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Clinical effects of *Lactobacillus reuteri* probiotic in chronic periodontitis – a systematic review

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Abstract. – OBJECTIVE: This systematic review examines the effectiveness of *Lactobacillus reuteri* as an adjunct to scaling and root planing in the treatment of chronic periodontitis.

MATERIALS AND METHODS: Scopus, PubMed, and Web of Science databases were searched according to specific inclusion and exclusion criteria in October 2022. Randomized control trials that evaluated the effects of *Lactobacillus reuteri* in patients with periodontitis were included. The primary outcome was pocket depth and clinical attachment levels, while the secondary outcome considered was bleeding on probing, microbial levels, and gingival index score. Study quality was assessed based on the Cochrane Handbook for Systematic Reviews of Interventions and the ROB2 tool.

RESULTS: A total of eleven studies that examined 369 subjects were included in the review. Adults in the age group of 18-70 years of age suffering from chronic periodontitis were evaluated. Eight out of the eleven studies reported statistically significant improvement in the intergroup pocket depths, whereas seven studies showed a statistically significant reduction in the clinical attachment levels in the probiotic group. Three studies showed no significant improvement in the pocket depth levels in the probiotic group as compared to the controls. Four studies showed no significant reduction in clinical attachment levels between the two groups. The overall risk of bias was high in four studies, while seven studies reported some concerns about the risk of bias.

CONCLUSIONS: Based on the limited evidence available, the adjunctive use of *Lactobacillus reuteri* to scaling and root planing may provide some additional benefit in improving periodontal parameters.

Key Words:
Lactobacillus reuteri, Periodontitis, Probiotics, Root planing, Scaling.

Introduction

Periodontitis is a multifactorial, inflammatory disease that affects tissues around the teeth, leading to advanced destruction of the periodontal ligament, attachment loss, pocket formation, gingival recession, and bone loss[1]. It is the most predominant human oral disease[2]. Former epidemiological trials have suggested that 15-30% of the global adult population suffers from periodontitis[3,4]. The Global Burden of Disease Study 2019 has estimated that nearly 1.1 billion people exhibit periodontitis, with the count progressively rising over the previous three decades[5]. The initiation and progress of periodontal disease are achieved through a dysbiosis of the commensal oral microbes present in dental plaque, which, upon interaction with the host immune response, triggers an immune inflammatory reaction[6]. This pathophysiological condition endures in spells of exacerbation and quiescence till either the plaque biofilm is eliminated, or the tooth exfoliates due to bone loss and subsequent mobility. Scaling and root planing (SRP) is the preliminary phase and is the gold standard of non-surgical periodontal therapy[7]. Thorough scaling and root planing effectively disrupt the plaque biofilm, eliminating accumulated plaque and calculus from both the tooth and root surfaces. It significantly reduces the total anaerobic colony-forming units (CFUs) and targets specific periodontal bacteria[8]. This restricts the progress of periodontal tissue destruction, alters the existing pathogenic microenvironment to a more symbiotic state, and initiates the resolution of inflammation. Advanced methods like metagenomics and culturomics are used in subgingival microbiota analysis and are also used to profile previously elusive microorganisms[9]. However, this state of symbiosis is only momentary as periodontopathogens rapidly recolonize the treated niches. Quirynen et al[10] have confirmed this temporary state of symbiosis even with the use of antibiotics or antiseptics. Thus, the idea of administering adjunctive probiotics or 'beneficial bacteria' has emerged as an attempt to overcome this limitation. The World Health Organization describes probiotics as live microbes

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that, when administered in sufficient quantities, confer a health benefit to the host[11]. The action of probiotics is via multiple mechanisms, such as biofilm disruption, metabolism of compounds, pH regulation, and exhibiting anti-inflammatory effects. It can directly interact with bacterial plaque, causing disturbance of biofilm formation by competing for binding sites on host tissues. It may produce several antimicrobial compounds, such as organic acids, hydrogen peroxide, peptides, bacteriocins, and anti-adhesion molecules. It may also modulate innate and adaptive immunity, with an alteration of cytokine production. Some probiotic species act by improving mucin production and barrier function, regulating host defense peptides, as well as aid in angiogenesis and wound healing[12]. The most common microbes used as probiotics are the Lactobacillus and Bifidobacterium genera[13]. Among the Lactobacillus species, *Lactobacillus reuteri* is known for its reuterin (beta-hydroxypropionaldehyde) forming ability. Reuterin can inhibit the overgrowth of pathogenic microbiota, including both gram-positive and gram-negative bacteria, viruses, and fungi. It also prevents the colonization of pathogenic microbes by interfering with the pathogen's adhesion to the host surface. This helps in the maintenance of a healthy microenvironment[14]. Furthermore, reuterin is known to have an immunomodulatory effect by suppressing inflammatory mediators, such as tumor necrosis factor-alpha by lipopolysaccharide-activated monocytoid cells. It also suppresses inflammatory markers, such as interleukin 1, interleukin 8, and matrix metalloproteinase 8 (*MMP8*). Saliva is a common habitat of this organism; however, it has also been detected in subgingival plaque samples, which indicates its potential for use as a probiotic for periodontitis.

