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Review article

Clinical, microbiological and immunological short, medium and long-term effects of different strains of probiotics as an adjunct to non-surgical periodontal therapy in patients with periodontitis. Systematic review with meta-analysis

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A R T I C L E I N F O

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A B S T R A C T

Introduction/objectives: Probiotics have been proposed as adjuncts to non-surgical periodontal therapy (NSPT), however, the effect of their use remains unclear. The aim of this systematic review and meta-analysis was to analyze the evidence regarding the use of probiotics as an adjunct to NSPT in patients with periodontitis at a clinical, microbiological and immunological level.
Data/sources: A comprehensive search to identify clinical studies investigating the use of probiotics as an adjunct to NSPT in patients treated for periodontitis was performed. The data were grouped according to probiotic strain, frequency, form and duration of the probiotic intake.
Study selection: A total of 25 articles were included, all articles analysed clinical parameters, 10 included also microbiological findings and only 4 had immunological findings. The difference in probing depth (PD) between the test and the control group was statistically significant in favour of the test group when the probiotics were in the form of lozenges, administered twice a day and when the strain was *L. reuteri*. In terms of Clinical Attachment Level (CAL) gain the difference was statistically significant in the short and in the medium term but not in the long term. Due to the heterogeneity of the data, it was not possible to compare trough a meta analysis the immunological and the microbiological findings that were therefore analysed only descriptively.
Conclusions: The use of probiotics as an adjunct to NSPT in patients with periodontitis appears to provide additional clinical benefits that depend on the duration, the frequency, the form and the strain of probiotic used.
Clinical significance: This review not only shows data on the efficacy of probiotics in non-surgical periodontal therapy, but provides important information on their effects over time and which forms of probiotic administration might be most clinically useful.

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Contents

1. Introduction

Periodontitis is an inflammatory disease that affects the supporting apparatus of the teeth, the periodontium. It can cause progressive destruction of the periodontal tissues leading to increased teeth mobility and ultimately to their loss [1]. Its etiology is multifactorial, however the main cause of its onset is dental plaque and the pathogenic microorganisms that populate it [2]. Bacteria considered to be periodontal pathogens are currently recognized as those of the so-called red complex, namely *Porphyromonas gingivalis*, *Tannerella forsythia*, and *Treponema denticola*, together with *Aggregatibacter Actinomicetemcomitans*. These bacteria, often normally present in clinically healthy oral cavity, when an imbalance increases their concentration in the gingival sulcus, can trigger and sustain periodontitis [2]. The treatment, therefore, at least in its early stage, consists in the elimination, or reduction below the pathogenic threshold, of the causative factor, namely the microorganisms responsible for its onset. The first phase of treatment, the so-called non-surgical periodontal therapy (NSPT), aims at eliminating plaque, calculus and all those factors that favour its accumulation, as well as the elimination or control, where possible, of the systemic risk factors [3]. The non-surgical phase of periodontal therapy is substantially mechanical, the so-called scaling and root planing (SRP), and can be performed with or without an adjunctive therapy. Because of bacterial nature of the pathology, this often consists of antibiotic therapy [4]. Antibiotics are strongly suggested for some specific manifestations of periodontitis such as the molar incisor pattern one (former aggressive), but also used, depending on the circumstances, in the other forms of the disease [5].

If antibiotic therapy aims at the elimination, mostly nonspecific, of bacteria, another type of co-adjuvant therapy has been proposed, the probiotic one, which has an almost opposite mechanism of action. Probiotic therapy, according to WHO definition, consists of "live microorganisms which, when administered in adequate amounts, confer a health benefit to the host", among these benefits we could briefly mention the competition with potentially pathogenic microorganisms or the interaction with their virulence factors, the stimulation of the host's immune response and the production of nutrients and cofactors [6].

In summary, if antibiotic therapy aims at counteracting dysbiosis by eliminating bacteria, pathogenic or not, probiotic therapy has aims at achieving the same goal by adding beneficial microorganisms to the flora.

The microorganisms most commonly used as probiotics are lactobacilli and bifidobacteria. The lactobacillus is a genus that includes bacteria that derive almost all of their energy from the fermentation of glucose and lactose to lactic acid (homolactic fermentation). Some bacteria belonging to the Lattobacillus are able to produce, through their metabolism, small quantities of H2O2 (antimicrobial agent) [7]. They are gram-positive, facultative anaerobes, non-spore-forming, non-motile and have only a rod-like shape. The Bifidobacterium is a genus that belongs to the phylum of Actinobateria, they are Gram positive, non-motile, non-spore-forming and non-filamentous and are a Y or V-shaped rod [8].

There are more and more studies reported in the literature that investigate the use of probiotics, of different nature but also of different forms and methods of administration, as additional therapy in the non-surgical and maintenance phase of periodontal therapy. The purpose of this systematic review and meta-analysis is to collect this evidence to highlight the effects of probiotics, at a clinical, microbiological and immunological level, when used in the treatment of periodontitis as an adjunct to non-surgical therapy.

2. Materials and methods

This systematic review was registered at the National Institute for Health Research PROSPERO, International Prospective Register of Systematic Reviews with the number CRD42021257782.

2.1. PICo criteria definitions

Participants: Patients suffering from periodontitis.
Intervention: Periodontal non-surgical therapy with adjunctive probiotics intake.

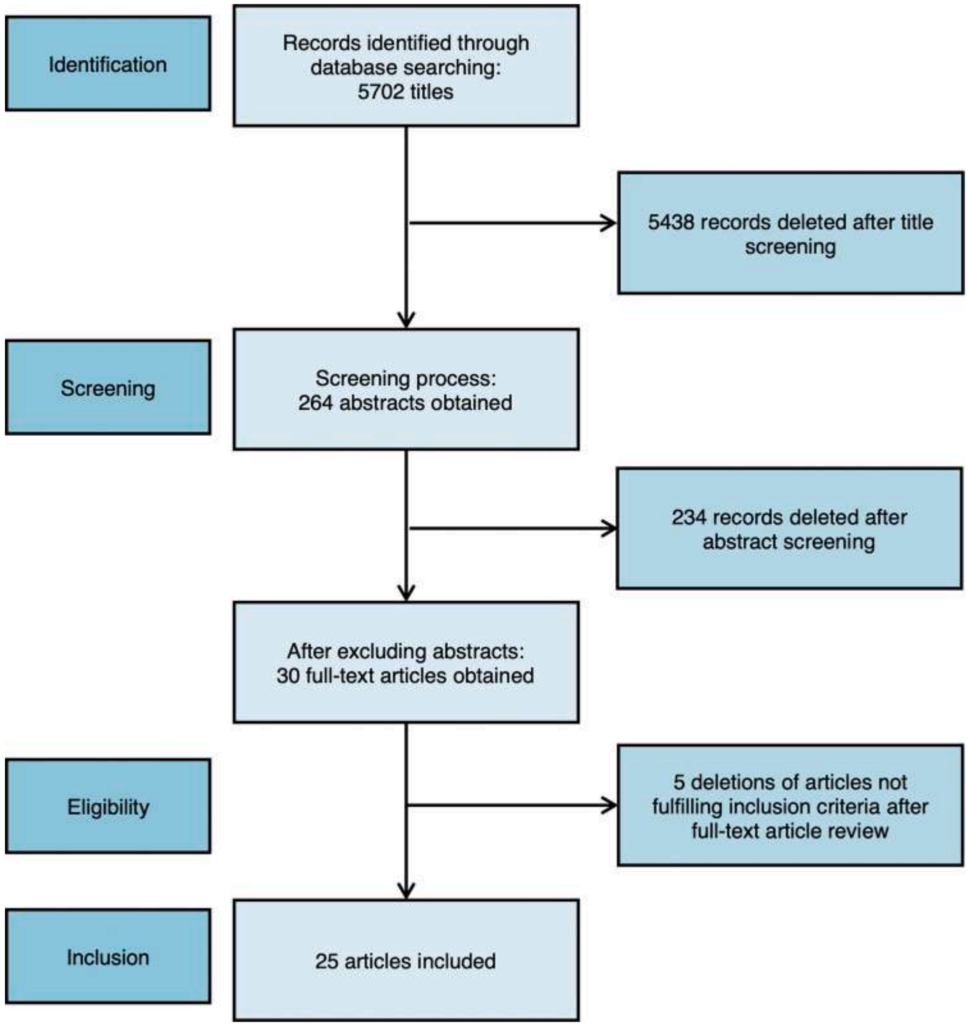


Fig. 1. PRISMA flow diagram of the study selection process.

