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Role of probiotics containing *Lactobacillus reuteri* in adjunct to scaling and root planing for management of patients with chronic periodontitis: a meta-analysis

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Abstract. – OBJECTIVE: Probiotics, nowadays are the effective in management of chronic periodontitis when used as an adjunct to non-surgical periodontal therapy. However, the beneficial effects of probiotics are varied with the nature of bacterial strain. Our meta-analysis aims at evaluating the magnitude of improvement in clinical and microbiological parameters, with administration of *Lactobacillus reuteri* alone in adjunct to scaling and root planning (SRP).

MATERIALS AND METHODS: A digitalized database search was made in MEDLINE (PubMed), Scopus, CENTRAL (Cochrane Registry of Trials), Web of Science, and EMBASE, to identify eleven randomized clinical trials published within last decade (2009-2019), with double blind, placebo controlled study design. The data extraction was carried out and subject to both qualitative and quantitative synthesis. The primary outcomes assessed were gain in clinical attachment level (CAL), reduction in probing pocket depth (PPD), and reduction in microbial levels.

RESULTS: The meta-analysis plots were used to assess all the clinical outcomes. The mean difference of reduction in PPD at 21 days (MD-0.61) and 3 months (MD-0.40), and CAL gain at 3 month (MD-0.30) showed favourable response in the sites treated with probiotics containing *Lactobacillus reuteri* in addition to SRP. The meta-plots for major peridonto-pathogens constructed at 21 days follow-up, showed short-term effective reduction.

CONCLUSIONS: Within the limits of the study, *Lactobacillus reuteri* containing probiotics showed a significant clinical and microbiological benefit, however, the favourable effect was shown to be short-term.

Key Words:

Lactobacillus reuteri, Scaling and root planing, Probiotics, Periodontitis, Systematic review, Meta-analysis.

Introduction

Periodontitis is denoted by overt inflammation of gingiva along with loss of clinical attachment and most importantly resorption of alveolar bone[1]. The primary etiological factor of initiation of periodontitis is devoted to the presence of pathogenic bacteria which induces connective tissue changes to further set in the extensive progression of periodontal disease[2]. Considering the traditional interventions to be effective, every effort is researched to provide improvement in periodontal therapies. Probiotics are nowadays in current research and plenty of trials have been conducted to prove same efficacy and its possible impact in improvement of periodontal disease and overall oral health[3-9].

Probiotics are the potentially beneficial bacteria, which, when administered in the host, show synergistic beneficial effects[10]. The benefits conferred by the probiotic strains are mostly delivered by few possible mechanisms. These include: (a) providing nutrients and cofactors, (b) competition with pathogens, (c) interaction with virulence factors of pathogens, and (d) stimulating the immune response of the host. The ability of the probiotics to carry out modifications in the pathogenicity of biofilm include the inhibition of proliferation and growth of micro-organisms and replacing them with beneficial ones[11]. The probiotic organisms, which prove beneficial ones, include *Lactobacillus* species, *Bifidibacterium* species, etc.[12]. The bacterial strains most widely used as probiotics, includes the species of *Lactobacillus,* and the genus *reuteri* is the most potent among all[13].

Lactobacillus reuteri is also known to have an immunomodulatory effect on the biofilm by suppressing human TNF production by lipopolysaccharide-activated monocytoid cells[14]. The admin-

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istration of *Lactobacillus reuteri* as a probiotic, showed low MMP-8 and high TIMP-1 levels, suggesting a reduction of the inflammation associated markers at the end of follow-up[15]. The strains of *Lactobacillus reuteri* synthesize an anti-microbial compound named reuterin (beta-hydroxypropionaldehyde), which has the ability to inhibit both gram negative and gram positive bacteria, along with other fungi, protozoal infections[16]. Reuterin prevents microbial colonization by interfering with pathogen's adhesion to host surface.

A numerous systematic review proves the role of probiotics to be beneficial when used in adjunct to scaling and root planning in treatment of chronic periodontitis. The majority of systematic reviews conducted in the last decade found the evidence available to be inconclusive for proving the effectiveness of probiotics in preventing or treating periodontal diseases[17-19].

Matsubara et al[20] performed a systematic review in 2016 on the role of probiotic bacteria in managing periodontal disease and included twelve Randomized Clinical Trials (RCTs) which used *Bifidobacterium* and *Lactobacillus* in probiotics. They concluded that the use of probiotics, especially *Lactobacilli*, have a favourable adjunctive effect when used with SRP and boost periodontal disease indices thereby reducing the need for antibacterial drugs. Martin-Cabezas et al[21] in 2016 also conducted a systematic review and meta-analysis on the clinical efficacy of probiotics as an adjunctive therapy to nonsurgical periodontal treatment of chronic periodontitis. They included only four RCTs in the systematic review and conducted a meta-analysis on three of them and advocated the use of *Lactobacillus reuteri* as an adjunct to SRP in deep periodontal pockets over short periods of time.