Although several studies[15,16] have been conducted on the use of probiotics in periodontal therapy, a majority of the available evidence fails to provide a conclusive verdict regarding the efficacy of probiotics in managing periodontal disease. Although *Lactobacillus reuteri* presents numerous advantages, its potential as an adjunctive therapy for improving periodontal disease is still uncertain, specifically in non-surgical periodontal therapy when used concomitantly with scaling and root planing. The clinical and microbiological effectiveness of *L. reuteri* as a complementary treatment option for periodontitis remains ambiguous, with current research indicating a significant yet transient impact[17,18]. Studies demonstrate a marked reduction in probing pocket depth and clinical attachment levels with the use of *L. reuteri* compared to the control group, while a few others report conflicting observations that reveal no substantial improvement in pocket probing depth[19-24] and clinical attachment levels[19,22,23]. Moreover, there is a lack of uniformity in the results of studies investigating the effects of *Lactobacillus reuteri* on bleeding on probing levels, with some demonstrating a significant decrease[20,24-26], while others showing contradictory findings[25,26]. Additionally, while most studies show a non-significant reduction in microbial levels[20,24] between groups, one study has reported contradictory results[21]. Evidence on the long-term benefits of *L. reuteri* as an adjunct to non-surgical periodontal therapy is inconsistent and conflicting. The aim of the present systematic review was to systematically assess the presently available evidence from randomized controlled clinical trials for the adjunctive effectiveness of *Lactobacillus reuteri* probiotics in treating chronic periodontitis.

Materials and Methods

Search Strategy

This systematic review was performed using the Preferred Reporting for Systematic Reviews and Meta-analysis (PRISMA) statement[27]. The research question was "Does treatment with *Lactobacillus reuteri* as an adjunct to scaling and root planing cause an improvement in the periodontal parameters?"

The electronic databases of PubMed, Scopus, and Web of Science were searched for eligible studies with no restrictions placed on the start date of 5-10-2022. Trial registers of US National Institutes of Health Ongoing Trials Register and the World Health Organization International Clinical Trials Registry platform, were searched in October 2022. Forward citation tracking was conducted using Google Scholar. Two authors (JR and KA) independently reviewed the search results for study selection. Duplicates and non-relevant articles were discarded. The researchers independently screened titles and abstracts of studies for eligibility and any disagreements were resolved through consensus with a third author (SP). The full text of relevant articles was examined for eligibility using the inclusion criteria. Manual supplementary searches of the references of the selected articles were conducted for additional eligible studies. The search strategy is depicted in **Supplementary Table I**. This review was submitted for registration in the International Prospective

Register of Systematic Reviews (PROSPERO Registration Number: CRD42023460029).

Inclusion Criteria

(P) Population: Subjects with chronic periodontitis;

(I) Intervention: Local or systemic administration of *Lactobacillus reuteri*;

(C) Control: Scaling and root planing;

(O) Outcome: Primary outcome – Pocket depth and attachment levels

Secondary outcome – Bleeding on probing, microbial levels, gingival index score

(S) Study type: Randomized control studies, controlled clinical trials, and cohort studies.

Exclusion Criteria

Case reports, systematic reviews, opinion articles, letters to the editor, and articles in languages other than English were excluded.

Data Extraction

Data extraction was independently conducted by two authors (CF and JR) and verified by a third author (JT) for accuracy. Characteristics of the study, along with the author's names, year of publication, country of origin, methodological aspects, sample size, treatment regimen, and duration, were extracted manually into a customized template.

Assessment of Study Quality

The quality of the selected studies was assessed using relevant guidelines from the Cochrane Handbook for Systematic Reviews[28]. Five specific domains were used to assess the external and internal validities of the studies, including randomizations, allocation concealment, blinding, missing outcome data, selective reporting, and other sources of bias. The response for each domain was either high, low, or unclear risk of bias. The absence of pertinent information regarding methodology in the selected study would result in a high risk of bias judgment for the particular domain. An unclear judgment was reserved for use in case of insufficient information. The overall risk of bias was determined using the highest level of risk observed under the domains. Agreement between the two raters was assessed overall using the kappa statistic.

Quality of Evidence for Outcomes in Summary of Findings Table

The primary outcome of pocket depth and clinical attachment level and the secondary outcome of bleeding on probing, microbial levels, and gingival index score were examined in the included studies. The Grades of Recommendation, Assessment, Development, and Evaluation (GRADE) tool was used to assess each outcome in the summary of findings table, as described in section 12.2 of the Cochrane Handbook for Systematic Reviews of Interventions[29]. The grading system was initially applied by one author (KA) and the evidence was then reviewed for each outcome by two other members (JT and SP) of the review team. The final rating was determined after the three reviewers reached a consensus. The certainty of the evidence was graded as high, moderate, low, and very low. Evidence for each outcome was graded as 'high quality' at the start in the case of RCTs. The evidence rating was downgraded by one level for serious or two levels for very serious concerns regarding the study limitations in risk of bias, inconsistencies in the outcomes, indirectness of evidence, imprecision of effect estimates, or publication bias.

