

Additional clinical benefits of probiotics as an adjunctive therapy to nonsurgical periodontal treatment of periodontitis: a systematic review and meta-analysis

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Abstract

Background: With more and more concerns concentrated on the novel therapy applying probiotics, we challenge its trustworthy clinical efficacy as an adjuvant to scaling and root planning (SRP) as compared with SRP alone or combined with placebo or other nonsurgical treatment applied as the initial therapy of periodontitis.

Methods: Electronic databases retrieval was performed until October 2017 under certain screening condition. Clinical randomized controlled trials (RCTs) to assess the efficacy of SRP + probiotic versus SRP were included. Primary outcome variables were PPD (pocket probing depth) reduction and CAL (clinical attachment level) gain. Nine publications were eligible for the systematic review and three were evaluated in the meta-analysis.

Results: Results demonstrated statistically significant bleeding on probing (BOP) percentage reduction (5.34, $p < 0.00001$) at short-term, but not a significant differences of overall PPD reduction (0.23mm, $p = 0.25$) for SRP + probiotic treatment versus SRP at short-term, and there was only a tendency ($p = 0.08$) with regard to overall CAL gain. However significant reduction of PPD and gain of CAL were observed when stratified for deep pockets (0.61mm, $p < 0.00001$) and for moderate pockets (0.37mm, $p = 0.006$) at short-term respectively.

Conclusions: Within the ranges of this study, the adjunctive use of probiotics seem to achieve short-term clinical benefits in the treatment of periodontitis. Conclusions must be treated with caution and future long-term RCTs are needed to testify the clinical application value of probiotics.

Background

Periodontitis is the irreversible chronic inflammatory disease with the initial etiologic factor-plaque biofilm destroying the periodontal support tissue and if untreated, leading to teeth loss, which makes it the unresolved challenge of the oral medicine [1]. It has been profound elucidated that the one of the pathologic characteristics of periodontitis is the dysfunctional host response to the oral biofilm that was changed attributing to both the amounts and types of bacteria [2, 3]. It has been universally acknowledged that non-surgical periodontal therapy is the gold-standard for the management of periodontitis which mainly consists of oral hygiene instructions and scaling and root planning (SRP) [4]. The target of this conventional treatment is to remove adherent and unattached bacterial biofilms as well as deposits of calculus thus reducing inflammation and pocket depths, and promoting periodontal reattachment [5-7]. Unfortunately, such therapeutic approaches are frequently observed with the outcomes of recolonization of treated sites by periodontopathogens, causing to the recurrence of the periodontitis [8]. To increase the efficacy of non-surgical periodontal treatment, adjunctive treatments have been applied such as the use of chlorhexidine, antibiotics, antiseptics or photodynamic therapy to achieve a better decontamination of the periodontal environment [9-11]. Although these non-surgical treatments have been confirmed to have some certain clinical effectiveness, efforts to improve periodontal therapies through complementary treatments are at research. Recently, probiotic therapy has gained increasing interests among the medical researchers.

Probiotics are living microorganisms, principally bacteria which, when administered in adequate amounts, confer a health benefit to the host [12]. Clinical advances have already been made in the prevention and treatment of systemic gastrointestinal diseases in which polymicrobial intestinal transplants have shown its potential in restoring a balance between the intestine and its microbial inhabitants [13, 14]. Probiotics can exert clinical benefits on the host with the promise to improve and maintain the symbiosis of polymicrobial communities through prevention of adhesion of pathogenic species, inhibition of bacterial growth, modulation of the mucosal immune system or the cell proliferation and improvement of intestinal barrier integrity [15]. Probiotics have been employed as effective adjuncts to control periodontal inflammation [16-19]. The therapeutic effects on the periodontitis have been highlighted in vitro and in vitro models [20-22]. However, this is a relatively new field, and data regarding the probiotics therapy in treating periodontitis is scarce.

The objective of this systematic review aims to analyze the available scientific evidence answering the following focused question developed in accordance with the recognized Patient, Intervention, Comparison and Outcome (PICO) format: 'What is the clinical efficacy of probiotic as an adjunctive therapy of SRP, in terms of PPD reduction and CAL gain, when compared with SRP alone or in combination with placebo or other adjunctive treatments in the management of periodontitis in humans?'

Methods

Protocol

This systematic review was prepared in accordance with the PRISMA guidelines [23], and the protocol was registered in the International Prospective Register of Systematic Reviews (PROSPERO, registration number CRD42017083840).

Search strategy

The following databases: MEDLINE, Cochrane Central Register of Controlled Trials and Science Direct databases were searched from January 2012 through October 2017. The following strategy was used in the search using Boolean operators and an asterisk symbol (*) as truncation, was employed to identify papers using Mesh, keywords and other free terms: (((Periodontitis OR Chronic periodontitis OR Aggressive periodontitis OR Periodontal disease OR Periodont* OR probing pocket depth OR periodontal pocket) AND (Intervention OR Therapy OR Treatment)) OR (Scaling and root planning OR SRP OR nonsurgical periodontal therapy OR non-surgical therapy OR Periodontal treatment OR Periodontal therapy) AND (Probiotic OR Probiotic* OR Probiotic therapy OR Probiotic effect OR Probiotic treatment)).

Only articles published in English language have been considered.

Selection criteria for the studies

Inclusion criteria

A study was considered eligible for inclusion in this systematic review if it met the following criteria:

1. Type of studies: Randomized clinical trials
2. Subjects: Anyone who received probiotics as a preventive or treatment agent for periodontitis.
3. Type of treatment intervention: Oral probiotic administration compared with placebo, or another active intervention. Randomized clinical trials were included when they (1) tested one or more probiotic agents as an adjunct to scaling and root planing (SRP) alone or with a placebo and (2) had a control group that received the same SRP as the treatment group. We considered any type of probiotic with any type of administration method.
4. Types of outcome measures: reported results in terms of PPD.