However, none of the previous systematic reviews have ever analyzed the evidence pertaining to adjunctive use of *Lactobacillus Reuteri* alone as probiotic agent, both in terms of clinical and microbiological improvements. The objective of this systematic review was to analyze the available scientific evidence on the effects of probiotics containing *Lactobacillus Reuteri* in adjunct to scaling and root planning for management of patients with chronic periodontitis.

Materials and Methods

This review was conducted according to the preferred reporting items for systematic review and meta-analysis (PRISMA) guidelines[22]. The protocol of the systematic review was prepared well ahead of search strategy and discussed among the reviewers to be clear enough on the search and selection criteria in order to reduce errors in the study selection.

Research Question

What is the effect of probiotics containing *Lactobacillus Reuteri* in adjunct to scaling and root planning for management of patients with chronic periodontitis?

Patient/Population: patients suffering from chronic periodontitis with 4 mm of attachment loss and pocket depth 4 mm demanding non-surgical periodontal therapy.

Intervention: probiotics containing *Lactobacillus Reuteri* administered orally as lozenges, tablets, mouthwashes, toothpastes, chewing gums etc. in adjunct to non-surgical periodontal therapy.

Comparison: placebo (mimicking the form of probiotics administered in exposure group) in adjunct to non-surgical periodontal therapy.

Outcomes: baseline and post-follow-up clinical outcomes (probing depth, clinical attachment level) and microbiological outcomes (bacterial counts).

Search Strategy

The search was carried out in various electronic databases, like PubMed, Scopus, EMBASE, CENTRAL (Cochrane Registry of Trials) and Web of Sciences (WoS) using the following search string: (Chronic Periodontitis OR Periodontal Disease OR Periodontal Pockets OR Attachment Loss OR Periodon*) AND (Probiotics OR Symbiotics OR Prebiotics OR Lactobacillus OR *L.Reuteri*).

The issues published for last decade in few reputed dental journals, like Journal of Periodontology, Journal of Clinical Periodontology, International Journal of Periodontics and Restorative Dentistry, Journal of Periodontal and Implant Sciences, Clinical Oral Investigations, Journal of Oral Sciences, were hand searched. The references of previously published systematic reviews along with other clinical studies were looked up on for any additional potentially eligible studies. Open grey literature of any unpublished trials and registry of clinical trials (clinical-trial.gov.in) were searched for trial protocols.

Selection Criteria

The following selection criteria were considered for inclusion of the studies:

- double blind, and placebo-controlled, RCTs published within last 10 years.

- presence of at least one test group in which probiotics is administered as an adjunct to scaling and root planning (SRP); along with an appropriate placebo controlled group, in which placebo is administered as an adjunct to SRP for the treatment of chronic periodontitis;
- patients included in the RCT should present with chronic periodontitis with ≥4 mm of attachment loss and pocket depth ≥4 mm demanding scaling and root planning;
- patients included in the RCT should have no systemic diseases or be on any long-term medication that could potentially influence the outcome of periodontal therapy.

Data Extraction

The data from the included studies were extracted meticulously by two independent reviewers) on an excel spreadsheet (Microsoft Word, Microsoft Inc., Radmond, WA, USA). The data related to demographic characteristics; study design and sample size; smoking status, type of probiotic administered; follow-up duration; source of funding and study setting; along with baseline and post-follow-up outcomes (probing depth, clinical attachment level, bleeding on probing scores). The authors of the included studies were contacted in case of missing data, or any lack of clarity in the information provided.

Outcomes

The primary outcomes preferably evaluated in this systematic review includes Reduction in Probing Pocket Depth (PPD), Gain in Clinical Attachment Level (CAL), and reduction in microbial levels of periodontopathogens (*Aggregatibacter actinomycetemcomitans, Prevotella intermedia, Porphyromonas gingivalis*) among all follow-up visits. The baseline and post follow-up measurements of all clinical and microbiological parameters from all included were extracted in mean and standard deviation (SD) values for facilitating quantitative data synthesis.

Data Synthesis

The data extracted for the clinical and microbiological parameters from all the included studies were subject to both qualitative and quantitative synthesis. The quantitative data extracted for different outcomes were constructed and subjected to meta-analysis in case of availability of at least 2 studies with similar outcome measurements at comparable follow-up period. A qualitative analysis was carried out, in case the meta-analysis could not be performed.

Risk of Bias (RoB) Assessment

The quality of the included studies were made by assessment of bias pertaining to randomization process and allocation concealment, blinding of participants, personnel or assessor, and any incomplete or selective outcome data reporting. The RoB assessments were judged independently by two reviewers and in case of any discrepancies and in case of doubt relating to the judgment, a third reviewer was consulted to arrive at a consensus. A graded response of options consisting of "high risk", "unclear risk", and "low risk" were considered for each of the domains.