Comparison: Periodontal non-surgical therapy alone.
Outcome: Change in clinical parameters (Probing Depth PD, Clinical Attachment Level CAL, Bleeding of Probing BoP).

2.2. Focused question

In patients affected by periodontitis, does non-surgical periodontal therapy with adjunctive intake of probiotics improve the clinical, microbiological or immunological outcomes compared to non-surgical periodontal therapy alone?

2.3. Search strategy

The data for this systematic review and meta-analysis were processed following PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) principles [9]; the introductory set of studies related to the topic "the adjunctive use of probiotics in the non-surgical therapy of patients affected by periodontal disease" was obtained through an electronic search of the MEDLINE/PubMed and Cochrane Oral Health Group databases.

Relevant articles published up to March 5th, 2020 were searched using the relevant keywords and respective Boolean logic operators (AND, OR, NOT) used in the above-mentioned databases: PubMed, EMBASE, Ovid MEDLINE, Web of Science. The relevant keywords were combined as follow for the search: ((((((((((((((("Chronic Periodontitis/immunology" OR "Chronic Periodontitis/microbiology")) OR "Dental Plaque Index") OR "Dental Plaque/microbiology") OR ("Gingival Crevicular Fluid/immunology" OR "Gingival Crevicular Fluid/metabolism")) OR "Gingival Diseases") OR "Gingivitis/microbiology") OR "Periodontal Index") OR "Periodontal Pocket/microbiology") OR ("Periodontal Diseases/immunology" OR "Periodontal Diseases/microbiology")) OR ("Periodontitis/immunology" OR "Periodontitis/microbiology")) OR ("Periodontium/immunology" OR "Periodontium/microbiology"))) OR (((((subgingiva*) OR gingiv*) OR periodont*) OR periopathogen*) OR periodontopath*)) OR (((dental OR tooth OR teeth OR oral*)) AND plaque))) AND (((((((("Probiotics") OR "Lactobacillus") OR "Lactobacillus brevis") OR "Lactobacillus casei") OR "Lactobacillus reuteri"[Mesh]) OR "Bifidobacterium")) OR ((probiotic* OR Lactobacill* OR Bifidobacter* OR bacill* OR Streptococcus thermophilus OR Saccharomyces))).

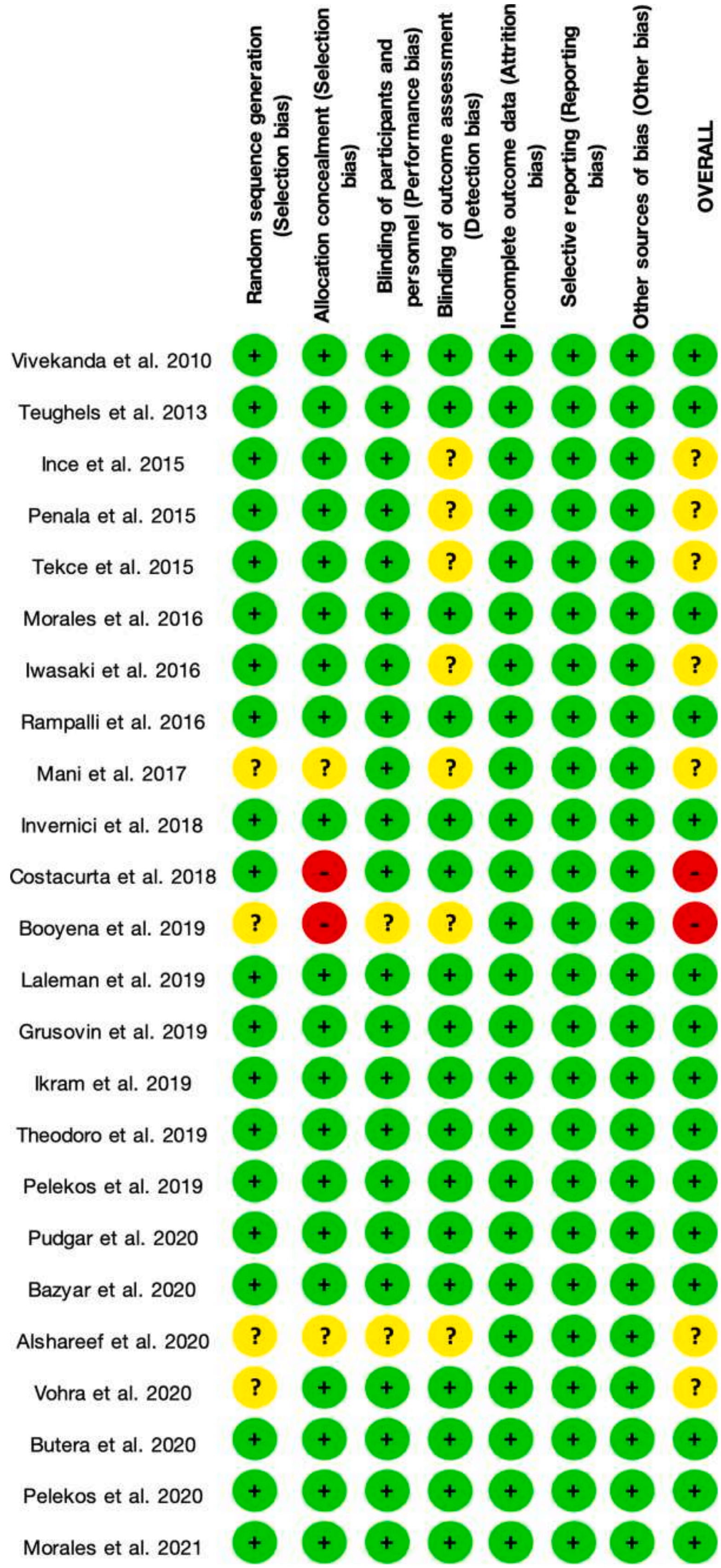
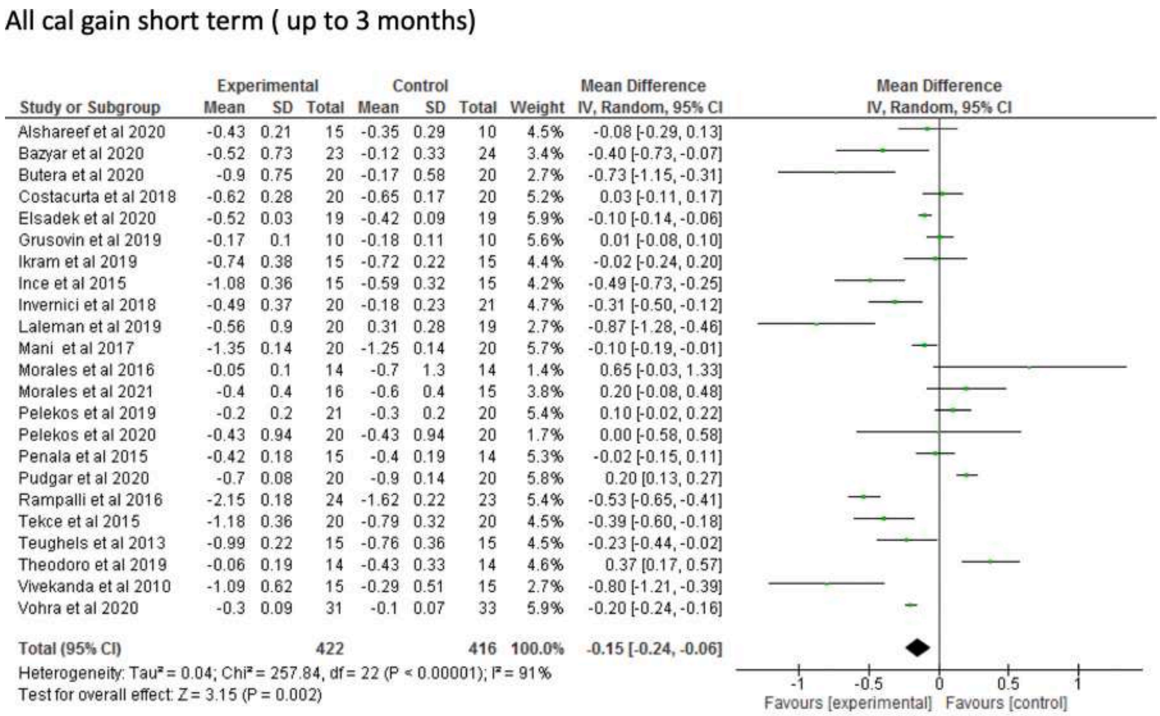