Exclusion criteria

Studies were excluded if they included patients with the habit of smoking and systemic disease or if they were duplicated or affiliated studies.

Article review and data extraction

Potential publications' titles and abstracts were scanned by two blinded authors independently (G.H.Q and C.X) and further defined whether or not consistent to inclusion. When studies appeared to meet the inclusion criteria or

information in the title and abstract was insufficient to reach an explicit decision, full texts were reviewed.

Disagreements were resolved through discussions between authors and when still couldn't reach a consensus, a third examiner was consulted. Lack of pertinent data, the relevant details were provided by contacting the authors of the identified articles. Kappa values, reflecting an almost perfect inter-author agreement, were equal to 0.89 ($p < 0.001$).

Basic information extraction from papers included the year and authors of publication, the type of study design, the population demographics, the definition of the periodontitis, the probiotic bacterial strains used, also their mode of administration such as the frequency and duration, and other associated treat models, the follow-up procedures, the adverse effects and the key parameters recorded, and finally, the outcome assessments. (Fig. 1 and table 1)

Search outcomes and evaluation

PPD and CAL parameters were evaluated as the primary outcomes. Secondary outcomes concluded BOP changes, plaque index (PI), gingival index (GI) or gingival bleeding index (GBI), oral malodor parameters, microbiological effects, the progression and prognosis of disease. Analysis and recording was implemented by one author (G.H.Q) and verified by another author (C.X).

Risk of bias assessment

Quality analysis according to the Cochrane Reviewers' Handbook [24] was performed to assess the risk of bias assessment. Discussing and resolving the differences within the reviewers.

Data items

The meta-analysis integrated the average difference between baseline and follow-up (3 months and 1 year) of PPD reduction (overall, stratified for moderate and deep pockets), CAL gain (overall, stratified for moderate and deep pockets) and reduction of percentage of sites with BOP.

Data synthesis

First, Q and I^2 test was conducted to check the heterogeneity between studies. P value of Q statistic < 0.1 or I^2 value higher than 40% was defined as an indicator of heterogeneity. Weighted mean differences (WMD) and 95% confidence interval (CI) were regarded as the differences of continuous outcomes between SRP + probiotic and SRP group for using either fixed or random models. Each included studies' mean differences and standard errors were enrolled. The mean difference can also be estimated by standard deviation using $rd = \sqrt{r1^2/n1 + r2^2/n2}$ formula since data were not presented in the form of mean differences. The meta-analysis were performed using Review Manager (Version 5.2. The Cochrane Collaboration, 2013, Oxford, UK).

Results

Study selection

Quickly browsing the search items, 185 potentially relevant papers were extracted. After looking through the titles and abstracts, 153 articles were excluded owing to duplicates. Then evaluating carefully the 14 remaining articles, five were excluded for not fulfilling the inclusion criteria (Appendix S1). Besides, since two studies [25, 26] were performed at the same centre and on the same date and after discussion, it came to the conclusion that the population enrolled in the study of Ince et al. was a subgroup of the population of the study of Tekce et al. Finally, a total of nine articles fulfilled the inclusion criteria and were concluded in this systematic review [27-35] and three were further conducted a meta-analysis. [27, 28, 36] (Fig. 1).

Study characteristics

The studies were all RCT published in the English language between 2013 and 2017. All of them were designed for comparison between groups and were conducted at a single centre. The follow-up period comprised 28 days [37], 2 months [32], 12 weeks [27, 30, 31], 24 weeks [34] and 1 year [28, 29, 35]. Studies have included 20 patients for Sajedinejad et al, 28 patients for Morales et al, 29 patients for Penala et al, 30 patients for three of them [27, 32, 35], 40 patients for Tekce et al, 48 patients for Laleman et al. (Table 1). All studies demonstrated the sample size calculation and all patients recruited were with no systemic diseases and habit of smoking and also with no history of antibiotic administration within 6 months before entering the experiment.

Treatment modalities

Oral hygiene instructions were provided in all the studies. SRP was always performed with ultrasonic scalers and manual instruments. *Lactobacillus reuteri* [25, 26, 38] two times per day, *Lactobacillus rhamnosus* SP1 [29] and *Lactobacillus plantarum* [30] one time per day, *Lactobacillus Salivarius*+*Lactobacillus reuteri* twice a day [31], *Lactobacillus brevis* CD2 twice per day [32], *L. salivarius* NK02 twice a day [33], and *Streptococcus oralis* KJ3+*Streptococcus uberis* KJ2+*Streptococcus rattus* JH145 [34] twice a day were evaluated for their probiotic effect against periodontitis. The organisms were orally administered either as lozenges, tablets, sachet, capsule, or mouthwashes or solution. The follow-up period ranged from 28 days to 360 days. The probiotic concentrations varied between 1×10^8 , 2×10^7 , 2×10^9 colony forming units (CFUs) per day.

Risk of bias across studies

All included studies included in this systematic review were performed quality analysis according to the Cochrane Reviewers' Handbook (Higgins & Green 2011) (Table 2), and risk of bias assessment showed that all the RCT were defined as a low risk of bias (Table 3).