Results

The initial search identified 298 records. After the removal of duplicates and screening of titles and abstracts for eligibility, the potentially relevant articles were identified. Full-text articles were selected for complete review. The Supplementary material, supporting information, and references associated with the selected articles were manually searched, but no further eligible studies were found. A total of eleven articles were selected for inclusion in this systematic review[30-40]. Intrarater agreement was high, with an overall agreement of 91% and a kappa statistic of 0.91 (95% confidence interval). The PRISMA flow diagram is shown in Figure 1. A total of eleven studies examined 369 subjects. The included studies were inconclusive about the number of teeth or sites examined. Adults in the age group of 18-70 years of age suffering from chronic periodontitis were evaluated. All studies are randomized controlled trials. Of these, nine studies had parallel arm study designs, whereas two studies were split mouth studies[30-40]. The majority of the studies were conducted in Asia (India, Hong Kong, Turkey and Pakistan), with other studies conducted in Europe (Italy and Switzerland). A summary of the selected trials is shown in Table I.

Characteristics of the Selected Studies

Nine studies had a parallel arm study design[31-35,37-39], while two studies were split mouth[30,40].

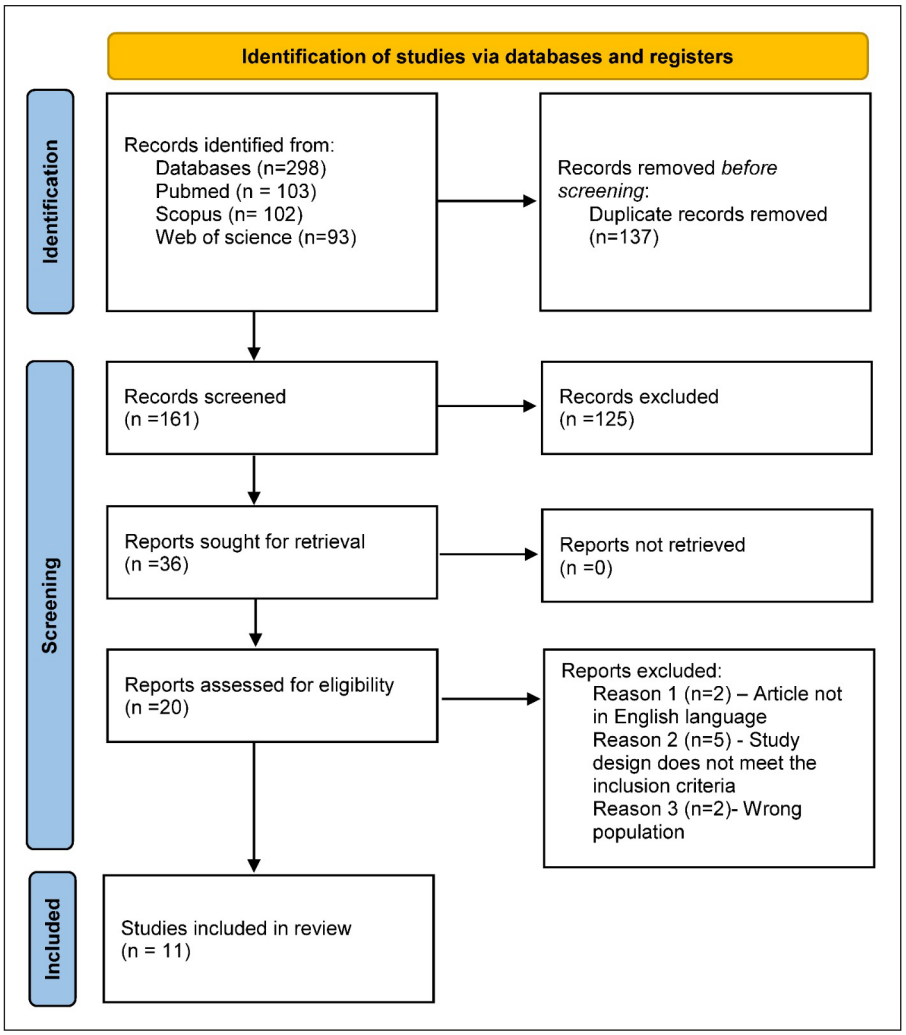

Figure 1. PRISMA flow chart.

All studies included systemically healthy subjects suffering from chronic periodontitis. Studies encompassed patients with chronic periodontitis with horizontal bone loss[32,33,36,39], with evidence of radiographic bone loss, patients with Stage II/Stage III and Grade A/Grade B chronic periodontitis[37], patients with generalized chronic periodontitis with information about supportive periodontal therapy, bleeding on probing, pocket probing depth, and clinical attachment level[34-39], and patients with chronic stages 3-4 periodontitis[40]. Some methodological insufficiencies were seen in the studies. One study did not mention subject dropouts[34]. All studies excluded smokers from their samples except for one study which had no mention of smokers[34]. Similarly, all studies excluded diabetics except for one study which had no mention of diabetics[34]. All studies revealed their gender distribution. All studies measured clinical attachment levels whereas one study measured relative attachment levels[33]. This study also performed only an intragroup comparison of plaque index, gingival index, and bleeding on probing levels without an intragroup comparison of these parameters[33]. Four studies[30,31,34,36] showed the use of 1×10^8 CFU colony-forming units of the strain *DSM17938* in combination with the *ATCC PTA 5289* strain of *Lactobacillus reuteri*. One study[37] used probiotic tablets containing *L. reuteri UBLRu-87*, 0.5 billion colony-forming units. Two studies[39,40] used 1×10^8 CFU colony forming units of *DSM17938* alone. One study[36] did not mention the strain and only mentioned that 5.9 billion colony-forming units were used. Two studies[32,35] provided no information regarding the strain as well as the colony-forming units. The majority of studies provided systemic administration of the probiotic in the form of lozenges and tablets. However, four studies provided local delivery of the probiotic, either in a powder form[35,38], or suspension/ solution form[39,40]. The

Table I. Summary of the selected studies.

