they fulfilled the inclusion criteria. The primary investigator performed SRP in all the patients using ultrasonic scaler (DTE D1 Ultrasonic scaler, Guilin woodpecker) and in deeper areas using hand instruments (Gracey curettes, Hu Friedy Mfg.) in a single session, following which patients were allocated randomly into both treatment groups, based on their sequence of reporting to the department by the study coordinator. Probiotic tablets (containing *L. reuteri* UBLRu-87, 0.5 billion CFU, Unique Biotech Ltd, Hyderabad, India) were distributed to the test group patients by the study coordinator and were guided to consume the probiotic chewable tablet once a day (evening) after the toothbrushing for a 1-month duration from initial SRP, whereas the control group received SRP alone. A single dose of probiotic tablet per day for test group patients in the present study was selected based on the evidence from studies by Vicario *et al*.[12,14] All patients were asked to abstain from the use of other probiotic and antibiotic medication during the study period. Taking intraoral photographs, recording all the clinical measurements, and plaque sample collection at baseline, and recall visits were performed by another blinded calibrated secondary examiner for both treatment groups [Figure 1]. Even though probiotic supplementation in the test group was for 1 month, the clinical and microbiological parameters were continued to evaluate till 3 months of therapy to assess how long do the probiotic effects persist in the periodontal environment. To evaluate the compliance and adverse effects in test group patients, a questionnaire [Figure 2] was also given to them by the study coordinator.

Clinical examination

The clinical parameters were recorded for the full mouth at baseline (before SRP), 1 month and 3 months using a periodontal probe (UNC-15, University of

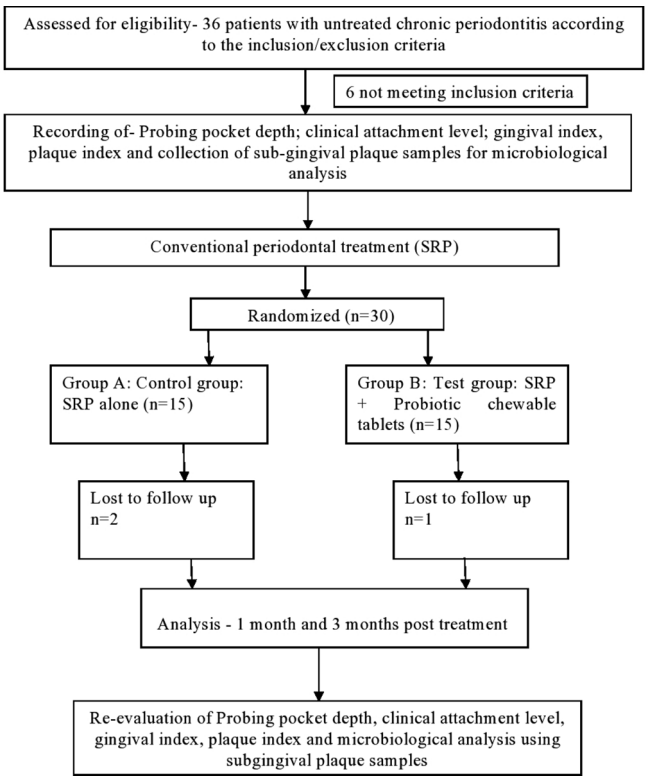

Figure 1: Consort flow chart showing patient inclusion and follow-up in the study

SL. NO	QUESTIONS	ANSWERS
1	Many patients have difficulties taking their medicines as the doctor recommended. In the past month, was there any day or period when you did not take the prescribed and distributed probiotic medication as recommended? a)Forgetfulness b)Drugs cause discomfort/ malaise/ dizziness/ fatigue	
2	If symptoms are present have you ever spoken with someone about these symptoms? Other doctors /Pharmacist /Nurse /Family/friends	
3	Do you think the symptoms you had may be caused by your medications?	
4	Have these symptoms led you to change the intake of medicines or stop taking them?	
5	Do the symptoms subside when you stop taking the prescribed medicines?	