Results

The systematic search in the digital databases and hand searching of related dental journals yielded a pool of 1145 reports, which when subjected to strict title and abstract evaluation by the independent reviewers, only 15 reports were identified for full-text evaluation. The systematic process of the study selection is summarized in the PRISMA flow chart provided in Figure 1.

Eleven studies[7,8,15,23-30] were included in this systematic review and meta-analysis, which met the selection criteria. Out of eleven, a total of eight studies[7,8,15,24-26,28,30] were subjected to quantitative analysis, and the rest three studies[23,27,29] were analyzed qualitatively. The general characteristics of all the included trials are showed in Table I. The reason for exclusion of the identified reports not meeting the selection criteria are provided in Table II.

Most of the included trials are judged to be of low to moderate risk of bias. A low or clear assessment was provided for all included trials considering proper reporting of randomization process, blinding, attrition and addressing selection bias. However, two of the trials did not mention the allocation concealment (Figure 2).

Three studies[23,27,29] could not be included for meta-analysis, hence they were considered for qualitative analysis. One study by Costacurta et al[27] had only measurements made at 1 month follow-up for assessing the reduction in clinical parameters with a high risk of bias for not mentioning the allocation concealment. On the other hand, Vicario et al[29] provides the data in percentage of sites showing reduction in PPD and gain in CAL, with no information of mean value at baseline and all follow-ups. Another study by Grusovin et al[23] could not be included for meta-analysis along with other included trials, as the report employs a specialized guided biofilm therapy (GBT) instead of SRP.

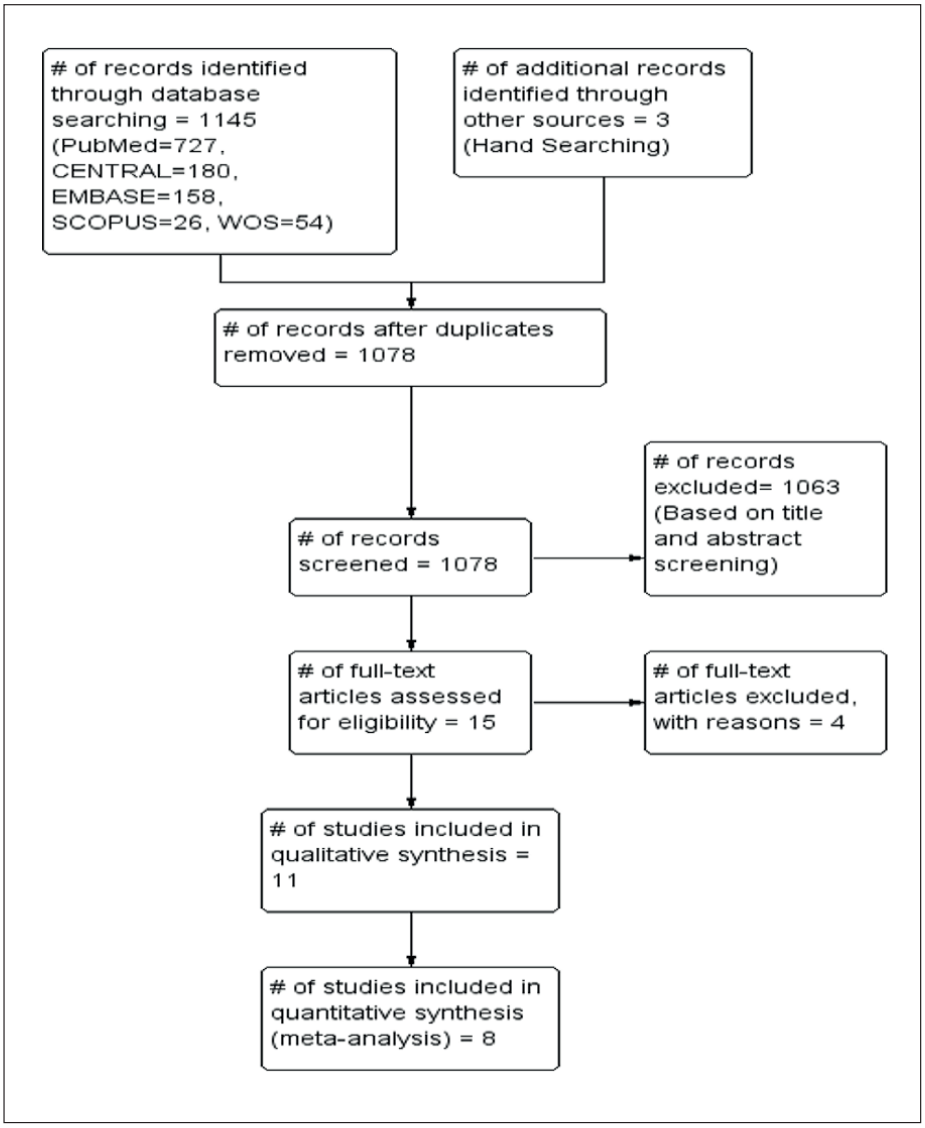

Figure 1. PRISMA flow chart for study selection process.