Author/ Year	Sample Size	Age group	Gender	Study Design	Intervention
Vivekananda et al[30] (2010)	39	34-50 years	Male: 9 Female: 11	Split-mouth, randomized clinical trial	*L.reuteri Prodentis lozenges* (1×10^8 CFU *DSM17938* 1×10^8 CFU *ATCC PTA 5289*)
Teughels et al[31] (2013)	30	Average age – in control group: 45.73 years – in test group: 46.60 years	Male: 15 Female: 15	Double-blind placebo-controlled parallel-arm	*L. reuteri* (1×10^8 CFU) for each of the strains *DSM17938* and *ATCC PTA5289.*
Tekce et al[33] (2015)	40	35-50 years	Male: 18 Female: 22	Randomised controlled trial parallel arm	*L. reuteri* containing lozenges
Inci et al[32] (2015)	30	35-50 years	Male: 17 Female: 13	Randomised controlled trial parallel arm	*L. reuteri* containing probiotics
Costacurta et al[34] (2018)	40	18-70 years	Male: 20 Female: 20	Randomised controlled trial parallel arm	*Lactobacillus reuteri DSM 17938* and *Lactobacillus reuteri ATCC PTA 5289*
Ikram et al[35] (2019)	28	Mean age in Group 1 was 40.14 ± 2.64 years. Group 2 was 41.78 ± 3.58 years	Male: 17 Female: 11	Randomised controlled trial parallel arm	*L. reuteri* containing probiotics
Pelekos et al[36] (2019)	49	53.7 ± 9.9 years in test group and 52.3 ± 10.5 years in control group	Male: 12 Female: 37	Double-blind, paralleled-arm, placebo-controlled and randomized clinical trial.	*L. reuteri DSM17938* and *L. reuteri ATCC PTA5289*
Jebin et al[37] (2021)	30	20-60 years	Male: 24 Female: 6	Double-blind, paralleled-arm, and randomized clinical trial.	Probiotic tablets containing *L. reuteri UBLRu-87*
Kumar et al[38] (2021)	48	18-65 years	Male: 24 Female:	Randomized controlled, 24 parallel-arm prospective interventional study	*L. reuteri* containing probiotics
El-bagoory et al[39] (2021)	12	35-55 years	Male: 3 Female: 9	Randomized controlled, parallel-arm prospective interventional study	*L. reuteri* containing probiotics
Sufaru et al[40] (2022)	40	Mean age: 48.65	Male: 19 Female: 21	Randomised controlled trial and Split mouth study	*Lactobacillus reuteri DSM 17938*

follow-up period varied between the studies with the shortest being one month[34]. This was followed by the trial by Vivekananda et al30 having a duration of 42 days. Two studies[31,35] had a follow-up of 12 weeks. Two studies[37,40] had a follow-up period of 3 months. Other two studies[36,39] had a follow-up period of 6 months, while the longest follow-up duration of 360 days was seen in studies by Tekce et al[33] and Inci et al[32]. Detailed summary of the characteristics of the selected trials is shown in **Supplementary Table II**.

Evaluation of Primary Outcome: (PD and CAL)

All studies evaluated the pocket depth and attachment levels. The majority of studies showed a statistically significant reduction in the probing pocket depth levels in the probiotic group as compared to the controls[30,32-35,37,39,40]. However, the studies by Teughels et al[31], Pelekos et al[36], and Kumar et al[38] showed no significant improvement in the pocket depth levels in the probiotic group as compared to the controls. The majority of studies also showed a statistically significant reduction in the clinical attachment levels in the probiotic group as compared to the controls[30,32,33,35,37,39,40]. Four studies[31,34,36,38] showed no significant reduction in the clinical attachment levels between the two groups.