Figure 2: Compliance and adverse effects of drug questionnaire for test group participants

North Carolina probe, Hu Friedy Mfg. Co. Inc.) which included plaque index (PI) (Silness and Loe, 1964),[15] gingival index (GI) (Loe and Silness, 1963),[16] PPD and CAL. Probing was done at 6 sites on each tooth. All measurements were rounded off to the nearest 0.5 mm. PPD reduction and the levels of *Porphyromonas gingivalis (Pg)* were considered as the primary outcome of the study.

Plaque sampling

For subgingival plaque sampling, a tooth was selected with PPD of 5–7 mm in at least 2 quadrants. Hence, per patient, a total of 2 sites were sampled. At baseline, 1 month and 3 months, samples were taken from the same sites. Before microbiological analysis, the samples from an individual were pooled. After PI was scored and preceding other clinical parameters, the plaque samples were collected from selected sites in each patient. Cotton rolls were used for the isolation of sites, and supragingival plaque removal. Using sterile curettes, subgingival samples were obtained from the gingival crevice. Plaque samples were immediately placed in sterile vials containing RNA later solution (RNA Stabilization reagent, QIAGEN GmbH, Germany) and were stored until further analysis at −20°C.

Microbiological examination

The collected subgingival plaque samples were submitted to qualitative real-time polymerase chain reaction (PCR) analysis of *Pg* in the test and control group, were as *L. reuteri* levels only in the test group. *Pg,* a red complex periodontal pathogen, was recognized as the keystone pathogen in the periodontal disease pathogenesis, which has the potential to alter the subgingival microbiota to a more virulent form. Hence for microbiological examination, *Pg* was selected for evaluation of *L. reuteri* effects on periodontal microbiota. DNA isolation was done using 1 ml of plaque sample, which was collected in a micro-centrifuge tube. Centrifugation of the sample was done at 12,000 rpm, for 10 min, the afloat was discarded. Pellet was incubated for 3 h at 55°C after the addition of 600 µl of extraction buffer. Phenol:Chloroform:Isoamyl alcohol (25:24:1) was added to the tube in equal volume and 30 s given for vortexing. Again centrifugation was done at 12,000 rpm for 10 min. It was then taken in a sterile microcentrifuge tube after removal of an aqueous phase and incubated at-20°C for 1 h after the addition of 0.6 volumes of isopropyl alcohol. The tubes were centrifuged for 10 min, at 10,000 rpm, after discarding the supernatant. After washing the pellet in 500 µl of 70% ethanol, it was dried and dissolved in 20 µl sterile distilled water and centrifuged at 10,000 rpm for 10 min. At −20°C, the samples were stored. A spectrophotometer was used to assess the concentration and purity of DNA. Perkin Elmer Primer Express® Software was used to design the primers for quantification analysis. Based on melting temperature, High-performance liquid chromatography was used to purify the synthesized primers. Applied Biosystems™ (A40393, Real-Time PCR Systems, Thermo Fisher SCIENTIFIC) Step One Real-Time PCR was used for quantification. All reagents were procured from Life Technologies. 0.005U AmpliTaq Gold, 1X Taq man-PCR buffer, 0.35 µl DNA template, 3 mM MgCl2, 0.2 mM each of dATP, dCTP, dGTP,0.4 mM dUTP, 0.002 U AmpErase UNG erase enzyme and 50–900 nM of oligonucleotide primer were contained in standard reaction volume 10 µl. UNG erase activation for 2 min at 50°C was the initial step of RT-PCR, followed by a 10 min hold at 95°C. Cycles (n = 40) consisted of a 30 s annealing/extension at 55°C preceded by a 15 s melt at 95°C. For extension, the final step was incubation for 30 s at 60°C. Against a serially diluted standard, all reactions were duplicated. Automatic detection by the system was set for threshold cycle analysis of all samples.

Statistical analysis

SPSS Version 23.0 (IBM. SPSS, Statistical Package for the Social Sciences software, Delaware, Chicago, IL) was used to analyze the collected data. Descriptive statistics frequency analysis, percentage analysis for categorical variables, and the mean and standard deviation for continuous variables were used to describe the data. Paired sample t-test and unpaired sample t-test were used to find the significant difference between the bivariate samples in paired groups and independent groups, respectively. To control the type I error for multivariate analysis, the repeated measures of ANOVA were used with Bonferroni correction. Fisher's exact was used to find the significance of categorical data. The probability value (P-value) of 0.05 was considered significant in all the statistical tools used in the present study.