Meta-Analysis

The data from the included trials were clubbed together and a meta-analysis was carried out for calculating the mean difference between the probiotics and placebo group for reduction of PPD, gain in CAL, and reduction in microbial levels, at all follow-up.

Reduction in Probing Pocket Depth (PPD)

At 21 days follow-up

Three studies[8,15,30] merged to compare the reduction in PPD between the groups at 21 days follow-up. The forest plot showed the mean difference of reduction in PPD at 21 day follow up between SRP + *L.Reuteri vs.* SRP + Placebo group suggestive of significant favourable response towards SRP + *L.Reuteri* group with MD 0.61 95% CI (0.52, 0.70), $p<0.0001$ (Figure 3).

At 3 months follow-up

Seven studies[7,8,15,24-26,28] merged to compare the reduction in PPD between the groups at 3-month follow-up. The forest plot showed the mean difference of reduction in PPD at 3 month follow up between SRP + *L.Reuteri vs.* SRP + Placebo group suggestive of significant favourable response towards SRP + *L.Reuteri* group with MD 0.40 95% CI (0.11, 0.68), $p=0.006$ (Figure 4).

Table I. General characteristics of included studies.

SL. No.	Study Design	Study Group	Experimental	Form of Delivery	Frequency	No.	Smokers	Age Range (in years)	M/F	Treated		Analyzed		Follow-up
										Exp	Ctr	Exp	Ctr	
1	Grusovin et al[23] 2019	DB, PC, RCT	GBT + *L. Reuteri*	Lozenges	Twice/day for 3 month	20	Yes	31-70	8/12	10	10	10	10	0, 90, 180, 270 days
2	Theodoro et al[7] 2019	DB, PC, RCT	SRP + *L. Reuteri*	Lozenges	Twice/day for 21days	34	Yes	30-56	19/15	17	17	14	14	0, 90 days
3	Pelekos et al[24] 2019	DB, PC, RCT	SRP + *L. Reuteri*	Lozenges	Twice/day for 28 days	87	No	18-59	27/60	28	59	21	41	0, 90, 180 days
4	Laleman et al[25] 2019	DB, PC, RCT	SRP + *L. Reuteri*	Tablets	Twice/day for 12 weeks	39	NR	NR	27/12	22	22	19	20	0, 90, 180 days
5	Ikram et al[26] 2019	DB, PC, RCT	SRP + *L.Reuteri*	Sachets (powder)	One Sachet for 12 weeks	28	No	38-45	17/11	14	14	14	14	0, 42, 84 days
6	Costacurta et al[27] 2018	DB, PC, RCT	SRP + *L. Reuteri*	Tablets	One/day for 1 month	40	NR	18-70	20/20	20	20	20	20	0, 30 days
7	Tekce et al[8] 2015	DB, PC, RCT	SRP + *L.Reuteri*	Lozenges	Twice/day for 3 weeks	40	No	35-50	18/22	20	20	20	20	0, 21, 90, 180, 360 days
8	Ince et al[15] 2015	DB, PC, RCT	SRP + *L.Reuteri*	Lozenges	Twice/day for 3 week	30	No	35-50	17/13	15	15	15	15	0, 21, 90, 180, 360 days
9	Teughels et al[28] 2013	DB, PC, RCT	SRP + *L.Reuteri*	Lozenges	Twice/day for 12 weeks	30	No	38-50	15/15	15	15	15	15	0, 21, 45, 60 days
10	Vicario et al[29] 2012	DB, PC, RCT	SRP + *L.Reuteri*	Tablets	One/day for 1 month	20	No	40-65	13/7	10	10	10	9	0, 30 days
11	Vivekananda et al[30] 2010	DB, PC, RCT	SRP + *L.Reuteri*	Lozenges	Twice/day for 21days	30	No	35-50	19/11	15	15	15	15	0, 21, 42 days

DB=Double Blind, PC=Placebo Controlled, RCT=Randomized Controlled Trial, SRP=Scaling and Root Planning, GBT=Guided Bone Therapy, L=Lactobacillus, NR=Not Reported.

Table II. Reasons for exclusion of excluded trials.

Author and Year	Reason for exclusion
Yuki et al[31] 2019	*Lactobacillus rhamnosus* as probiotic strain
Paul et al[32] 2019	*Lactobacillus brevis* as probiotic strain
Imran et al[33] 2015	*Lactobacillus casei* as probiotic strain
Mayanagi et al[34] 2009	*Lactobacillus salivarius* as probiotic strain

At 6 months follow-up

Four studies[8,15,24,25] merged to compare the reduction in PPD between the groups at 6 month follow-up. The forest plot showed the mean difference of reduction in PPD at 6 month follow up between SRP + *L.Reuteri vs.* SRP + Placebo group could not achieve a significant favourable response towards SRP + *L.Reuteri* group with MD 0.56 95% CI (-0.06, 1.18), p=0.08, suggesting failure of *L.Reuteri* containing probiotics in maintaining its long term effect (Figure 5).