Evaluation of Secondary Outcome: (BOP, Microbial Levels, and GI Score)

Nine studies[31-36,38-40] evaluated bleeding on probing. Of these, five studies[33-35,39,40] showed a statistically significant reduction in the bleeding on probing levels in the scaling plus probiotic group as compared to the other groups at the end of the recall period. Two studies[36,38] showed no statistically significant difference in the bleeding

on probing levels on the intergroup comparison. One study[31] evaluated the percentage of sites with gingival bleeding which, on the intergroup comparison, was found to be non-significant at 9 weeks but significantly lower at 12 weeks in the probiotic group. One study[33] only performed intra group comparison of bleeding on probing levels which was found to be statistically significant in both groups at all time intervals. No intergroup comparison was performed. Five studies[30,31,33,37,39] evaluated the levels of microbes. Vivekananda et al[30] showed a significantly reduced microbial count for *Aggregatibacter actinomycetemcomitans* (p=0.005), *Porphyromonas gingivalis* (p=0.005) and *Prevotella intermedia* (p=0.05) in the use of scaling in combination with probiotics as compared to the other groups. Teughels et al[31] evaluated the levels of *Aggregatibacter actinomycetemcomitans*, *Fusobacterium nucleatum*, *Porphyromonas gingivalis*, *Prevotella Intermedia*, and *Tannerella forsythia*. Of these, the *Porphyromonas gingivalis* counts were significantly lower in the probiotic group at both 9 as well as 12 weeks. However, no statistically significant difference was found in the levels of other bacteria at all time points. Ince et al[32] found a significant difference in the intergroup percentage of obligate anaerobes at 21, 90, and 180 days but no significant difference in the intergroup percentage of obligate anaerobes at 360 days. The other two studies[37,39] also showed a statistically significant reduction in the *Porphyromonas gingivalis* levels favoring the probiotic group as compared to the other groups. (p=0.001). Among the eleven studies examined, seven studies[30-33,37-40] demonstrated a statistically significant decrease in the gingival index (GI) score within the experimental group. This group consisted of individuals who underwent scaling and root planing (SRP) and were exposed to different interventions, including probiotics, lozenges, or localized administration of *Lactobacillus reuteri*, and findings were compared to control groups[30-33,37,38,40]. The group receiving Prodentis alone had a statistically significant reduction in gingival bleeding compared to the group receiving SRP alone. The study found that the use of SRP resulted in a decrease in gingival bleeding. However, the administration of the probiotic led to an improvement in gingival bleeding[30]. An independent study[31] demonstrated notable improvements in clinical parameters. The group that underwent scaling and root planing (SRP) along with probiotic supplementation exhibited a more substantial decrease in pocket depth (p<0.05) and a significant increase in attachment (p<0.05), particularly in moderate and deep pockets. Additionally, a decrease in *Porphyromonas gingivalis* was noted. The GI score in the test group was significantly lower (p<0.05) compared to the control group throughout all time periods after the treatment. The study conducted by Sufaru et al[40] showed that the local administration of *L. reuteri DSM17938* in conjunction with normal non-surgical therapy resulted in significant improvements in periodontal attachment and reduction in gingival hemorrhage among patients diagnosed with stage 3-4 periodontitis.

Quality Assessment

In terms of the overall risk of bias, there were significant concerns regarding the risk of bias in a majority of studies. The overall risk of bias was high in four studies[34-36,40]. Seven studies[30-33,35-39] reported some concerns with the risk of bias. Eight studies[30-33,35-38] showed double blinding. No information about blinding was provided by two studies[34,40], while the information by El Bagoory et al[39] was inconclusive. The summary assessment of the risk of bias is presented in Figure 2[41].

Certainty of Evidence

Our review examined eleven studies with 369 samples. Based on GRADE, the overall quality of evidence for both outcomes in this study was low. This suggests limited confidence in estimating the effect of *Lactobacillus reuteri* on clinical periodontal parameters of probing depth, attachment level, and bleeding on probing as well as on periodontal microbial levels. The serious risk of bias in the studies raises doubts regarding the magnitude of the effect of the interventions examined. The reasons for downgrading the study were due to methodological insufficiencies, i.e., the risk of bias. All the involved studies were at either some concerns or a high risk of bias. Table II shows the summary of the findings.

Discussion

The initial therapy of periodontitis focuses on the reduction of the microbial load, which is primarily achieved by scaling, root planing, and oral hygiene instructions[42,43]. However, recolonization commences shortly after this mechanical debridement, engendering the need for adjunctive therapeutic approaches such as antimicrobial agents, lasers, probiotics, and photodynamic therapy, to reduce this recolonization[44,45]. The possible ap-