Results

Thirty, systemically healthy, mild-to-moderate CP patients (with age between 20 and 60 years, including 24 males and 6 females) were included in the study of which three patients were dropped out of the study due to personal reasons. Hence, the final statistical analysis was performed using 14 test group patients and 13 control group patients.

Clinical parameters

The baseline clinical parameters and the *Pg* levels for the patients were comparable for both treatment groups ($P > 0.05$) [Table 1]. The mean PI, GI, PPD, and CAL values at baseline, 1 month, and 3 months intervals for both groups are shown in Table 2 ($P \leq 0.05$). Within the test group and control group, the changes in PI, GI, PPD, and CAL from baseline were significant ($P \leq 0.05$) at 1 month and 3 months period. Inter-group comparison of the clinical parameters is demonstrated in Table 3 ($P \leq 0.05$). After treatment, the clinical parameters were significantly reduced in the test group at all-time points compared to the control group. PPD outcome measures at baseline and 3 months in study groups using *t*-test were demonstrated in Table 4 ($P \leq 0.05$). Both groups demonstrated a statistically significant decrease in the number of deeper probing depth sites (5–7 mm) and an increase in the number of shallow probing depth sites (≤4 mm) when compared to baseline at 3 months evaluation.

Table 1: Baseline characteristics of participants in study groups (mean±standard deviation)

	SRP + probiotic group (*n*=14)	SRP alone group (*n*=13)	*P*
Male/female	11/3	10/3	1.01¶
Age (years)	37.5±7.12	37.8±7.90	0.906*
PI (score)	1.69±0.36	1.80±0.32	0.452*
GI (score)	1.89±0.26	1.90±0.27	0.940*
PPD (mm)	5.27±0.49	5.20±0.40	0.691*
CAL (mm)	3.99±0.56	4.17±0.20	0.283*
P. gingivalis (mean log CFU/ml)	6.60±0.63	6.43±0.64	0.493*

*Student *t*-test $P>0.05$ not significant, ¶Chi- square test $P>0.05$ not significant. PI: Plaque index; GI: Gingival index; PPD: Probing pocket depth; CAL: Clinical attachment level; *P. gingivalis: Porphyromonas gingivalis*; SRP: Scaling and root planning

Microbiological parameters

Figure 3a depicts the changes in the *Pg* levels in both the groups at baseline, 1 month, and 3 months of the study period. Treatment led to a significant reduction in the *Pg* levels from baseline to 3 months in both the treatment groups ($P \leq 0.05$). The inter-group comparison suggested that the test group was better for all time intervals compared to the control group. Figure 3b demonstrates the *L. reuteri* levels in subgingival plaque samples of the test group from baseline to 3 months. There was a statistically significant difference in levels of *L. reuteri* from baseline to 3 months in the test group ($P \leq 0.05$).

Need for surgery

The "need for surgery" outcome measure was evaluated according to Cionca *et al.*[17] based on the PPD data, in both the study groups. At 3 months of evaluation, patients in the SRP + Probiotic group had significantly lesser sites "in need of surgery" compared with the SRP alone group ($P \leq 0.05$) when compared to baseline. The reduction in total number of sites in need for surgery at 3 months evaluation was 24.28 ± 3.33 in SRP + probiotic group and 14.16 ± 3.12 in SRP alone group.

Discussion

The present clinical trial was aimed to assess the clinical as well as microbiological effects of probiotic chewable tablets containing, *L. reuteri* UBLRu-87 as an adjunct to

Table 2: Intra- group comparison of clinical and microbiological parameters in scaling and root planning + probiotic group and scaling and root planning alone group

	SRP + probiotic group (*n*=14)				SRP alone group (*n*=13)			
	Baseline	1 month	3 months	*P*	Baseline	1 month	3 months	*P*
PI (score)	1.69±0.36	0.79±0.23*	0.95±0.19¶	0.0005†	1.80±0.32	1.11±0.12*	1.28±0.23¶	0.0005†
GI (score)	1.89±0.26	0.81±0.14*	1.02±0.16¶	0.0005†	1.90±0.27	1.17±0.18*	1.36±0.18¶	0.0005†
PPD (mm)	5.27±0.49	4.31±0.52*	3.6±0.56¶	0.0005†	5.20±0.40	4.80±0.42*	4.35±0.38¶	0.0005†
CAL (mm)	3.99±0.56	3.43±0.41*	2.97±0.35¶	0.0005†	4.17±0.20	3.84±0.23*	3.50±0.21¶	0.0005†
P.gingivalis (mean log CFU/ml)	6.60±0.63	3.35±0.34*	3.94±0.33¶	0.0005†	6.43±0.64	3.84±0.66*	4.54±0.49¶	0.0005†