The effect of probiotics in periodontitis

PPD reduction

These studies [29, 33, 35] showed a significant difference on overall PPD reduction ($p < 0.05$) favoring the superior clinical efficacy of the SRP + probiotic treatment when compared to the SRP. One study [30] clarified that there only was significantly greater PPD reduction in teeth with an initial PD ≥ 4 mm in favor of the SRP+probiotic group ($p < 0.05$). Oppositely, four researches [27, 29, 34, 36] did not elucidate the significant differences between them ($p > 0.05$). The overall mean PPD reduction ranged from 0.6 mm (± 0.3) to 1.74 mm (± 0.62) in test group compared with 0.4 mm (± 0.4) to 1.52 mm (± 0.38) in control group at the end of follow-up visit [25, 29, 39]. Additionally, the study of Piyush Shah et al found no significant differences between SRP+ probiotic, SRP+ probiotic+ doxycycline, SRP+ doxycycline [19]. In four studies [27-29, 36], the results of treatment were analyzed on the basis of initial PPD of which the depth was further stratified as moderate or deep accordingly. In moderate pockets, a reduction of 1.84 mm (± 0.22) for SRP + probiotic and of 1.72 mm (± 0.17) for SRP + placebo were observed at 3 months by Teughels et al, which was in accordance with Penala et al where there were a reduction of PPD of 1.89 mm (± 0.25) in the SRP + probiotic group and a PPD reduction of 1.36mm (± 0.65) in the SRP + placebo group. As for deep pockets, the study of Teughels et al showed a PPD reduction of 2.88mm (± 0.35) for SRP + probiotic and of 2.25 mm (± 0.27) for SRP + placebo at 3 months, which was confirmed by Penala et al reporting a PPD reduction of 2.56mm (± 0.98) in the SRP + probiotic group and of 2.12 mm (± 0.70) for SRP + placebo. Such comparative clinical improvement was still maintained for both moderate and deep pockets after 1 year [28, 29].

CAL/attachment gain

Two studies [28, 35] showed a significant difference on overall attachment gain ($p = 0.001$) favoring the superior clinical efficacy of the SRP + probiotic treatment when compared to the SRP. However, no significant difference was manifested in five included studies [27, 29, 31, 32, 39]. The overall mean CAL gain ranged from 0.07 mm (± 0.5) to 1.39 mm (± 0.26) in test group compared with 0.09 mm (± 0.8) to 0.99 mm (± 0.22) in control group at the end of the follow-up visit [26, 27, 29]. Researches published by Teughels et al and Penala et al, CAL gain was stratified separately for moderate and deep pockets. Whereas only Teughels et al elucidated a more pronounced CAL gain in both moderate (1.01 mm \pm 0.59 and 1.42 mm \pm 0.27 respectively; $p = 0.014$) and deep pockets (0.68mm \pm 0.85 and 1.47 mm \pm 0.71 respectively; $p = 0.007$) in favor of the SRP + probiotic treatment. Besides, the situation of recession formation tended to be improved in the SRP + probiotic group ($p = 0.089$). Whilst these differences were not confirmed in the study of Penala et al, but the test group tend to show more favorable results than the control group.

BOP, GBI, GI and PI changes

Bleeding on probing (BOP), GBI reduction, Gingival index and PI were more important in the SRP + probiotic group in all the included studies but with no significant inter-group differences [27, 29-33, 39], except two studies [28, 35]. BOP, GI and PI were significantly lower in the SRP + probiotic group than in the SRP + placebo group at all time points in the two studies [28, 35].

Other outcomes measures

Five studies [27, 28, 32-34] assessed the microorganism index. Sajedinejad et al demonstrated that the reduced bacterial colony count of *A. actinomycetemcomitans* in the test group was significant less pronounced compared to that of the control group whereas this reduction is all significant between three groups in the study of Piyush Shah et al with no inter-group differences. Teughels et al and Laleman et al both manifested that in saliva, *P. intermedia* numbers in the SRP + P group were significantly ($p < 0.05$) lower at week 12 when compared to the SRP group.

Additionally, Teughels et al further unleashed that there was significantly ($p < 0.05$) larger reductions in *P. gingivalis* numbers in the subgingival, supragingival and saliva samples in the SRP + P group over the 12 week period, when compared to the SRP group. What's more Tekce et al stressed a significant decrease in proportions of obligate anaerobes at days 21, 90 and 180 of SRP+probiotic group. When considering the need of surgery which was defined as persisting pockets >4 mm with BOP [40], Tekce et al showed significant differences between groups ($p < 0.05$) in the percentage of sites and teeth and also the number

of patients needed for surgery through 1 year follow-up. Interestingly, owing to different follow-up periods, discrepancies existed between Teughels et al who found that after 3 months' observation, only in deep sites and the number of patients that there was significant difference between groups. Three included studies [25, 29, 38] in which the periodontal risk assessment tool [41] was conducted, reported that after receiving additional probiotics treatment, more patients were classified as low risk and fewer patients as high risk for disease progression. Moreover, the study of Penala et al analyzed that there was a statistically significant reduction in halitosis scores ($p < 0.05$) at 1 month and 3 months in test group when compared to placebo, and Ince et al illustrated that decreased GCF MMP-8 levels and increased TIMP-1 levels were more significant important up to day 180 ($p < 0.05$) in experimental group.

Synthesis of the results

PPD reduction

Inter-study heterogeneity appeared significant regarding overall PPD reduction ($c2 = 20.86$, $p < 0.001$, $I^2 = 90\%$) and PPD reduction at 3 months in moderate pockets ($c2 = 4.26$, $p = 0.004$, $I^2 = 77\%$). The meta-analysis failed to show a significantly different overall PPD reduction (0.23mm, 95% CI [-0.61, 0.16], $p = 0.25$) at short term between SRP + probiotic and control (Fig. 2A). With regard to moderate pockets, the results also didn't favor the adjunctive administration of probiotics with a significant PPD reduction at 3 months (0.29mm, 95% CI [-0.69, 0.11], $p = 0.15$). Whereas in deep pockets there was more pronounced PPD reduction at 3 months in favor of SRP + probiotic treatment (0.61mm, 95% CI [-0.82, -0.40], $p < 0.00001$). (Fig. 2B, C)

Clinical attachment level

Inter-study heterogeneity appeared significant regarding the overall CAL gain ($c2 = 9.07$, $p = 0.01$, $I^2 = 78\%$) and CAL gain in deep pockets at 3 months ($c2 = 5.84$, $p = 0.02$, $I^2 = 83\%$). The meta-analysis failed to show a significantly pronounced overall CAL gain at short term (0.2mm, 95%CI [-0.43, 0.03], $p = 0.08$). However, the overall CAL gain tended to be greater (0.20 mm) in the SRP + probiotic group (Fig. 3A). When categorized into two subtypes, there was a significant higher CAL gain in moderate pockets of SRP+probiotic group (0.37mm, 95%CI [-0.69, -0.11], $p = 0.006$) but this change was not profound in deep pockets (0.32mm, 95%CI [-1.21, 0.57], $p = 0.48$) at 3 months favoring the SRP + probiotic group (Fig.3B, C).