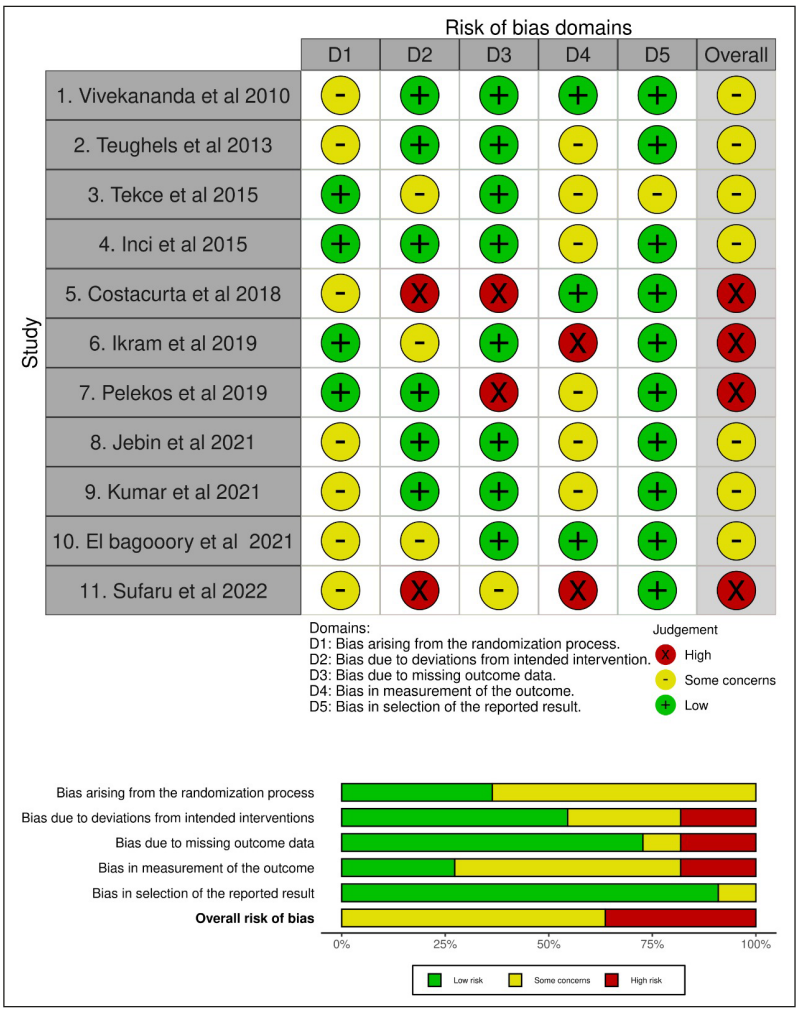

Figure 2. Summary of risk of bias assessment.

Table II. Summary of findings table.

Quality assessment						Summary of findings		
Outcome	Risk of bias	Incon-sistency	Impre-cision	Indirect-ness	Public-ation bias	Impact	No of partici-pants (Studies)	Certainty of evidence (GRADE)
Pocket depth and clinical attachment level	Serious[a]	Not serious	Not serious	Not Serious	Not serious	Our confidence in the effect estimate is limited: the true effect may be substantially different from the estimate of the effect	369 (11)	Low
Bleeding on probing	Serious[a]	Not serious	Not serious	Not Serious	Not serious		308 (9)	Low
Microbial levels	Serious[a]	Not serious	Not serious	Not Serious	Not serious		142 (5)	Low

[a]Three studies showed some serious concern with allocation concealment and two studies showed some concern with blinding.

plication of several probiotics in periodontics has increasingly been widely encouraged[46]. This review aimed to systematically review presently available randomized controlled clinical trials for the clinical effects of *Lactobacillus reuteri* probiotic in the treatment of chronic periodontitis. The

use of *L. reuteri* as an adjunct to scaling and root planing has demonstrated predominantly favorable outcomes, with the primary measures of pocket depth and clinical attachment levels being evaluated. However, the studies were not unanimous in their conclusions. Eight out of eleven studies found that the probiotic group had a reduction in the probing pocket depth levels[30,32-35,37,39,40]. Seven out of eleven studies[30,32,33,35,37,39,40] reported that the probiotic group had a reduction in clinical attachment levels. Dissenting with this view, three out of eleven studies showed no improvement in the pocket depth levels in the probiotic group compared to the controls[31,36,38]. Four out of eleven studies[31,34,36,38] showed no significant reduction in the clinical attachment levels between the two groups. Bleeding on probing, microbial levels and GI score were the secondary outcomes assessed. Bleeding on probing is one of the techniques for evaluating the periodontal condition and disease progression. It is the first sign of inflammation inside the connective tissue. The results of the eleven studies that examined the secondary outcome were not unanimous. Of the nine studies that evaluated bleeding on probing[31-36,38-40], only five studies period[33-35,39,40] showed a statistically significant reduction in the bleeding on probing levels in the scaling plus probiotic group compared to the other groups at the end of the recall. Five studies[30,31,33,37,39] also evaluated the levels of microbes, which mainly showed ameliorated results. Among the examined studies, seven revealed a significant decrease in gingival index (GI) scores within the experimental group, which received various interventions, including Prodentis probiotics. The combination of Prodentis with scaling and root planing (SRP) notably improved gingival bleeding and periodontal attachment, indicating the potential synergistic effects of probiotic therapy in periodontitis management[30-33,37,38,40].

The conflicting results in the studies included in this review are borne out in the literature. Eight out of eleven studies[30,32-35,37,39,40] showed a statistically significant reduction in the pocket depth levels in the probiotic group compared to the controls. This observation is in broad agreement with six other studies[19-24] linking *Lactobacilus reuteri* with significantly reduced probing depth. A non-randomized controlled trial by Szkaradkiewicz et al[19] involving the use of tablets, containing *L. reuteri* strain-producing hydrogen peroxide (1×10^8 CFU *L. reuteri ATCC PTA 5289*, Prodentis) demonstrated a significantly reduced probing depth in the probiotic group as compared to the controls. Penala et al[20], in their randomized, double-blinded trial evaluated the effectiveness of subgingival delivery of *L. reuteri* and probiotic mouthwash, with control subgingival delivery of placebo and placebo mouthwash. They found significantly reduced probing depth in moderate pockets, the probiotic group as compared to the placebo group[20]. Additionally, Galofre et al[21] in their study on peri-implantitis subjects too, found significantly reduced pocket depths (p=0.036) after the use of *L reuteri* as compared to the placebo group. Schlagenhauf et al[22] and Laleman et al[24] also showed similar results. This observation also follows the double-blind trial by Grusovin et al[23] which demonstrates a significantly reduced pocket depth in the probiotic group at the one-year follow-up as compared to the control. The study by Vicario et al[47] demonstrated significantly reduced pocket depths on the intragroup comparison but did not evaluate intergroup probing depth levels.