Fig. 2. Risk of bias according to Cochrane Collaboration tool for RCTs (RoB2).



Study or Subgroup	Experimental Mean	Experimental SD	Experimental Total	Control Mean	Control SD	Control Total	Weight	Mean Difference IV, Random, 95% CI
Alshareef et al 2020	-0.43	0.21	15	-0.35	0.29	10	4.5%	-0.08 [-0.29, 0.13]
Bazyar et al 2020	-0.52	0.73	23	-0.12	0.33	24	3.4%	-0.40 [-0.73, -0.07]
Butera et al 2020	-0.9	0.75	20	-0.17	0.58	20	2.7%	-0.73 [-1.15, -0.31]
Costacurta et al 2018	-0.62	0.28	20	-0.65	0.17	20	5.2%	0.03 [-0.11, 0.17]
Elsadek et al 2020	-0.52	0.03	19	-0.42	0.09	19	5.9%	-0.10 [-0.14, -0.06]
Grusovin et al 2019	-0.17	0.1	10	-0.18	0.11	10	5.6%	0.01 [-0.08, 0.10]
Ikram et al 2019	-0.74	0.38	15	-0.72	0.22	15	4.4%	-0.02 [-0.24, 0.20]
Ince et al 2015	-1.08	0.36	15	-0.59	0.32	15	4.2%	-0.49 [-0.73, -0.25]
Invernici et al 2018	-0.49	0.37	20	-0.18	0.23	21	4.7%	-0.31 [-0.50, -0.12]
Laleman et al 2019	-0.56	0.9	20	0.31	0.28	19	2.7%	-0.87 [-1.28, -0.46]
Mani et al 2017	-1.35	0.14	20	-1.25	0.14	20	5.7%	-0.10 [-0.19, -0.01]
Morales et al 2016	-0.05	0.1	14	-0.7	1.3	14	1.4%	0.65 [-0.03, 1.33]
Morales et al 2021	-0.4	0.4	16	-0.6	0.4	15	3.8%	0.20 [-0.08, 0.48]
Pelekos et al 2019	-0.2	0.2	21	-0.3	0.2	20	5.4%	0.10 [-0.02, 0.22]
Pelekos et al 2020	-0.43	0.94	20	-0.43	0.94	20	1.7%	0.00 [-0.58, 0.58]
Penala et al 2015	-0.42	0.18	15	-0.4	0.19	14	5.3%	-0.02 [-0.15, 0.11]
Pudgar et al 2020	-0.7	0.08	20	-0.9	0.14	20	5.8%	0.20 [0.13, 0.27]
Rampalli et al 2016	-2.15	0.18	24	-1.62	0.22	23	5.4%	-0.53 [-0.65, -0.41]
Tekce et al 2015	-1.18	0.36	20	-0.79	0.32	20	4.5%	-0.39 [-0.60, -0.18]
Teughels et al 2013	-0.99	0.22	15	-0.76	0.36	15	4.5%	-0.23 [-0.44, -0.02]
Theodoro et al 2019	-0.06	0.19	14	-0.43	0.33	14	4.6%	0.37 [0.17, 0.57]
Vivekanda et al 2010	-1.09	0.62	15	-0.29	0.51	15	2.7%	-0.80 [-1.21, -0.39]
Vohra et al 2020	-0.3	0.09	31	-0.1	0.07	33	5.9%	-0.20 [-0.24, -0.16]
Total (95% CI)			**422**			**416**	**100.0%**	**-0.15 [-0.24, -0.06]**

Heterogeneity: Tau² = 0.04; Chi² = 257.84, df = 22 (P < 0.00001); I² = 91%
Test for overall effect: Z = 3.15 (P = 0.002)