Bleeding on probing

The meta-analysis favored SRP + probiotic treatment with a higher percentage reduction of BOP at short-term (5.34%, 95% [-6.31, -4.36], $p < 0.00001$) in a fixed effect model (Fig. 4).

Compliance and adverse effects of probiotics

None of the RCT included in this review reported the adverse effects of probiotic administration and patients all showed good compliance with probiotics application.

Publication bias

Due to the small number of trials included in this review, it was not possible to statistically assess publication bias.

Discussion

As the mainstay of the management of periodontitis is the reduction and elimination of specific periodontal pathogens (periodontopathogens/pathobionts) and SRP always being a primary step in the treatment [42], further antimicrobial agents can also be used in conjunction with mechanical procedures to reduce the pathogenic microbial burden and provide a satisfactory clinical outcome particularly in chronic situations [43, 44]. However, there is evidence that periodontopathogens, such as *Tannerella forsythia* and *A. actinomycetemcomitans*, remain in periodontal pockets after nonsurgical therapy [45, 46], and bacterial recolonization occurs even shortly after scaling and root planning and the emergence of antibiotic resistance in these pathobionts have provoked human's concerns. Under the circumstances of that, it's urgently to call for new approaches for the management of periodontitis. These limitations of conventional periodontal therapy gave rise to many attempts to introduce one rather incredible or innovative approach that has been attempted during the last few decades as an alternative for the adjuvant treatment of periodontitis through administration of probiotics to manage a number of infectious diseases, so that the disease-causing pathogens are inhibited, promoting the development of a healthy flora, thus leading to restoration of health [47-49]. Probiotics therapy has gained great success in conquering intestinal inflammatory diseases and increasingly occupies the dominant position in selecting therapies to treat some certain intestinal abnormalities [50]. On the basis that the oral cavity is closely connected with the intestinal canal and they share plenty of similarities, it's well-founded to postulate that the probiotics can also be applied to treat dental inflammatory diseases.

This systematic review and meta-analysis was aimed to unveil whether adjunct use of probiotics has additional clinical efficacy in the treatment of periodontitis. Our findings were in favor of administration of *Lactobacillus* as an adjuvant to SRP compared to SRP therapy alone. All the studies assessed had a low risk of bias and the studies incorporated in the meta-analysis share similar characteristics. Moreover, the results showed a statistically significant bleeding on probing (BOP) reduction [-5.34%, 95%CI (-6.31, -4.36), $p < 0.00001$] for SRP + probiotic treatment versus SRP at short-term, but not a significant differences of overall PPD reduction [0.23mm, 95%CI (-0.61, 0.16), $p = 0.25$] for SRP + probiotic treatment versus SRP at short-term, and only a tendency ($p = 0.08$) has been observed in terms of overall CAL gain. Whereas results were significant when the reduction of PPD was stratified for deep pockets [0.61mm, 95%CI (-0.82, -0.40), $p < 0.00001$] and the gain of CAL was stratified for moderate pockets [0.37mm, 95%CI (-0.64, -0.11), $p = 0.06$]. As this is a relatively new area of dental field, only a handful of RCTs that confirmed to the well-defined exclusion and inclusion criteria. On analysis of the data from the 9 selected studies, it is safe to conclude that 5 *Lactobacillus* sp., i.e. and 3 *Streptococcus* sp., i.e. that have been selected as probiotics to explore whether they can improve the clinical parameters of periodontitis in the targeted patient cohorts. Interestingly, two studies unraveled that *L. rhamnosus* SP1 and *Streptococci* sp. [29, 39]

had no additional clinical effects as an adjuvant therapy to SRP, demonstrating the species specificity of probiotics in managing periodontitis. Based on these findings, we further guess that whether another extensively researched probiotics Bifidobacterium can be administrated to treat periodontal diseases. Fortunately, there are some in vitro studies have evidenced that Bifidobacterium sp. can modify immunoinflammatory and microbiologic parameters and promote a protective effect against experimental periodontitis [51-53]. However in vivo studies are needed to purport its clinical benefits.

Considering that applying probiotics can result in the reduction of burden of periodontopathic organisms such as *A. actinomycetemcomitans*, *P. intermedia* and *P. gingivalis* [27, 28, 32-34] and the concentrations of proinflammatory factors [35], however, the mechanisms underlying the observed salutary effect of probiotics are unclear as yet. According to the ecological plaque hypothesis and the changes of the periodontal microenvironment [48], the possible mechanisms may be the potential of probiotics to change the overall composition of the periodontal biofilm in favor of commensals and alleviate the dysbiosis caused by periodontopathogens (the so called 'red complex bacteria') and competitive capture of adhesion sites and nutrient marasmus [54, 55]; modulation of the immune system [56, 57]; modulation of cell proliferation and apoptosis [58]; production of antimicrobial substances, such as lactic acid, hydrogen peroxide, and reuterin [54, 59]; and modulation of the pH and/or the oxidation of the microenvironment [60].

Now that probiotics have potential in improving clinical performances and bring the same clinical outcomes as antibiotics, it's worthy to make it a regular periodontal therapy with no adverse effects and great patient compliance. However, it's disappointing to see that the probiotics therapy investigated in the selected studies varies in forms of administration, dosage, frequency, and duration. Hence, future studies should agree on a standardized protocol, such as the period, the dosage, the frequency and the form of probiotic administration so as to yield more predictable clinical outcomes.