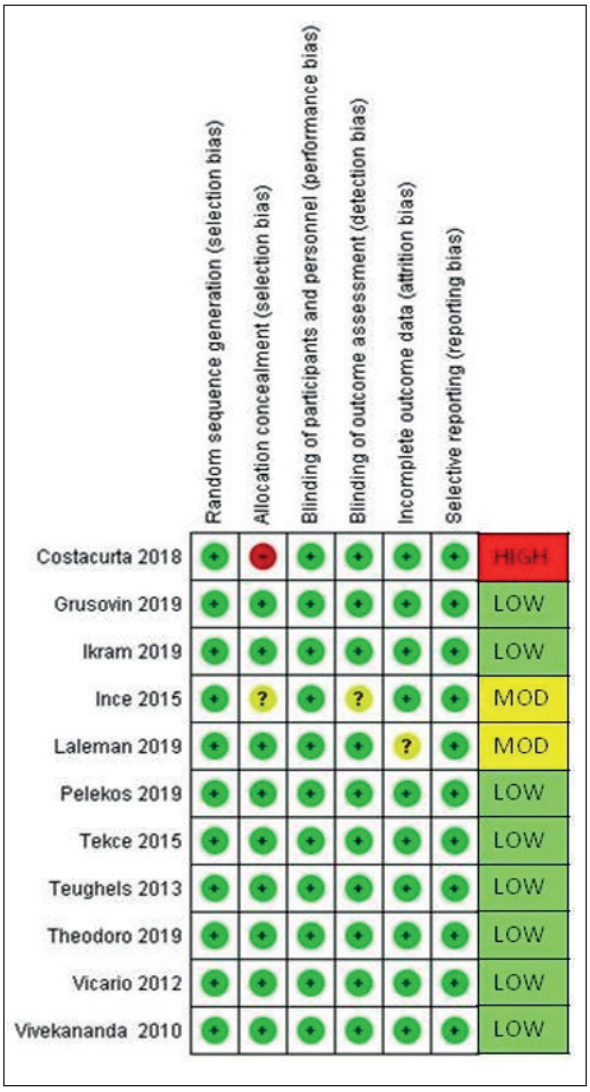

Figure 2. Risk of Bias (RoB) Assessment.

Gain in Clinical Attachment Level (CAL)

At 3 months follow-up

Seven studies[7,8,15,24-26,28] merged to compare the gain in CAL between the groups at 3-month follow-up. The forest plot showed the mean difference of reduction in PPD at 3 month follow up between SRP + *L.Reuteri vs.* SRP + Placebo group suggestive of significant favourable response towards SRP + *L.Reuteri* group with MD 0.30 95% CI (0.15, 0.45), p=0.0001 (Figure 6).

At 6 months follow-up

Four studies[8,15,24,25] merged to compare the gain in CAL between the groups at 6 month follow-up. The forest plot showed the mean difference of gain in CAL at 6 month follow-up between SRP + *L.Reuteri vs.* SRP + Placebo group could not achieve a significant favourable response towards SRP + *L.Reuteri* group with MD 0.08 95% CI (-0.12, 0.28), p=0.45, suggesting failure of *L.Reuteri* containing probiotics in maintaining its long term effect (Figure 7).

Reduction in microbial levels (mean difference in log10 values)

The reduction in microbial counts expressed in mean and standard deviation in log10 values, at the end of 21 days follow-up between both groups showed favourable response for SRP + *L.Reuteri* group compared to placebo group.

The sub-group analysis carried out according to the various periodontopathogens, also showed a significant favourable response (p<0.0001) for group treated with SRP + *L.Reuteri*, with the MD 0.89 95% CI (0.61, 1.17) for *Aggregatibacter actinomycetemcomitans*, MD 0.87 95% CI (0.65, 1.06) for *Porphyromonas gingivalis*, and MD 0.69 95% CI (0.37, 1.02) for *Prevotella intermedia* (Figure 8).

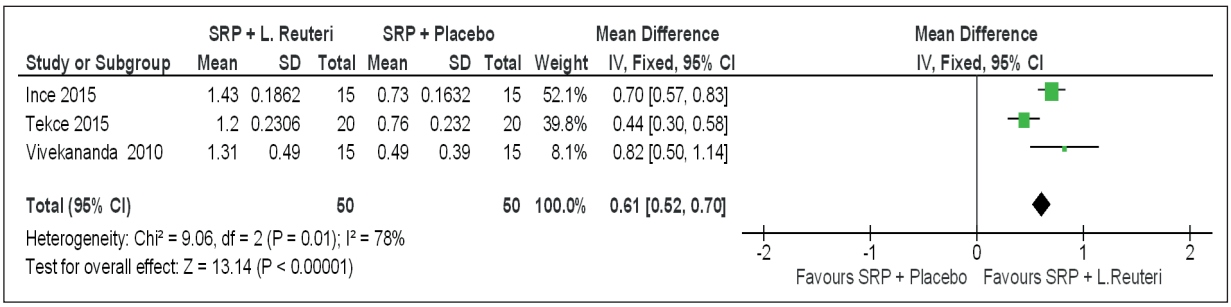

Figure 3. Reduction in PPD at the end of 21 days.