**P*≤0.05 using Paired *t*-test within the study groups (Baseline - 1 month), ¶*P*≤0.05 using Paired *t*-test within the study groups (Baseline - 3 month), †Significant difference from within the groups from baseline to 3 months using repeated measures of ANOVA with Bonferroni correction (*P*≤0.05). PI: Plaque index; GI: Gingival index; PPD: Probing pocket depth; CAL: Clinical attachment level; *P. gingivalis: Porphyromonas gingivalis*; SRP: Scaling and root planning

Table 3: Inter group comparison of changes in clinical parameters from baseline to 3 months

	Baseline to 1 month			1 month to 3 months			Baseline to 3 months		
	SRP + PROB	SRP alone	*P*	SRP + PROB	SRP alone	*P*	SRP + PROB	SRP alone	*P*
PI (score)	0.90±043	0.68±0.18	0.0005*	−0.16±0.33	−0.16±0.48	0.012*	0.74±0.41	0.51±0.16	0.0005*
GI (score)	1.07±0.32	0.73±0.15	0.0005*	−0.20±0.24	−0.19±0.01	0.0005*	0.86±0.35	0.54±0.18	0.0005*
PPD (mm)	0.96±0.37	0.39±0.11	0.0005*	0.70±0.06	0.44±0.14	0.0005*	1.66±0.36	0.84±0.13	0.0005*
CAL (mm)	0.56±0.36	0.33±0.07	0.0005*	0.45±0.6	0.33±0.01	0.0005*	1.02±0.39	0.66±0.06	0.0005*

*Student *t*-test; statistically significant between the groups (*P*≤0.05). PI: Plaque index; GI: Gingival index; PPD: Probing pocket depth; CAL: Clinical attachment level; SRP: Scaling and root planning

Table 4: Probing depth outcome measures at baseline and 3 months in study groups

	SRP + Probiotic (*n*=182)	SRP alone (*n*=181)	*P*
Number of sites with PPD ≤4 mm			
Baseline	155.57±9.80	156.7±9.32	0.621
3 months	179.86±3.54*	170.7±3.2¶	0.0005†
Number of sites with PPD 5-7 mm			
Baseline	26.57±9.61	25.48±9.52	0.921
3 months	2.29±0.99*	11.32±0.89¶	0.0005†

*$P \leq 0.05$ using Paired *t*-test in the test group (Baseline - 3 month), ¶$P \leq 0.05$ using Paired *t*-test in the control group (Baseline - 3 month), †Student *t*-test between the groups ($P \leq 0.05$). PPD: Probing pocket depth, SRP: Scaling and root planning