Conclusions

To conclude, under the limitations of the restricted RCTs regarding to the the effect of adjunctive probiotics to SRP in the management of periodontitis, our systematic review and meta-analysis manifests that adjunctive use of probiotics to SRP promises effective clinical benefits in the management of periodontitis, reducing periodontal pathogenic bacteria, and improving the clinical outcomes at short-term with probiotic strain specificity. As probiotic therapy could be an attractive supplementary adjunct for traditional therapies in managing periodontal disease, further long-term RCTs are warranted.

Abbreviations

SRP, scaling and root planning; PPD, periodontal probing depth; BOP, bleeding on probing; CAL, clinical attachment level; CP, chronic periodontitis; AgP, aggressive periodontitis; AAP, advanced adult periodontitis; FMD, full-mouth disinfection; GBI, Gingival Bleeding Index; GI, Gingival Index; M/F, Male/Female; N, Population; OHI, oral hygiene instructions; PI, Plaque Index; PDI, Periodontal disease index; RCT, randomized clinical trial; REC, gingival recession; MGI, modified gingival index; BANA, N-benzoyl-DL-arginine-naphthylamide; ORG, organoleptic scores; NK, not known; L, low risk bias; WMD, Weighted mean differences; CI, confidence interval; CFUs, colony forming units

Declarations

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

GHQ, CX, LL, WXQ and XY adapted the economic model for this analysis. GHQ and CX wrote the first and subsequent versions of the manuscript. All the authors contributed to fruitful discussion of the results and to the review of the manuscript. XY is the guarantor of the overall content of the paper. All authors have read and approved the manuscript, and ensure the integrity and accuracy.

Ethics approval and consent to participate

No applicable

Consent for publication

No applicable

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Conflict of interest

None of the authors of this review declares a conflict of interest or obtained any kind of financing or support from any company related to the production of probiotics.

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Tables

Table 1 Characteristics of included studies

Study	Type	Clinical parameters	N M/F Age	CP definition	Treatment	Probiotic Administration	Adverse effects
et al. 5) [26]	RCT Placebo Parallel	PI, GI, BOP, PPD, attachment level 6sites/tooth	30 CP 17/13 35-50	Radiographically detected horizontal bone loss with presence of at least 2 teeth with one approximal site with PPD of 5-7 mm and a GI of ≥ 2 in each quadrant	OHI (2 groups) SRP + placebo (control) SRP + probiotic (test) (2 sessions of SRP)	Lactobacillus reuteri 2 lozenges per day for 3 weeks Started at onset of initial therapy 1x10 ⁸ CFU per lozenge	No
ghels et al. 3) [27]	RCT Placebo Parallel	PI, GI, BOP, PPD, REC, attachment level	30 CP 15/15 45.73/46.6	Severe generalized adult periodontitis (Van der Velden, U., 2005): ≥ 14 teeth affected and bone loss $> 1/2$ of the root length or attachment loss ≥ 6 mm	OHI (2 groups) SRP (FMD) + placebo (control) SRP (FMD) + probiotic (test)	Lactobacillus reuteri 2 lozenges per day for 12 weeks 1x10 ⁸ CFU per lozenge	No

ales et al 6) [29]	RCT Placebo Parallel	PI, GI, BOP, PPD, attachment level. 6sites/tooth	28 CP 14/14 36-60	Chronic periodontitis was defined as having at least five teeth with periodontal sites with pocket probing depth (PPD) \geq 5mm and clinical attachment loss (AL) \geq 3 mm, 20% bleeding on probing (BOP) and extensive radiographically determined bone loss	OHI (2 groups) SRP + placebo (control) SRP + probiotic (test) (4-6 sessions of SRP)	Lactobacillus rhamnosus SP1 1 sachet taken orally every day for 3 months Started at the last session of SRP 2 ¹⁰ 7 CFU per sachet	No
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aki et al 6) [30]	RCT Placebo Parallel	PI, GI, BOP, PPD 6sites/tooth	36CP 13/23 67.0-6 .2	Having one or more initial periodontal pockets \geq 4mm	OHI (2 groups) SRP + placebo(control) SRP + probiotic (test) (4 sessions of SRP)	Lactobacillus plantarum 1 capsule per day for 12 weeks. Started at onset of initial therapy	No
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ila et al 6) [36]	RCT Placebo Parallel	PI, MGI, BI, BOP, PPD, attachment level, BANA,ORG. 6sites/tooth	29CP NK 25-59	chronic periodontitis clinically evident with at least 4 teeth showing probing depth (PD) \geq 5 mm, clinical attachment level (CAL) \geq 4 mm	OHI (2 groups) SRP + placebo(control) SRP + probiotic (test) (2 sessions of SRP)	Probiotic (Lactobacillus Salivarius and Lactobacillus reuteri) mouthwash for 15 days after SRP and underwent subgingival delivery of probiotic solution at baseline (immediately after SRP), 1 week, 2 weeks, and 4 weeks 2 ¹⁰ 9 CFU per strain	No
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sh 1 al. 3) [32]	RCT Parallel Placebo	PI, GI, BOP, PPD, attachment level 6sites/tooth	30AgP 14/16 14-35	having sites with a probing depth and a loss of the clinical attachment level which were \geq 5 mm, and with a radiographic evidence of bone loss	SRP+OHI (3 groups) Group A - probiotic alone, Group B - a combination of the probiotic with doxycycline, Group C - doxycycline alone	Those in the group A consumed lozenges of the probiotic, Inersan® (Lactobacillus brevis CD2) twice (1-0-1) every day for fourteen days. Those in the group B consumed lozenges of the probiotic, Inersan® twice (1-0-1) and a doxycycline tablet once (0-1-0) every day for fourteen days. Those in the group C consumed a doxycycline tablet once (0-1-0) every day for fourteen days. Started at onset of initial therapy 1'108 CFU per lozenge	No
dinejad al. 7) [33]	RCT Parallel Placebo	PI, PDI, GI, BOP, PPD 6sites/tooth	20CP 6/14 31-59	moderate to severe chronic periodontitis with PPD \geq 4 mm, CAL \geq 3 mm, Bone loss \geq 3 mm	OHI (2 groups) SRP + placebo(control) SRP + probiotic (test)	Twenty milliliters of L. salivarius NK02 mouthwashes was used twice a day for 28 days Started at onset of initial therapy 1'108 CFU	No
man al. 5) [39]	RCT Parallel Placebo	PI, GI, REC BOP, PPD 6sites/tooth	48AAP 26/22 37-58	With moderate (initial PPD between 4 and 6 mm) and deep (initial PPD \geq 7 mm) pockets	OHI (2 groups) SRP + placebo(control) SRP + probiotic (test) (1 sessions of SRP)	probiotic tablet containing Streptococcus Oralis KJ3, Streptococcus uberis KJ2 and Streptococcus rattus JH145 dissolve on their tongue twice a day for 3 months.Started at onset of initial therapy 1'108 CFU per strain	No