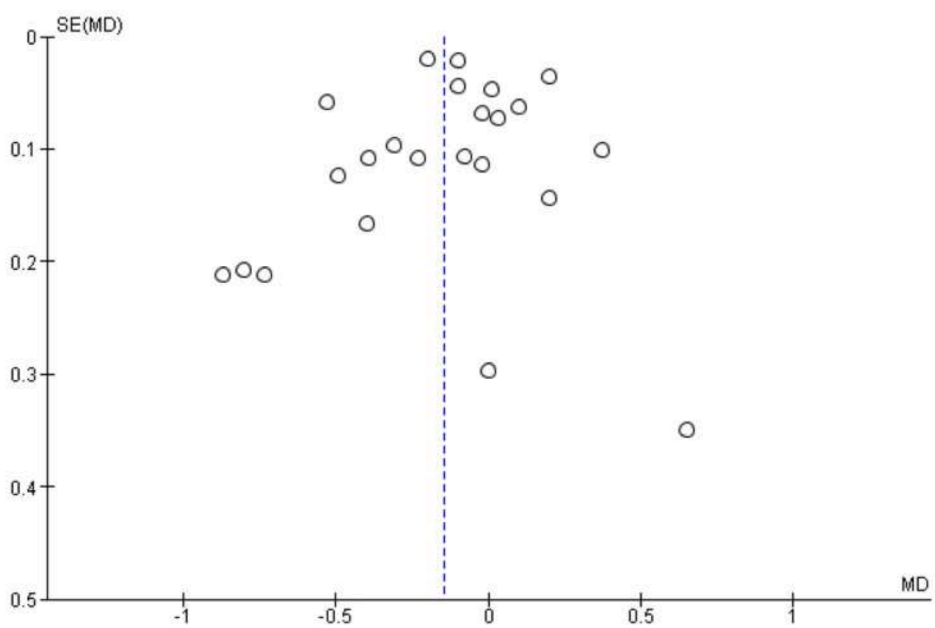
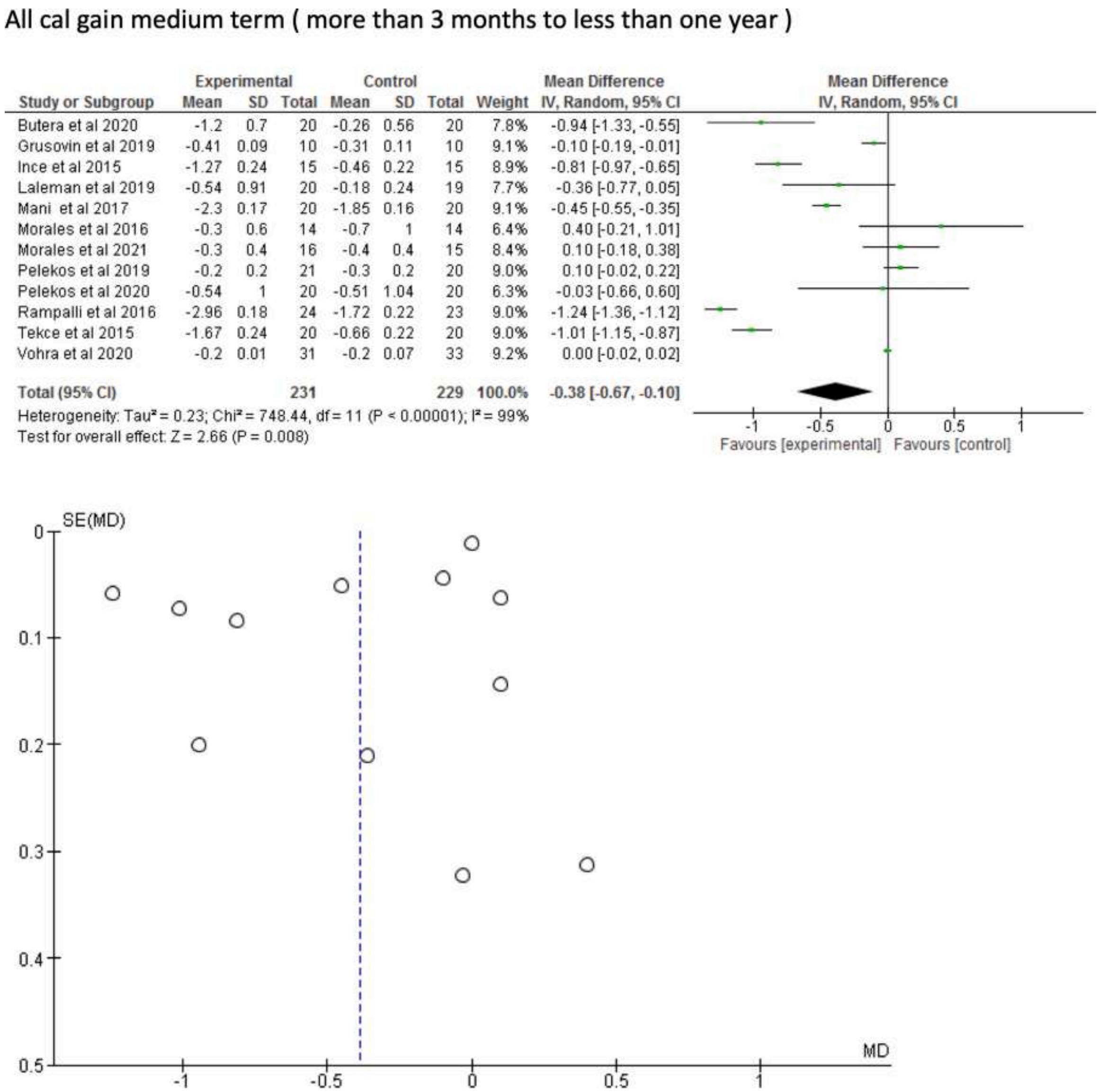


Fig. 3. Forest plot and funnel plot for CAL gain short term (up to 3 months).


All cal gain medium term (more than 3 months to less than one year)

Study or Subgroup	Experimental Mean	Experimental SD	Experimental Total	Control Mean	Control SD	Control Total	Weight	Mean Difference IV, Random, 95% CI
Butera et al 2020	-1.2	0.7	20	-0.26	0.56	20	7.8%	-0.94 [-1.33, -0.55]
Grusovin et al 2019	-0.41	0.09	10	-0.31	0.11	10	9.1%	-0.10 [-0.19, -0.01]
Ince et al 2015	-1.27	0.24	15	-0.46	0.22	15	8.9%	-0.81 [-0.97, -0.65]
Laleman et al 2019	-0.54	0.91	20	-0.18	0.24	19	7.7%	-0.36 [-0.77, 0.05]
Mani et al 2017	-2.3	0.17	20	-1.85	0.16	20	9.1%	-0.45 [-0.55, -0.35]
Morales et al 2016	-0.3	0.6	14	-0.7	1	14	6.4%	0.40 [-0.21, 1.01]
Morales et al 2021	-0.3	0.4	16	-0.4	0.4	15	8.4%	0.10 [-0.18, 0.38]
Pelekos et al 2019	-0.2	0.2	21	-0.3	0.2	20	9.0%	0.10 [-0.02, 0.22]
Pelekos et al 2020	-0.54	1	20	-0.51	1.04	20	6.3%	-0.03 [-0.66, 0.60]
Rampalli et al 2016	-2.96	0.18	24	-1.72	0.22	23	9.0%	-1.24 [-1.36, -1.12]
Tekce et al 2015	-1.67	0.24	20	-0.66	0.22	20	9.0%	-1.01 [-1.15, -0.87]
Vohra et al 2020	-0.2	0.01	31	-0.2	0.07	33	9.2%	0.00 [-0.02, 0.02]
Total (95% CI)			**231**			**229**	**100.0%**	**-0.38 [-0.67, -0.10]**

Heterogeneity: Tau² = 0.23; Chi² = 748.44, df = 11 (P < 0.00001); I² = 99%
Test for overall effect: Z = 2.66 (P = 0.008)

Fig. 4. Forest plot and funnel plot for CAL gain medium term (more than 3 months, less than 1 year).

Two independent reviewers (NAV, FA) screened all of the titles, abstracts and then the full text of the studies according to the inclusion and exclusion criteria.

2.4. Inclusion criteria

Studies were included if the following a priori criteria were met:

- Prospective cohort human studies, randomized clinical trials
- A follow-up period of at least 4 weeks from NSPT
- At least 10 patients included
- Studies in which data about either clinical or microbiological or immunological outcomes were clearly reported