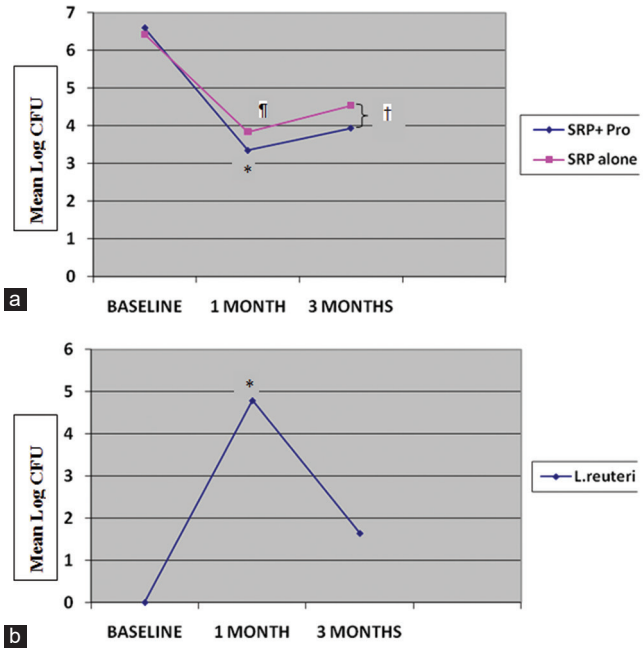

Figure 3: (a) Inter- group comparison of *P.gingivalis* levels at baseline, 1 month and 3 months in study groups using student t test. *Significant difference in Test group when compared with baseline values (Repeated Measures ANOVA, Bonferroni, $P \leq 0.05$). ¶Significant difference in Control group when compared with baseline values (Repeated Measures ANOVA, Bonferroni, $P \leq 0.05$). †Significant difference among groups in the same time point (Student t test, $P \leq 0.05$). (b) *L.reuteri* levels in subgingival plaque samples of SRP + Probiotic group at baseline, 1 month and 3 months. *Significant difference in L reuteri levels in Test group when compared with baseline value (Repeated Measures ANOVA, Bonferroni, $P \leq 0.05$)

conventional periodontal therapy and to evaluate whether periodontal pockets can be colonized by *L. reuteri* in CP patients. This study showed that consuming *L. reuteri,* along with SRP facilitated a better improvement in clinical parameters compared to SRP alone in 3 months treatment period. Corresponding to improvement in clinical parameters, microbiological parameters also showed significant improvement with a reduction in *Pg* levels and the increase in *L. reuteri* levels on 1 month and 3 months evaluation compared to baseline ($P \leq 0.05$).

Regarding the primary study outcome, significantly greater PPD reductions and clinical attachment gain were noticed in the SRP + Probiotic group at all assessment time points compared to baseline. In the probiotic and SRP alone groups, reduction in PPD was detected as 1.66 mm and 0.84 mm and clinical attachment gains were 1.02 mm and 0.66 mm, respectively. In moderate deep pockets (4–6 mm), the literature reported a PPD reduction between 0.5 and 2.2 mm at 12 months after SRP.[18] In the present study, the control group demonstrated a mean PPD reduction in agreement with the previous literature.[18]

In the present study, at 3 months evaluation, in the SRP + Probiotic group, statistically significant PPD reductions were observed, particularly in deep pockets, and significantly lower percentages of sites with a residual pocket depth of ≥5 mm were evident. This superior results in the probiotic group could be due to the additional effects of increased proportions of *L. reuteri* in the subgingival environment following probiotic administration along with mechanical debridement and supragingival plaque control of the patients. The PPD reduction in the present study, favoring the probiotic group which is >1 mm, might be assigned not only to the marked antimicrobial activity of probiotics but also due to their immune-modulatory effects leading towards anti-inflammatory action.

Antimicrobial substances such as reuterin and reutericyclin, produced by *L. reuteri* can suppress an extensive range of pathogens by induction of oxidative stress in cells and also by the ability to prevent the binding of periopathogens to host tissue. These possible mechanisms constitute the basis of the direct or indirect anti-plaque properties of *L. reuteri*.[19] The anti-inflammatory effects of the probiotics and the resolution of the inflammation could be one of the possible reasons for the significant improvements in PPD and attachment levels. PI and GI parameters demonstrated the anti-plaque and anti-inflammatory effects of probiotic chewable tablets. The intergroup comparison of the PI and GI mean scores were statistically significant and were in support of the probiotic group ($P \leq 0.05$).

These observations were commensurable to previous literature, which revealed a statistically significant PPD reduction after the probiotic usage. Tekce *et al.*[10] assessed the efficacy of *L. reuteri* probiotic lozenges as an adjunct to SRP in periodontitis. They included periodontitis patients with horizontal bone loss and presence of at least two teeth with one site PPD of 5–7 mm and GI ≥2 per quadrant. The measured clinical parameters were significantly lower in the probiotic group compared to the control group at all

evaluation time points following treatment. But, conflicting results, reported by Iniesta *et al*.[12] and Iniesta *et al*.,[12] which failed to show any advantages of probiotics, in terms of changes in PI, GI, mean PPD, and CAL. Variation in the study designs, probiotic strains, study population, probiotic delivery systems, dosage, and frequency may be the reasons for these disputable observations.[20] Teughels *et al*.[9] in his study straight away after full-mouth disinfection, used *L. reuteri* lozenges for 3 months, 2 times a day, that is 10^8 CFU/day. Vivekananda *et al*.[21] used a similar method with an irregularity that probiotic tablets were initiated by patients 21 days after SRP and with no added oral disinfection.