de et al. 5) [28]	RCT Placebo Parallel	PI, GI, BOP, PPD, attachment level	40 CP 18/22 35-50	Radiographically detected horizontal bone loss with presence of at least 2 teeth with one approximal site with PPD of 5-7 mm and a GI of ≥ 2 in each quadrant	OHI (2 groups) SRP + placebo (control) SRP + probiotic (test) (2 sessions of SRP)	Lactobacillus reuteri 2 lozenges per day for 3 weeks Started at onset of initial therapy 1'108 per lozenge	No
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BOP, bleeding on probing; *CAL*, clinical attachment level; *CP*, chronic periodontitis; *AgP*, aggressive periodontitis; *AAP*, advanced adult periodontitis; *FMD*, full-mouth disinfection; *GBI*, Gingival Bleeding Index; *GI*, Gingival Index; *M/F*, Male/Female; *N*, Population; *OHI*, oral hygiene instructions; *PI*, Plaque Index; *PDI*, Periodontal disease index; *PPD*, periodontal probing depth; *RCT*, randomized clinical trial; *REC*, gingival recession; *MGI*, modified gingival index; *BANA*, N-benzoyl-DL-arginine-naphthylamide; *ORG*, organoleptic scores; *NK*, not known

Table 2 Summary of risk of bias in individual studies

Item	Ince et al. (2015) [26]	Tekce et al. (2015) [28]	Teughels et al. (2013) [27]	Morales et al. 1. [29]	Iwasaki et al. (2016) [30]	Penala et al. (2016) [36]	Piyush Shah et al. (2013) [32]	Sajedinejad et al. (2017) [33]	Laleman et al. (2015) [39]
Sequence generation	Computer-based randomization program	Computer-based randomization program	Block randomization	Computer-based randomization program	Computer-based randomization program	Computer-based randomization program	Block randomization	Block randomization	Block randomization
Allocation concealment	Identical bottles	Identical bottles	Coded bottles	Identically numbered, sealed envelopes	Identically numbered, sealed envelopes	Identically labeled containers	Identical lozenges	Identical mouthwashes	Coded bottles
Examiner blinding	Double blinded	Double blinded	Double blinded	Double blinded	Double blinded	Double blinded	Double blinded	Double blinded	Double blinded
Incomplete outcome data	No missing outcome data	No missing outcome data	No missing outcome data	No missing outcome data	No missing outcome data	No missing outcome data	No missing outcome data	No missing outcome data	No missing outcome data
Selective outcome reporting	All outcomes stated in the material & method section were analysed and presented	All outcomes stated in the material & method section were analysed and presented	All outcomes stated in the material & method section were analysed and presented	All outcomes stated in the material & method section were analysed and presented	All outcomes stated in the material & method section were analysed and presented	All outcomes stated in the material & method section were analysed and presented	All outcomes stated in the material & method section were analysed and presented	All outcomes stated in the material & method section were analysed and presented	All outcomes stated in the material & method section were analysed and presented
Other sources of bias	Supported by BioGaia (Sweden)	Founded by BioGaia (Sweden)	Grant of BioGaia (Sweden)	Approved by the Local Ethical Committee of the Faculty of Dentistry at University of Chile					Funded by grants of Pierre Fabre Medicament Castres, France

Table 3 Risk of bias summary

	Ince et al. (2015) [26]	Tekce et al. (2015) [28]	Teughels et al. (2013) [27]	Morales et al. (2016) [29]	Iwasaki et al. (2016) [30]	Penala et al. (2016) [36]	Piyush Shah et al. (2013) [32]	Sajedinejad et al. (2017) [33]	Laleman et al. (2015) [39]
andom sequence generation (selection bias)	L	L	L	L	L	L	L	L	L
location concealment (selection bias)	L	L	L	L	L	L	L	L	L
inding of participants and personnel (performance bias)	L	L	L	L	L	L	L	L	L
inding of outcome assessment (detection bias)	L	L	L	L	L	L	L	L	L
complete outcome data (attrition bias)	L	L	L	L	L	L	L	L	L
lective reporting (reporting bias)	L	L	L	L	L	L	L	L	L
ther bias	L	L	L	L	L	L	L	L	L