2.5. Exclusion criteria

- Retrospective cohort studies
- Pre-clinical studies

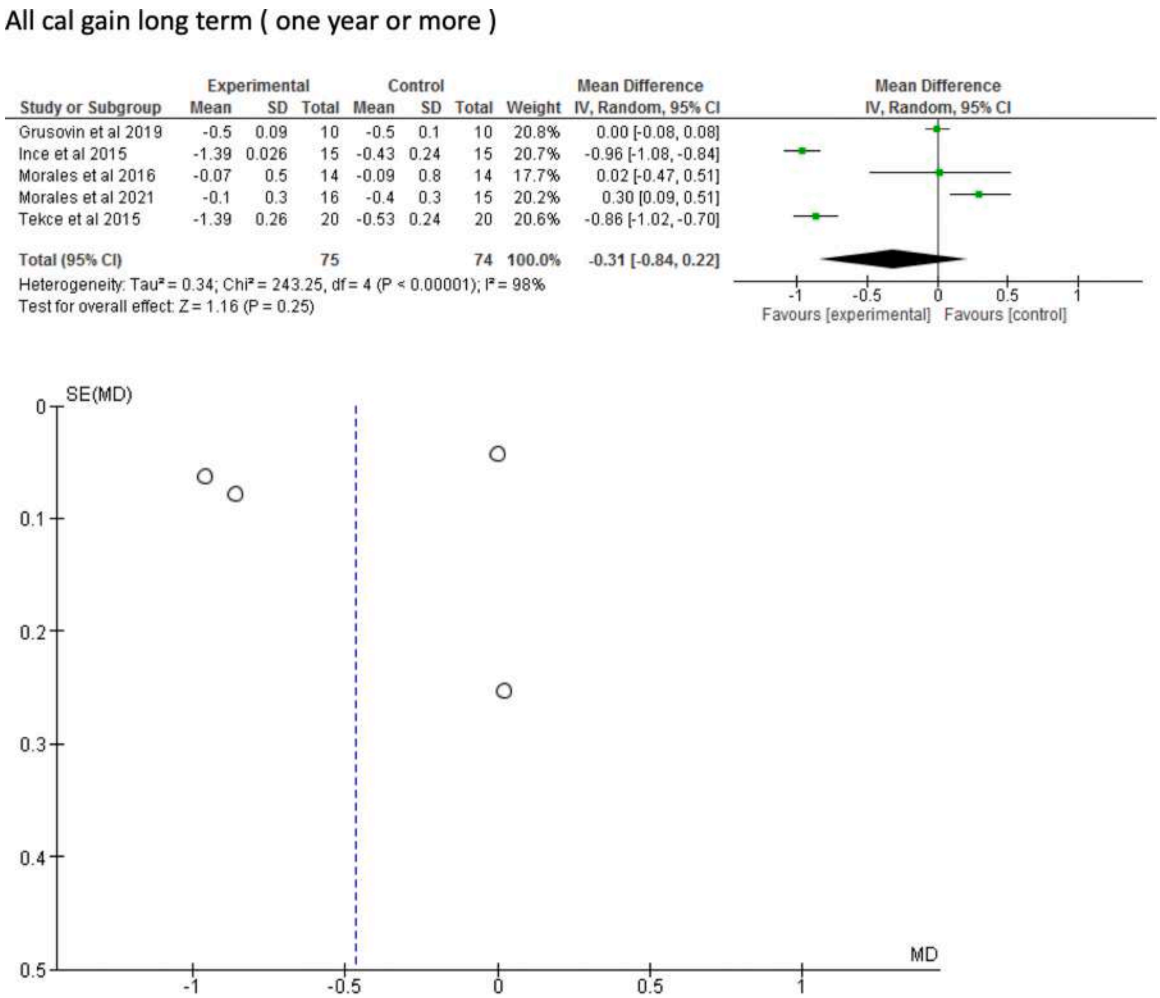


Fig. 5. Forest plot and funnel plot for CAL gain long term (1 year or more).

- Animal studies
- Case reports
- Repeated reports of the same study/author

2.6. Quality assessment

Three authors (NAV, FA, EB) independently assessed the studies in terms of the inclusion, relevance, eligibility, and risk of bias following the Cochrane Collaboration tool (Higgins et al., 2011) for the randomized studies and the Newcastle-Ottawa tool for prospective cohort studies; any disagreement was resolved by consensus of reviewers (NAV, FA, EB) and statistics researcher (ZN).

2.7. Data extraction and collection process

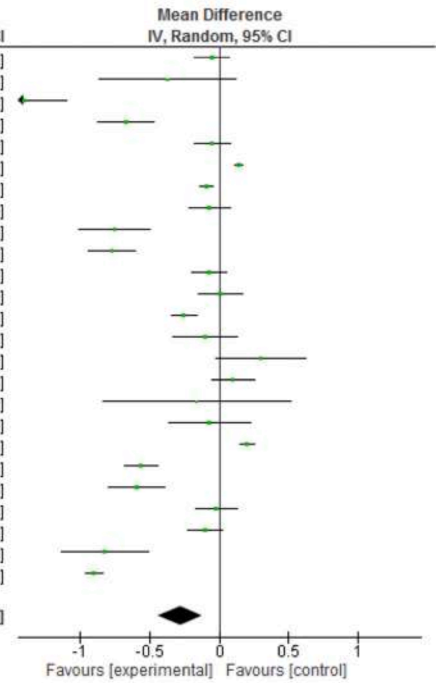
Following the screening process, full-text versions of included articles were read by the authors, data were extracted independently, and any conflict was resolved among the authors and confirmed by the statistician. Information was extracted from each included trial about the number of patients at the beginning and at the end of the study, setting, drop out, presence and number of smokers, presence and number of diabetic patients, case definition use for the diagnosis, probiotic strain used, frequency of administration and form, type of intervention, total follow up, time points of follow up, CAL gain, PD reduction, microbiological and immunological findings.

All PD reduction short term (up to 3 months)

Study or Subgroup	Experimental Mean	Experimental SD	Experimental Total	Control Mean	Control SD	Control Total	Weight	Mean Difference IV, Random, 95% CI
Alshareef et al 2020	-0.36	0.09	15	-0.31	0.19	10	4.2%	-0.05 [-0.18, 0.08]
Bazyar et al 2020	-0.82	0.93	23	-0.45	0.77	24	3.0%	-0.37 [-0.86, 0.12]
Booyena et al 2019	-3	0.3	10	-1.6	0.39	10	3.7%	-1.40 [-1.70, -1.10]
Butera et al 2020	-1	0.22	20	-0.33	0.4	20	4.1%	-0.67 [-0.87, -0.47]
Costacurta et al 2018	-0.65	0.25	20	-0.6	0.16	20	4.2%	-0.05 [-0.18, 0.08]
Elsadek et al 2020	-0.48	0.02	19	-0.62	0.06	19	4.4%	0.14 [0.11, 0.17]
Grusovin et al 2019	-0.18	0.008	10	-0.09	0.08	10	4.3%	-0.09 [-0.14, -0.04]
Ikram et al 2019	-1.96	0.14	15	-1.89	0.26	15	4.2%	-0.07 [-0.22, 0.08]
Ince et al 2015	-1.6	0.39	15	-0.85	0.32	15	3.9%	-0.75 [-1.01, -0.49]
Invernici et al 2018	-0.52	0.32	20	0.25	0.22	21	4.1%	-0.77 [-0.94, -0.60]
Iwasaki et al 2016	-0.09	0.15	19	-0.02	0.22	17	4.2%	-0.07 [-0.19, 0.05]
Laleman et al 2019	-0.43	0.23	20	-0.44	0.28	19	4.2%	0.01 [-0.15, 0.17]
Mani et al 2017	-1.4	0.16	20	-1.15	0.13	20	4.3%	-0.25 [-0.34, -0.16]
Morales et al 2016	-0.5	0.2	14	-0.4	0.4	14	4.0%	-0.10 [-0.33, 0.13]
Morales et al 2021	-0.5	0.4	16	-0.8	0.5	15	3.7%	0.30 [-0.02, 0.62]
Pelekos et al 2019	-0.4	0.2	21	-0.5	0.3	20	4.2%	0.10 [-0.06, 0.26]
Pelekos et al 2020	-1.24	1.09	20	-1.08	1.09	20	2.4%	-0.16 [-0.84, 0.52]
Penala et al 2015	-0.76	0.43	15	-0.69	0.38	14	3.8%	-0.07 [-0.36, 0.22]
Pudgar et al 2020	-0.8	0.03	20	-1	0.12	20	4.3%	0.20 [0.15, 0.25]
Rampalli et al 2016	-2.47	0.19	24	-1.91	0.23	23	4.2%	-0.56 [-0.68, -0.44]
Tekce et al 2015	-1.44	0.33	20	-0.85	0.32	20	4.1%	-0.59 [-0.79, -0.39]
Teughels et al 2013	-1.41	0.25	15	-1.39	0.15	15	4.2%	-0.02 [-0.17, 0.13]
Theodoro et al 2019	-0.25	0.19	14	-0.15	0.15	14	4.2%	-0.10 [-0.23, 0.03]
Vivekanda et al 2010	-1.31	0.49	15	-0.49	0.39	15	3.7%	-0.82 [-1.14, -0.50]
Vohra et al 2020	-3.6	0.13	31	-2.7	0.12	33	4.3%	-0.90 [-0.96, -0.84]
Total (95% CI)			**451**			**443**	**100.0%**	**-0.28 [-0.43, -0.12]**