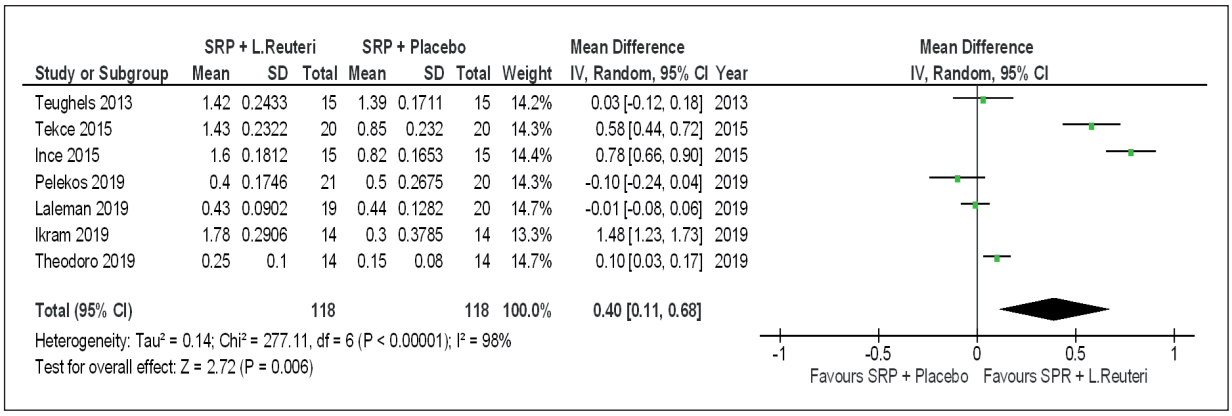

Figure 4. Reduction in PPD at the end of 3 months.

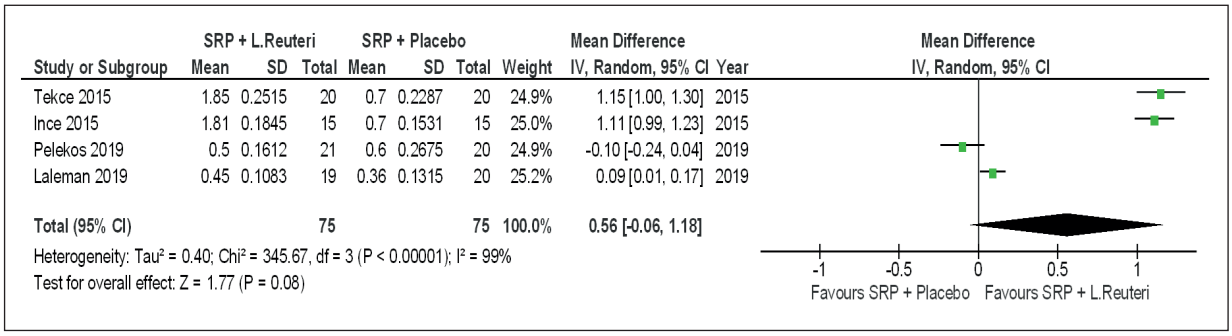

Figure 5. Reduction in PPD at the end of 6 months.

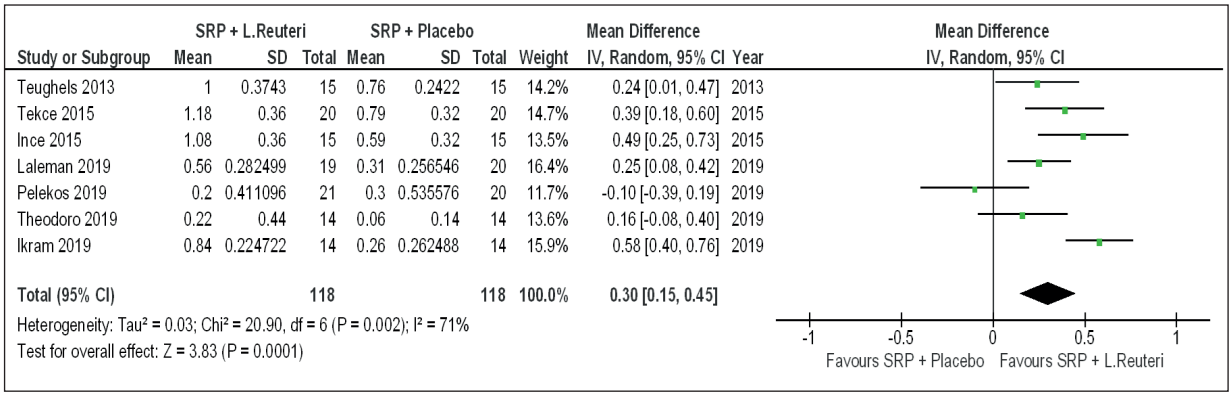

Figure 6. Gain in CAL at the end of 3 months.