L, low risk bias

Figures

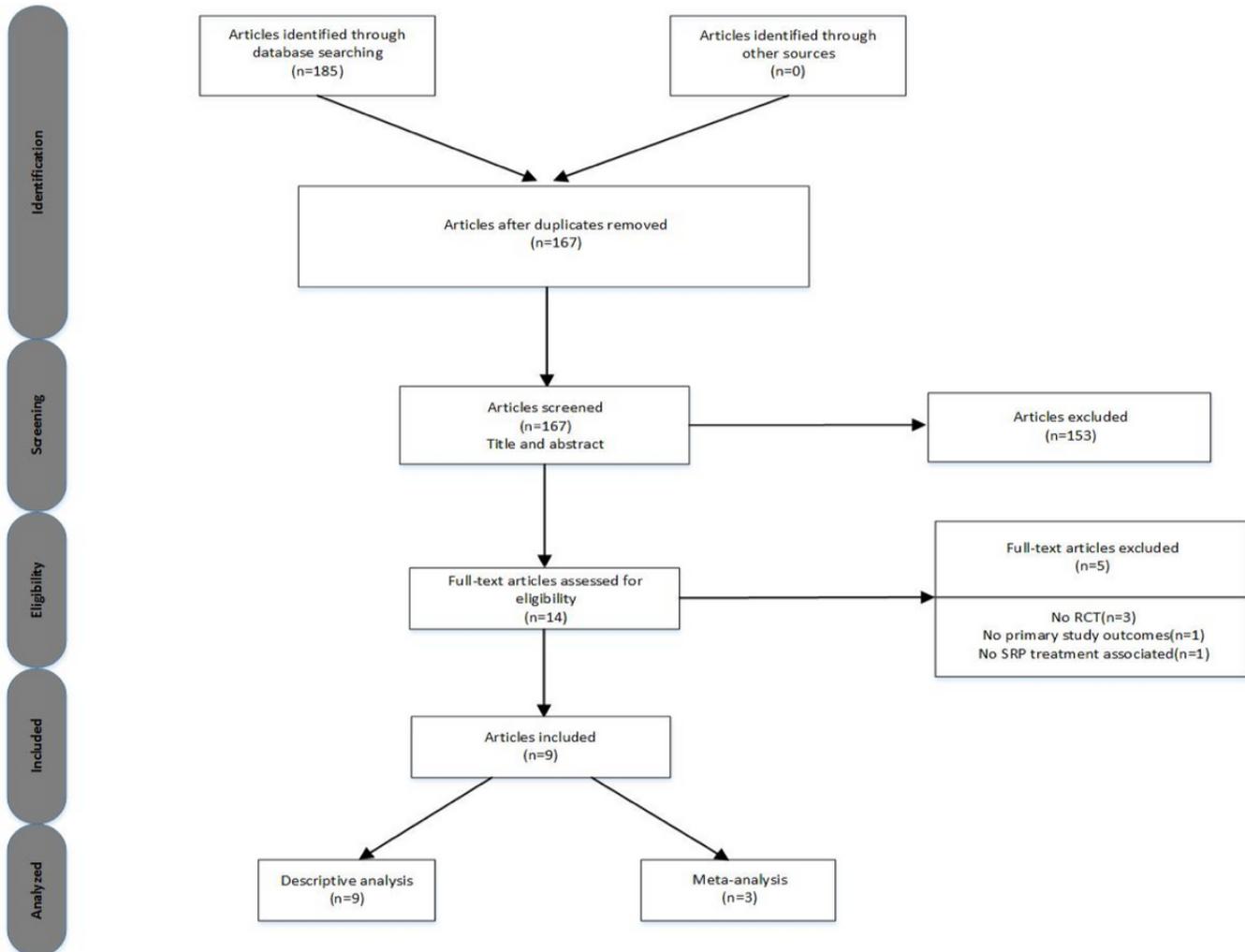
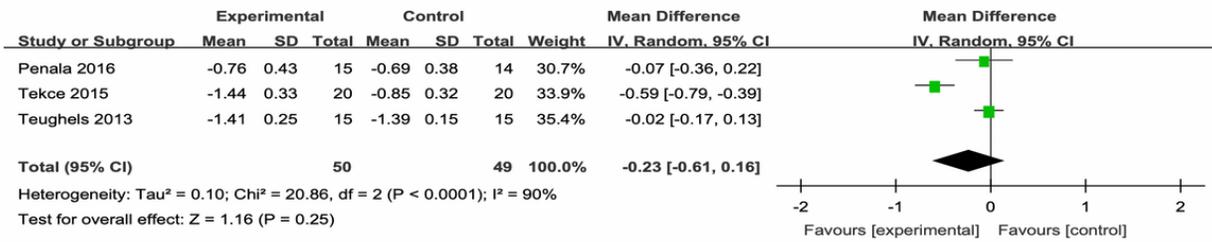
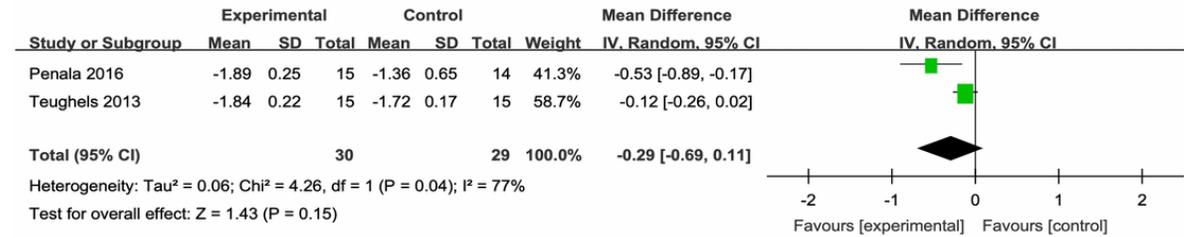