Heterogeneity: Tau² = 0.14; Chi² = 1346.59, df = 24 (P < 0.00001); I² = 98%

Test for overall effect: Z = 3.51 (P = 0.0004)



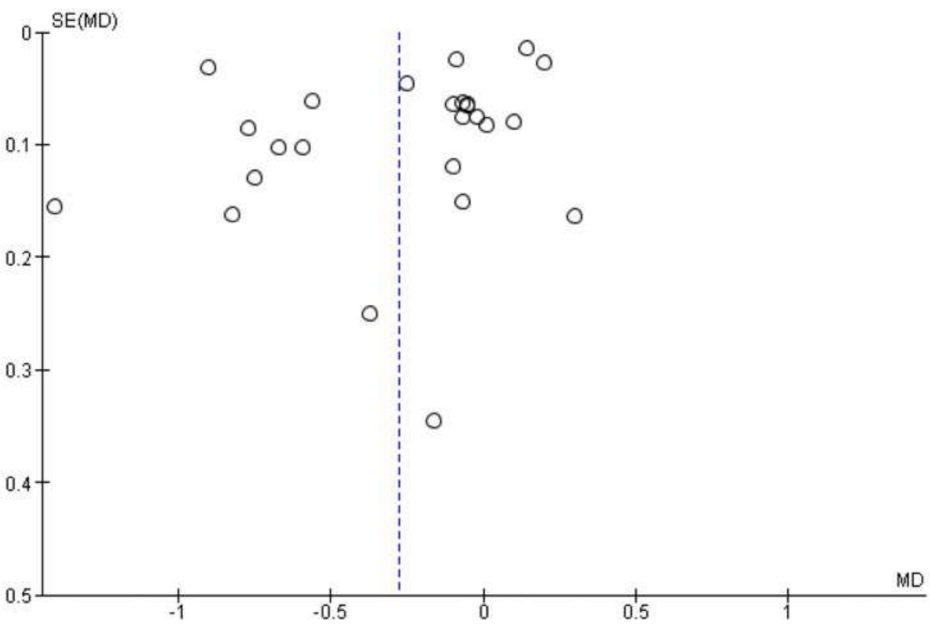


Fig. 6. Forest plot and funnel plot for PD reduction short term (up to 3 months).

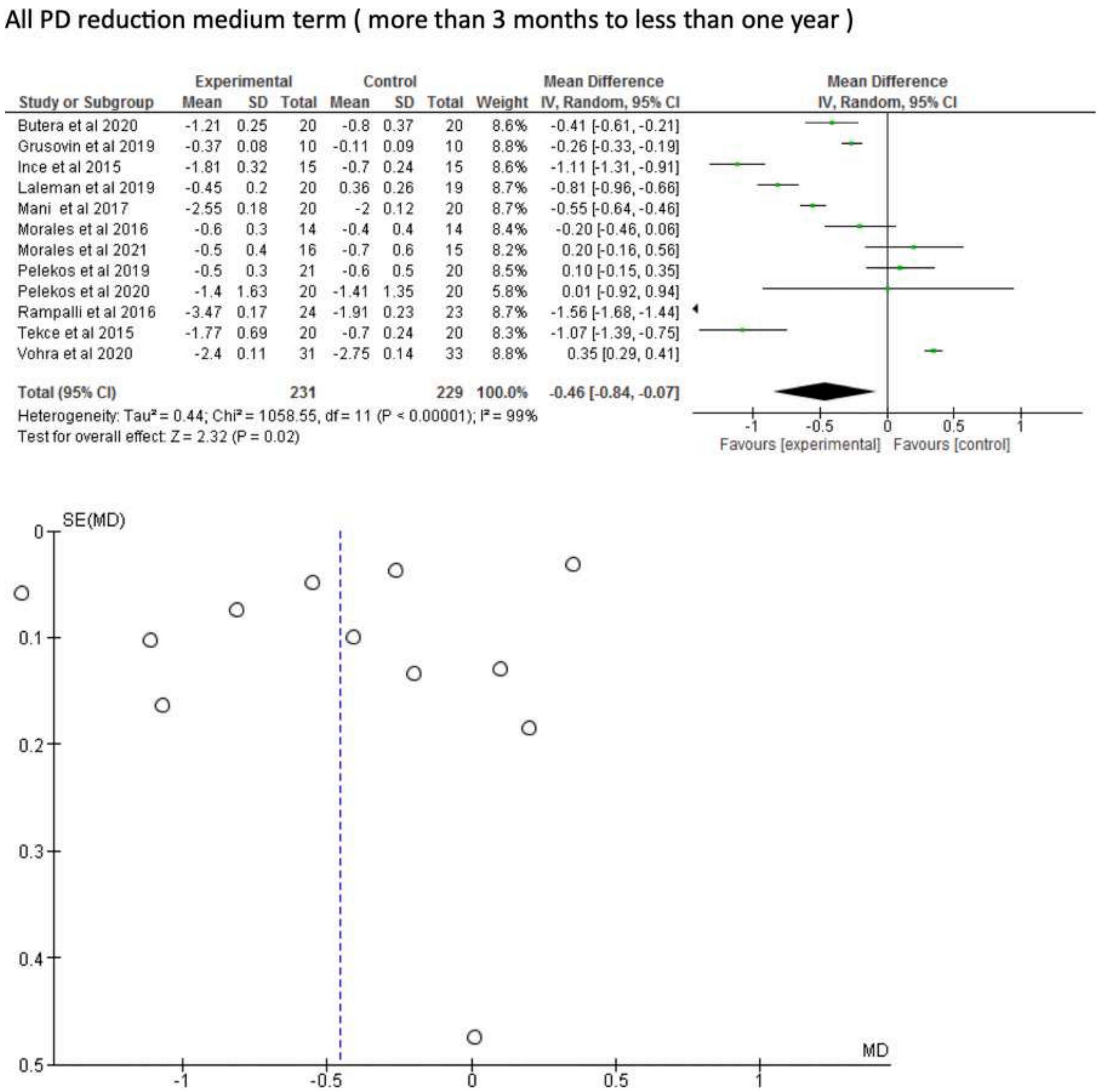


Fig. 7. Forest plot and funnel plot for PD reduction medium term (more than 3 months, less than 1 year).

The primary (PD change and CAL change) and secondary outcomes (microbiological findings, inflammatory mediators findings) were classified as follows:

2.7.1. PD change

Considering PD as the distance from the soft tissue (gingiva or alveolar mucosa) margin to the tip of the periodontal probe during usual periodontal diagnostic probing, PD change is the difference between PD before NSPT and PD after NSPT expressed in mm.