The outcomes examined in this review showed divergent results. Three out of eleven studies[31,36,38] showed no significant improvement in the pocket depth levels in the probiotic group as compared to the controls. This observation is in agreement with previous studies by Theodoro et al[25] on smokers and by Vorah et al[26] on shamma users. Seven out of eleven studies[30,32,33,35,37,39,40] in the present review showed a statistically significant reduction in the clinical attachment levels in the probiotic group as compared to the controls. This finding mirrors the results of previous studies[19,22,23]. A non-randomized controlled trial by Szkaradkiewicz et al[19] involving the use of tablets, containing *L. reuteri* strain-producing hydrogen peroxide (1×10^8 CFU *L. reuteri ATCC PTA 5289*, Prodentis) demonstrated significantly improved clinical attachment levels in the probiotic group as compared to the control. These findings are also consistent with the study by Schlagenhauf et al[22] in sea sailors and with Grusovin et al[23] in their double-blind randomized controlled trial with a one-year follow-up period. However, four studies[31,34,36,38] showed no significant reduction in the clinical attachment levels between the two groups. These results corroborate previous research by Penala et al[20], Laleman et al[24], Theodoro et al[25], and Vohra et al[26]. Assessing bleeding on probing, five out of nine studies[31-36,38-40] showed a statistically significant reduction in the bleeding on probing levels in the scaling plus probiotic group as compared to the other groups at the end of the recall period[33-35,39,40].

This was as per three other studies in the literature[22,23,25]. The study by Vicario et al[47] demonstrated significantly reduced bleeding on probing levels on the intragroup comparison but did not evaluate intergroup probing depth levels. Contrarily, two studies in the present review[36,38] showed no statistically significant difference in the bleeding on probing levels on the intergroup comparison. This is also consistent with the results of three other studies in previous literature[21,24,26]. Galofre et al[21], in their study on peri-implantitis subjects, found a significant difference in the bleeding on probing levels on the intergroup comparison at 30 days. However, no significant intergroup difference in the bleeding on probing levels was seen at 90 days (p=0.031). Likewise, studies done by Laleman et al[24] and Vohra et al[26], also correlated with this finding. Five of the eleven studies[30,31,33,37,39] in the present study also evaluated the levels of microbes, which mainly showed ameliorated results. Inter-group comparison by Penala et al[20] revealed a significant reduction in BANA scores indicative of reduced red complex microorganisms in the test group at one month as compared to the control group. However, there was no statistically significant inter-group difference at three months. Galofre et al[21] showed a statistically significant intergroup difference in the *Porphyromonas gingivalis* levels. However, it was not significant for other microbes, such as Aggregatibactor actinomycetum commitans, *Prevotella Intermedia*, Campylobactor rectus, *Fusobacterium nucleatum*, *Tanarella forcithia*, *Treponema denticola*, and *Eikenella corrodens*[21]. Similarly, Laleman et al[24] showed no significant intergroup difference in the microbial levels. The findings of this review are somewhat limited due to the considerable risk of bias and conflicting experimental results regarding the magnitude of the intervention. Overall, there is low-level evidence from eleven studies that *Lactobacillus reuteri* can improve periodontal clinical parameters. Heterogeneity in the study designs precluded us from performing a meta-analysis.

Overall Completeness and Applicability

The results of this review cannot be generalized due to the limited number of articles evaluating the effectiveness of the probiotic *Lactobacillus reuteri* as an adjunct to conventional scaling and root planing in the treatment of chronic periodontitis. This review includes only randomized controlled trials. Randomized controlled trials provide the most consistent corroboration of the effectiveness of interventions because the processes utilized during randomized controlled trials minimize the risk of confounding factors that can influence the results. Thus, the observations made by a randomized controlled trial are likely to be closer to true effect than the observations made by other research techniques[48]. In the selected studies, the sample size in the reviewed articles was limited. Studies by Ikram et al[35] and El Bagoory et al[39] had a low sample size of twelve patients and twenty-eight subjects respectively. Additionally, the follow-up period varied between the studies, with the shortest being one month34. This was followed by the trial by Vivekananda et al[30], having a duration of 42 days. Two studies have a follow-up of 12 weeks[31,35]. However, the literature reveals that a pronounced incidence of pocket closure is after 3 to 6 months of healing after periodontal therapy[49].