Figure 1

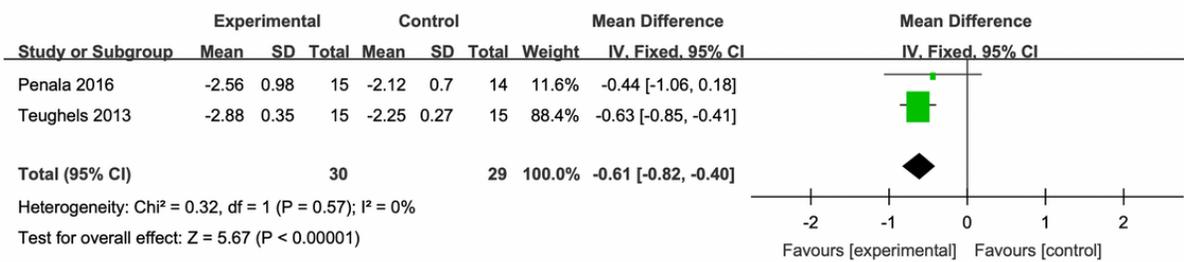
Flow chart of the study



A



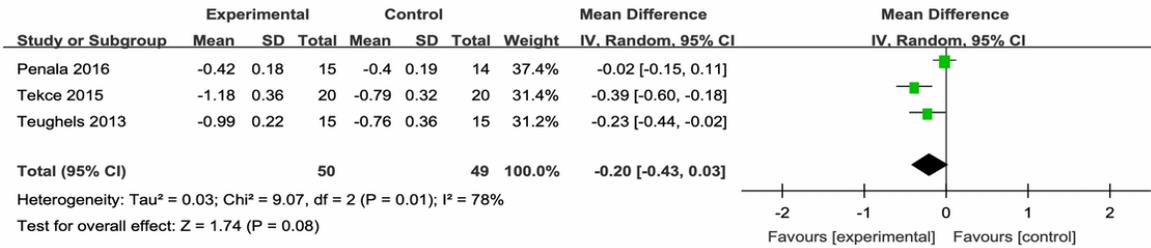
B



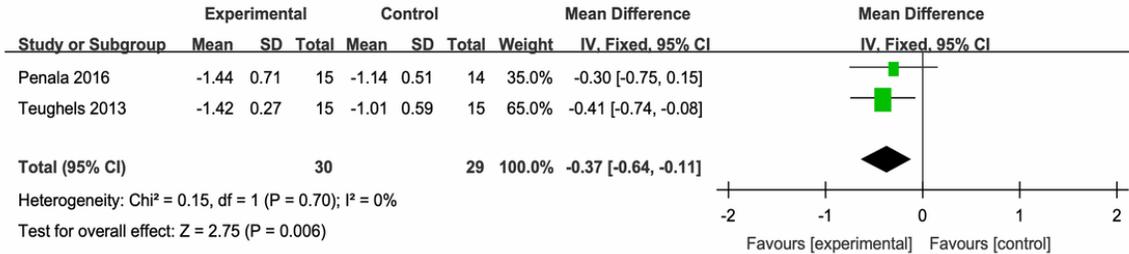
C

Figure 2

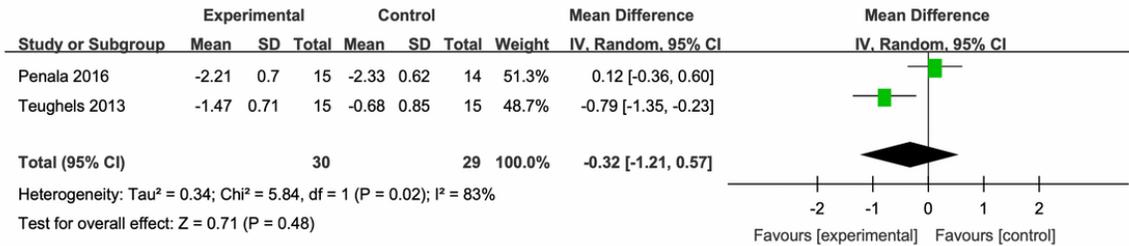
(A) Forest plot of overall PPD reduction at short-term; (B) Forest plot of PPD reduction at 3 months in moderate pockets; (C) Forest plot of overall PPD reduction at 3 months in deep pockets



A



B



C

Figure 3 (A) Forest plot of overall CAL gain at short-term; (B) Forest plot of CAL gain at 3 months in moderate pockets; (C) Forest plot of CAL gain at 3 months in deep pockets

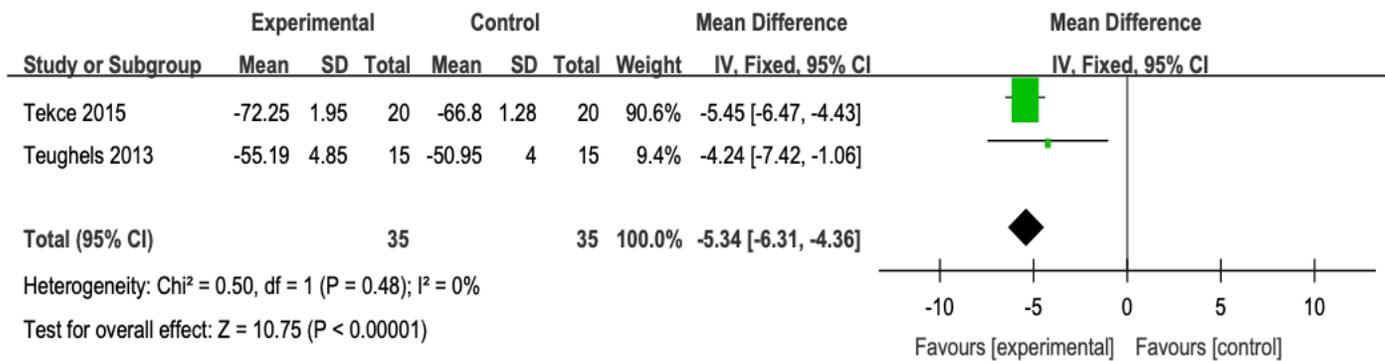


Figure 4 Forest plot of overall percentage of BOP reduction at short-term