2.7.2. CAL gain

Considering CAL as the distance from the cemento-enamel junction to the tip of the periodontal probe during usual periodontal diagnostic probing, CAL change is the difference between CAL before NSPT and CAL after NSPT expressed in mm.

2.7.3. Microbiological findings

Quantity (absolute or difference between time points) or frequency of detection of periodontal pathogenic or related microbiological species.

2.7.4. Inflammatory mediators findings

Quantity (absolute or difference between time points) of inflammatory biomarkers.

The following sub-analyzes were performed:

- Form of administration of probiotics (lozenges, capsules, other)
- Frequency of administration (daily, twice a day, etc.)
- Type of probiotic (*lactobacillus reuteri* or others)

All clinical parameter results were further sub-analyzed based on short (up to 3 months), medium (> 3 months to < 1 year) and long (> 1 year) term change.

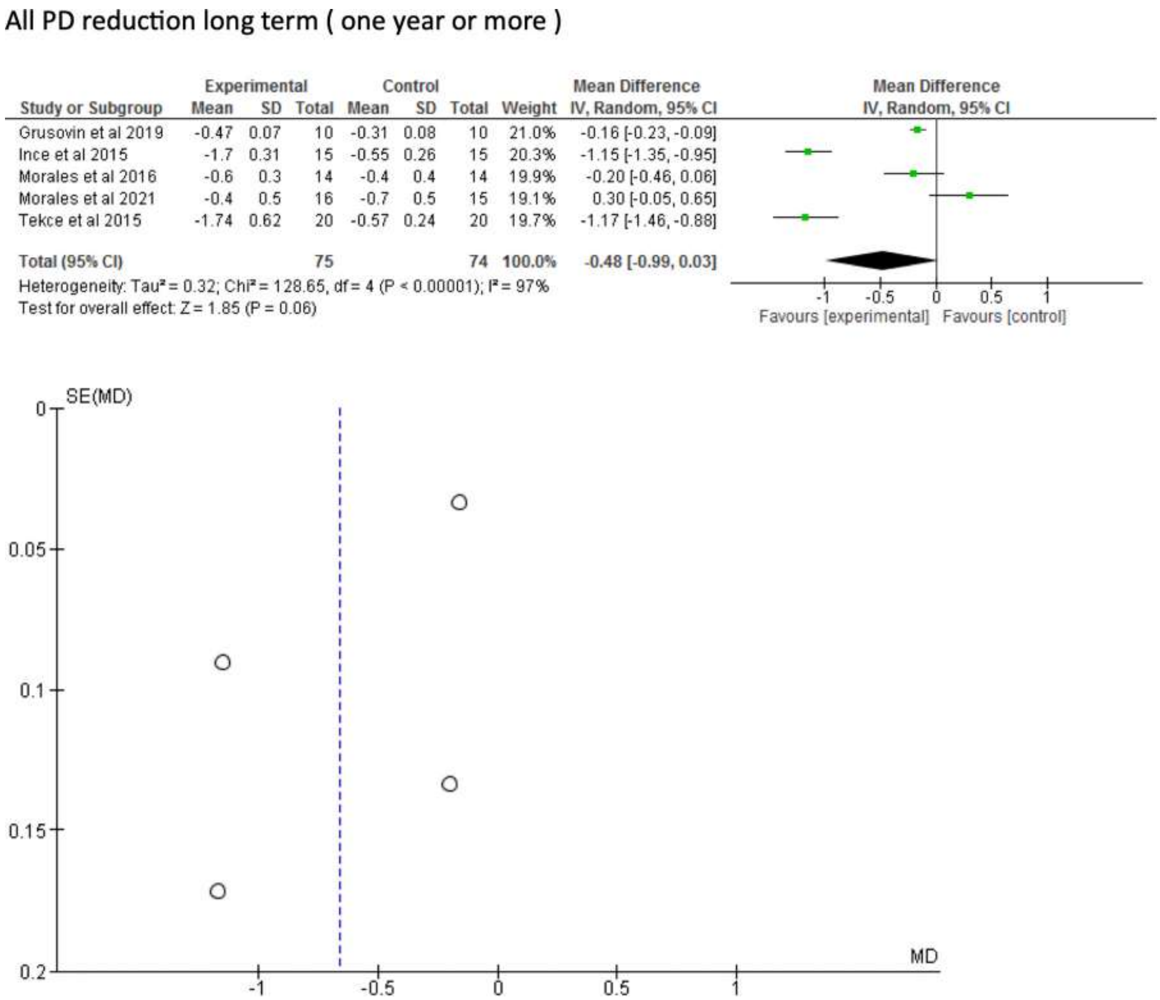


Fig. 8. Forest plot and funnel plot for PD reduction long term (1 year or more).

2.8. Statistical analysis

A random effects analysis model was performed to calculate the effect size. All the analyses were performed based on duration: short term (up to 3 months), medium term (more than 3 months to less than one year), and long term (one year or more). Subgroups analyses were implemented based on probiotic strain (*Lactobacillus reuteri* or others), frequency (once or twice a day), or form (lozenges, capsule or others) to minimize the heterogeneity. The heterogeneity was evaluated using the Chi [2] and I^2 tests. P value less than 0.05 was considered statistically significant. The publication bias was assessed using funnel plots, Beggs's and Egger's tests. A meta-analysis was conducted using the RevMan software (The Cochrane Collaborative, v 5.4, Cochrane IMS).

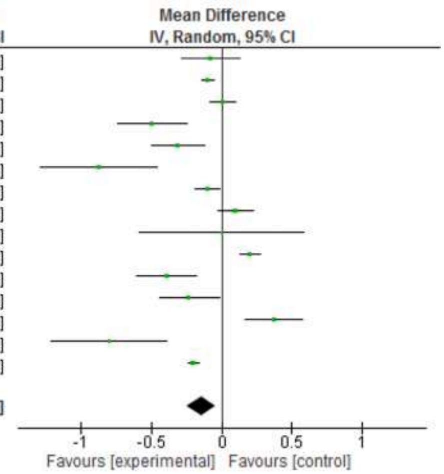
3. Results

The selection process of the articles, summarized in the PRISMA flow chart (Fig. 1), produced 5702 articles which, after the screening of the titles, were reduced to 264 abstracts. After evaluating the latter, 234 were excluded thus leading to 30 articles that were evaluated by reading the full text and only 25 [10–34] were useful for the extraction of data as they met the inclusion and exclusion criteria. All funnel plots resulting from Beggs's and Egger's tests show no publication bias (Figs. 3–34). All 25 articles reported about clinical parameters, of these, 10 studies reported about microbiological findings, 4 studies reported about immunological findings and one study reported both about microbiological and immunological findings.