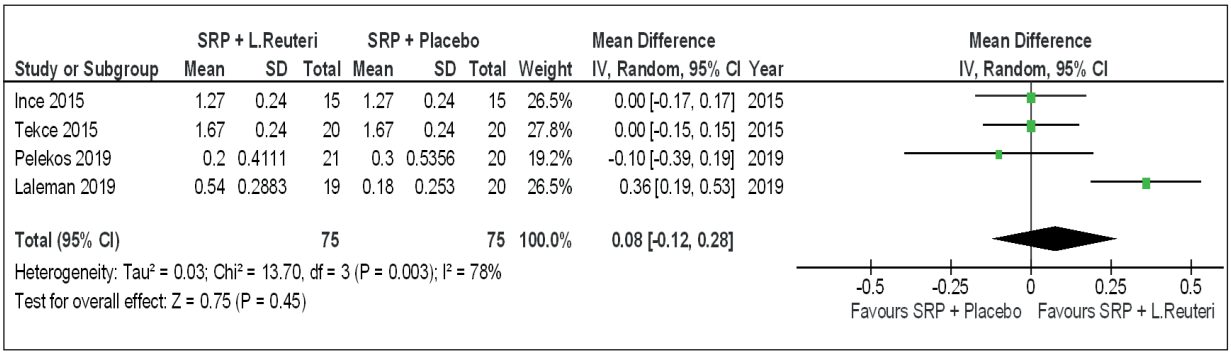

Figure 7. Gain in CAL at the end of 6 months.

Discussion

This systematic review and meta-analysis aimed at evaluating the effects of probiotics containing *Lactobacillus Reuteri* in adjunct to scaling and root planning for management of patients with chronic periodontitis. The literature available to evaluate the clinical and microbiological benefits of administration of probiotics in addition to SRP are plenty, but inconclusive. A systematic review and meta-analysis on the use of probiotics for managing caries and periodontitis was published by Gruner et al[19] in 2016 and concluded that probiotics positively affected the indicators of gingival inflammation, though the evidence was considered inconclusive for supporting probiotic therapy as treatment of periodontal disease.