Periodontal therapy is largely influenced by risk factors, such as diabetes, smoking, and cardiovascular diseases[50]. Smoking may cause a less favorable outcome to non-surgical periodontal treatments and hinder the outcome of the study[51,52]. Similarly, both periodontitis and cardiovascular diseases are of inflammatory origin and share comparable risk factors[53]. All studies excluded smokers from their samples except for one study, which had no mention of smokers[34]. Similarly, all studies excluded diabetics except for one study[34]. While the majority of studies (nine) had a parallel arm study design[31-35,37-39], two studies were split mouth[30,40]. Split-mouth study designs offer advantages compared to parallel-arm trials. In split-mouth trials, the intervention effect estimates benefits from reduced variability among subjects, potentially increasing statistical power, as each subject essentially serves as their own control, minimizing confounding factors. However, the split-mouth design may lead to biased intervention effect estimates such as cross-over effects[54,55]. The *Lactobacillus reuteri* used in the studies were of dissimilar strains. It is suggested that the initially used *L. reuteri ATCC 55730* was originally isolated from breast milk and may be present in humans on the lining of the gastric body and antrum, duodenum, and ileum. However, *L. reuteri ATCC 55730* was found to exhibit a potentially transferable resistance trait for tetracycline[56] and, therefore, most studies of the present review utilized *L. reuteri DSM 17938*, a strain without undesired resistance. Moreover, the majority of studies provided systemic administration of the probiotic, while some studies provided local delivery of the probiotic. Both these modes

of administration have different benefits. Systemic administration destroys microbial reservoirs present in the saliva, tonsils, and connective tissues, which can be a source of recurrence. It is also less time-consuming and more cost-effective. On the other hand, local delivery makes available a higher drug concentration, does not need to be administered every day, is efficacious, and has fewer adverse effects[57,58]. However, studies[59,60] comparing the efficacy of local and systemic drug delivery in subjects with chronic periodontitis have shown no statistically significant difference. Although *Lactobacillus reuteri* is very commonly used as an adjunct to non-surgical periodontics, in a large period of more than a decade (2008 to 2022), only eleven studies have examined the effect of the diode laser on red-complex bacteria[17,61]. Thus, more well-designed randomized controlled trials with larger sample sizes are needed.

Quality of the Evidence

The certainty of the evidence was downgraded by two steps, once for bias and once due to the inconsistency in the studies. We found the quality of evidence for the outcome to be of low quality primarily due to the risk of bias. The overall risk of bias was high in four studies[34-36,40]. Seven studies[30-33,37-39] reported some concerns with the risk of bias. Only five of the eleven studies[32,35,37-39] followed the CONSORT guidelines. Several studies lacked clarity in reporting key information required for quality assessment. No information about blinding was provided by two studies[34,40], while the information by El Bagoory et al[39] was inconclusive. The low quality of evidence is insufficient to enable robust conclusions to be drawn. A sensitive and wide-ranging search strategy was employed to identify studies for inclusion in this review. No restriction was placed on the publication date and multiple authors independently assessed eligibility using well-defined inclusion criteria to minimize any selection bias.

Limitations and Future Directions

Despite the authors' best efforts, this review has some limitations. A majority of the papers were published before 2000 and lacked definite information that is vital for quality assessment. This led to a response of unclearness in several domains during risk of bias assessment. Only English language studies were considered for inclusion. This review may not be exhaustively comprehensive due to the exclusion of articles published in other languages. Further high-quality trials using multiple assessment protocols are necessary before definitive universal guidelines can be issued.

The findings of this systematic review contribute valuable insights into the potential effectiveness of *Lactobacillus reuteri* as an adjunct to scaling and root planing in managing chronic periodontitis. However, it is important to acknowledge certain limitations within this study. First, the heterogeneity in study designs, patient populations, *Lactobacillus reuteri* strains, and administration methods presents a challenge in synthesizing the results for a comprehensive meta-analysis. Furthermore, the relatively small sample sizes and varying follow-up durations in the included studies warrant caution in generalizing the findings to broader populations. Future research endeavors should focus on conducting more extensive randomized controlled trials with rigorous methodological standards, standardized reporting, more extended follow-up periods, and well-defined patient groups. Investigating the optimal strain, dosage, and administration method of *Lactobacillus reuteri*, as well as exploring its potential synergistic effects with other therapeutic modalities, could provide more conclusive evidence of its efficacy in non-surgical periodontal therapy. Moreover, studies evaluating the long-term benefits and potential adverse effects of *Lactobacillus reuteri* supplementation are crucial to establishing its role as a safe and effective adjunctive treatment option for chronic periodontitis.

Conclusions

This systematic review evaluates the evidence for the effectiveness of the probiotic *Lactobacillus reuteri* in the treatment of chronic periodontitis. It assesses its effect on clinical periodontal parameters such as pocket depth, clinical attachment levels, bleeding on probing, plaque index, gingival index, microbial levels, recession, and matrix metalloprotein levels. Within the limitations of the systematic review, there is low-level evidence that the adjunctive use of *Lactobacillus reuteri* to scaling and root planing may provide some additional benefit in terms of improvement in periodontal parameters. Further well-designed trials adhering to reporting guidelines and using objective measures are necessary before outlining universal guidelines for best practice.

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Conflicts of Interest
The authors declare no conflict of interest.

Authors' Contributions
J. Ram: Conceptualization, Methodology, Software.
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S. Bhandi: Visualization, Investigation.
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