Teughels *et al*.[9] showed that when probiotics were used as supplementary to full-mouth disinfection protocol, there was a statistically significant depletion in moderate and deep pockets. Vicario *et al*.[14] also reported comparable results. One month's consumption of probiotics without conventional intervention showed significant variation in the percentage of sites with 4–5 mm PPD, which supported the probiotic group. It can be postulated from these results, that probiotics might be a useful adjuvant for the nonsurgical periodontal therapy of pockets ≥4 mm in patients with CP.

The need for surgery outcomes was assessed in the present study at baseline and 3 months following therapy in both study groups. Remaining pockets ≥5 mm with bleeding on probing was defined as the need for surgery outcome.[17] The reduction in total number of sites in need for surgery at 3 months evaluation, in the present study, was 24.28 ± 3.33 in SRP + probiotic group and 14.16 ± 3.12 in SRP alone group. The patients in need for surgery were more in SRP alone group ($P \leq 0.05$) compared to the test group. In literature for probiotic therapy, only two studies have examined the need for surgery outcome measures.[9,10] Tekce *et al*. showed that after 1 year of follow-up, significant differences between groups in terms of percentage of sites, percentage of teeth, and number of patients for whom surgery was needed.[10] Fascinatingly, Teughels *et al*. found a significant reduction only in deep probing sites in "need for surgery" at 3 months evaluation.[9] Differences in follow-up periods (1 year/12 week)[9,10] could be the reason for these disparities.

To assess whether *L. reuteri* can colonize the subgingival environment following probiotic therapy, *L. reuteri* levels were estimated in the SRP + Probiotic group from baseline to 3 months post-therapy. There was no detection of *L. reuteri* at baseline. A 1-month evaluation, the highest levels of *L. reuteri* levels were estimated (4.79 ± 1.47 mean log CFU) in the subgingival plaque implying the colonization in the subgingival area by *L. reuteri*. At 3 months evaluation, *L. reuteri* levels were dropped to 1.64 ± 1.39 mean log CFU, an observation that may lead to reconsider the prescription of the supplement by clinicians suggesting the short term effects of probiotics on the periodontal environment.[10] These results indicate that the probiotic usage was correlated with modification in the subgingival microbiota, mainly related with a decrease in the number of target periodontal pathogens, like *Pg* levels in SRP + Probiotic group (2.66 ± 0.42 mean log CFU) compared to SRP alone group (1.89 ± 0.31 mean log CFU) over 3 months study period. These observations are per an existing similar study, which reported a statistically significant lowering of subgingival microbiota and in the number of selected five pathogens (*Aggregatibacter actinomycetemcomitans, Prevotella intermedia, Pg, Treponema denticola and Tanerella forsythia*) after 4 weeks of using probiotic *L. salivarius* WB.[11] Emphasize has to be given that interpretation of microbiological results should be done with caution, due to their intrinsic variability and dosage of the probiotic tablets administered.[22] Besides, the intake of *L. reuteri* tablets culminates in the subgingival colonization of this bacterium, as revealed by qPCR analysis.

One of the main shortcomings of the present study was that it was short term study, and the effect of probiotics on recolonization of periodontal pathogens could have been observed over a much longer period. Hence, further longitudinal trials are necessary to assess the re-colonization pattern of periodontal pockets by pathogens as well as the probiotic organism. Another limitation of the present study was that there was no placebo intervention in the control group.

Within the limitations of the present study, it can be implicated that adjunctive probiotic therapy, along with scaling and root planning, provides additional benefit over SRP alone on clinical and microbiological parameters of CP patients over 3 months duration. During the study period, no adverse effects were reported by any test group patients or observed by the clinicians, while some patients reported improvement in bowel movements and appetite, thus proving the safety of the probiotic chewable tablets containing *L. reuteri*.[9,10,23]

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(UBL chewable tablets) as per the requirements, but they were not involved in the data analysis.

Conflicts of interest

There are no conflicts of interest.

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