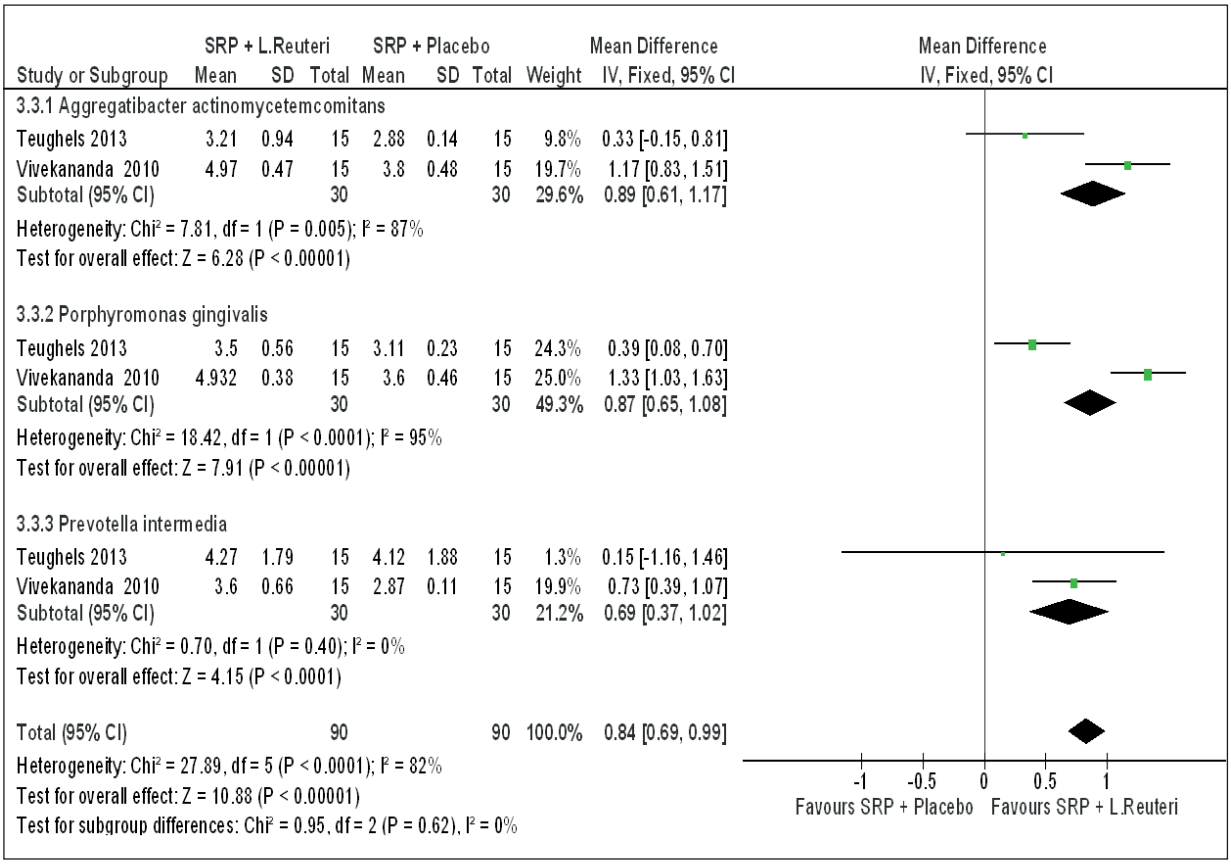

Figure 8. Reduction in microbial levels (mean difference in log10 values) at the end of 21 days follow-up.

The present systematic review reviewed only the trials evaluating the effect of probiotics containing *L.Reuteri* in adjunct to SRP for management of chronic periodontitis. A total of eleven trials were analyzed and all of them indicated the beneficial effects of the same in treatment of chronic periodontitis. Most of the trials were found to have low risk of bias, however, few of trials failed to mention regarding the proper allocation concealment and blinding.

The meta-analysis comparing the reduction of PPD and gain in CAL showed significant favourable response for the group treated with *Lactobacillus reuteri* containing probiotics in addition to SRP at an early follow-up of 3 months. However, the response could not be favourable at 6-month follow-up, suggesting the effect of probiotics could not be maintained for a long term, in most of the included trials the probiotics were administered twice daily for a maximum period of 3 weeks.

The present systematic review also showed a reduction in microbial count at the end of 21-day follow-up. The possible mechanism of such effect of the probiotics is poorly understood in oral environment. A reduction in the colony counts of the periodonto-pathogenic bacteria may be possibly due to the capacity of the probiotic species to compete with the pathogenic bacteria for nutrition, as well as surface adhesion[35]. They also secrete anti-bacterial agents which are capable of promoting the normal epithelial barrier against pathogenic bacterial invasion[36]. Furthermore, production of substances which have an immunomodulatory effect by stimulating the dendritic cells and, as well as the destruction of the pathogenic bacteria, may account for the probiotic action. One of the clinical trials, comparing the effectiveness of systemic antibiotic therapy (amoxillin plus metronidazole) to that of probiotics containing *Lactobacillus reuteri*, showed similar improvement in all clinical periodontal parameters. This indicates that both adjunctive therapeutic agents showed similar efficacy in resolving inflammation and improving periodontal outcomes[37].

The systematic review by Seminario-Amez et al[38] published in 2017, included 12 RCTs reported an improvement of clinical parameters, like bleeding on probing, probing depth, and gingival index, similar to the present study. They did not find any significant difference in the periodontal bacterial counts unlike our study. Recently, Ikram et al[39] published a systematic review and meta-analysis which included seven RCTs on use of probiotics as an adjunct to SRP and there was sufficient heterogeneity in PPD reduction and CAL gain between the studies, though the overall results indicated that adjunctive probiotics may lead to supplementary advantages of CAL gain in chronic periodontitis patients.

Almost all the included studies demonstrated supplementary advantage of using probiotics containing *L.Reuteri* with SRP but at the same time, certain things have to be considered while interpreting these favourable results. The form of probiotic drugs, their composition, stain of the bacteria used, dosage, frequency of use, as well the follow-up period, is not standardized across the RCTs. Moreover, it is well known that local drug delivery in periodontal disease has shown promising results[40]; hence, the administration of probiotics containing *L.Reuteri* using the same route may have a better result. From the included studies neither a threshold dosage for the probiotics nor a specific follow-up period could be recommended as a standard of practices while administering adjunctive probiotics containing *L.Reuteri*.

The clinical benefit of treatment should be judged against its adverse effects and the preferences of the patients should be taken into account[41]. Patient-reported outcomes (PROs) were included in one of the study[23] and showed that taking the lozenges was well accepted by the patients.

Conclusions

Within the limits of the study, *Lactobacillus reuteri* containing probiotics showed a significant clinical and microbiological benefit, however, the favourable effect was shown to be short-term. Further trials with larger sample size, local delivery of such probiotics with a specific dosage and time, is recommended for proving the long-term benefit.

Author Contributions

DS conceived and designed the study. DS and XL collected the data and performed the literature search. DS was involved in the writing of the manuscript. All authors have read and approved the final manuscript.

Conflict of Interests

The authors declare that they have no conflict of interests.

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