Lozenges cal gain short term (up to 3 months)

Study or Subgroup	Experimental Mean	Experimental SD	Experimental Total	Control Mean	Control SD	Control Total	Weight	Mean Difference IV, Random, 95% CI
Alshareef et al 2020	-0.43	0.21	15	-0.35	0.29	10	6.5%	-0.08 [-0.29, 0.13]
Elsadek et al 2020	-0.52	0.03	19	-0.42	0.09	19	8.9%	-0.10 [-0.14, -0.06]
Grusovin et al 2019	-0.17	0.1	10	-0.18	0.11	10	8.4%	0.01 [-0.08, 0.10]
Ince et al 2015	-1.08	0.36	15	-0.59	0.32	15	6.0%	-0.49 [-0.73, -0.25]
Invernici et al 2018	-0.49	0.37	20	-0.18	0.23	21	6.9%	-0.31 [-0.50, -0.12]
Laleman et al 2019	-0.56	0.9	20	0.31	0.28	19	3.6%	-0.87 [-1.28, -0.46]
Mani et al 2017	-1.35	0.14	20	-1.25	0.14	20	8.5%	-0.10 [-0.19, -0.01]
Pelekos et al 2019	-0.2	0.2	21	-0.3	0.2	20	8.0%	0.10 [-0.02, 0.22]
Pelekos et al 2020	-0.43	0.94	20	-0.43	0.94	20	2.3%	0.00 [-0.58, 0.58]
Pudgar et al 2020	-0.7	0.08	20	-0.9	0.14	20	8.7%	0.20 [0.13, 0.27]
Tekce et al 2015	-1.18	0.36	20	-0.79	0.32	20	6.5%	-0.39 [-0.60, -0.18]
Teughels et al 2013	-0.99	0.22	15	-0.76	0.36	15	6.5%	-0.23 [-0.44, -0.02]
Theodoro et al 2019	-0.06	0.19	14	-0.43	0.33	14	6.7%	0.37 [0.17, 0.57]
Vivekanda et al 2010	-1.09	0.62	15	-0.29	0.51	15	3.7%	-0.80 [-1.21, -0.39]
Vohra et al 2020	-0.3	0.09	31	-0.1	0.07	33	8.9%	-0.20 [-0.24, -0.16]
Total (95% CI)			**275**			**271**	**100.0%**	**-0.14 [-0.24, -0.04]**

Heterogeneity: $Tau^2 = 0.03$; $Chi^2 = 178.69$, df = 14 (P < 0.00001); $I^2 = 92\%$
Test for overall effect: Z = 2.72 (P = 0.007)



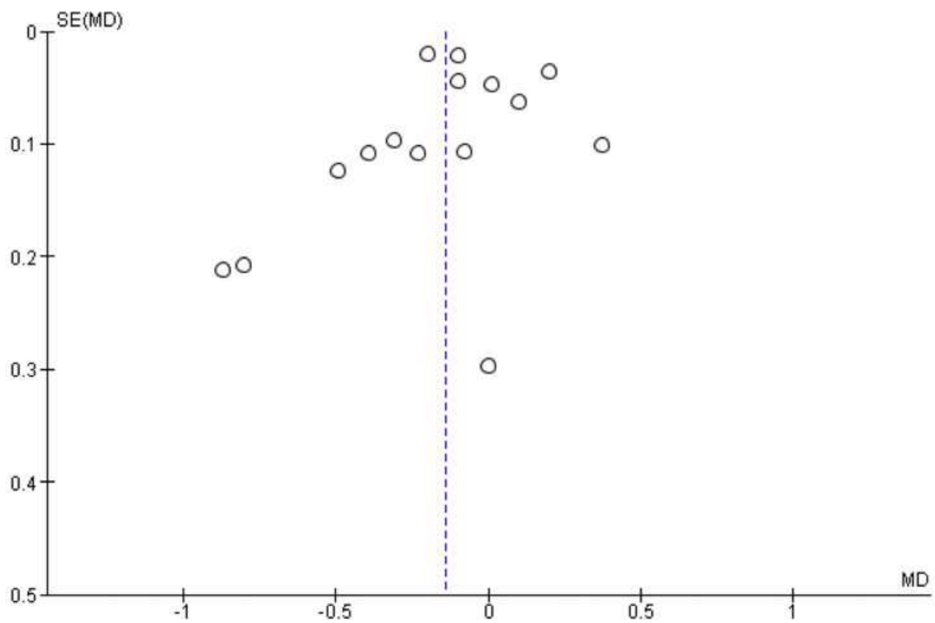


Fig. 9. Forest plot and funnel plot for CAL gain (lozenge form) short term (up to 3 months).

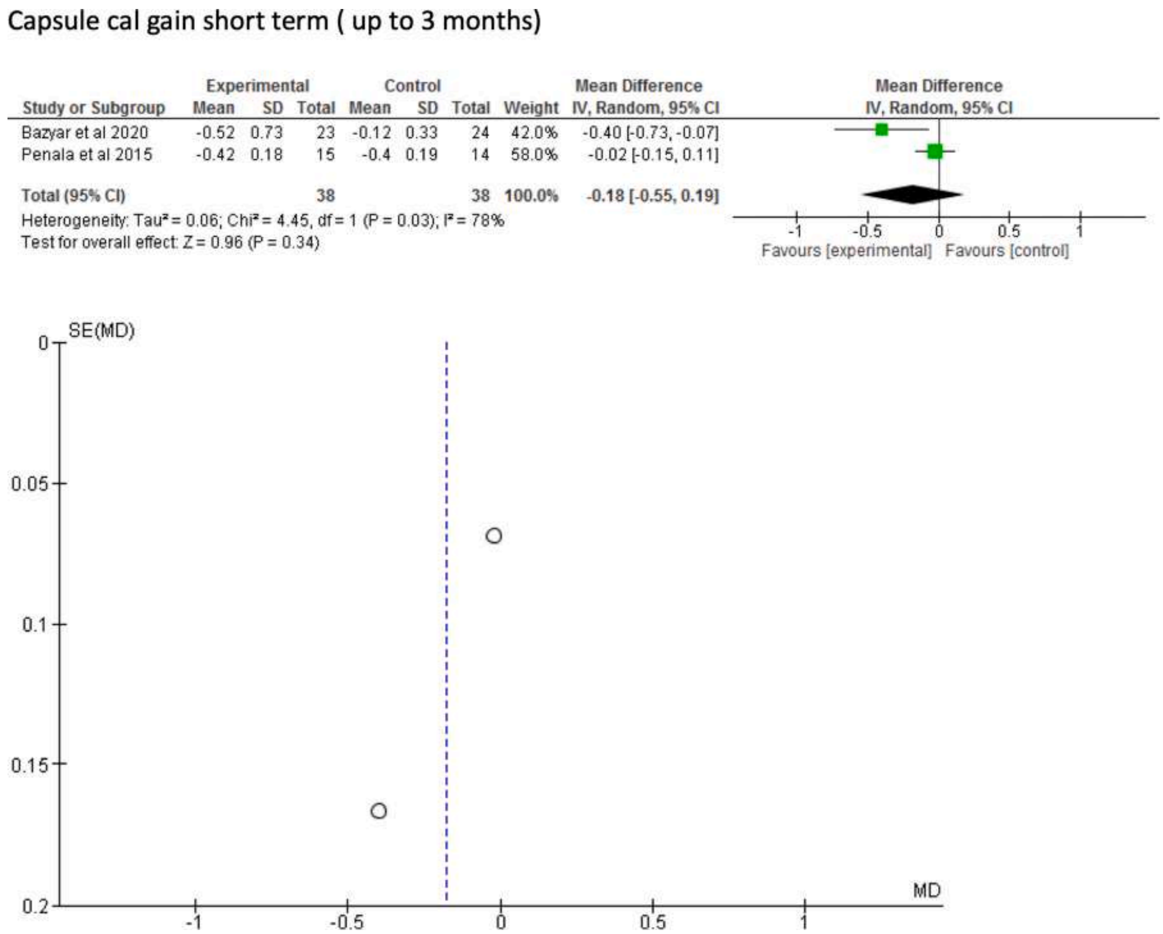


Fig. 10. Forest plot and funnel plot for CAL gain (capsule form) short term (up to 3 months).

Studies by Morales et al. of 2018 and 2021 [34,35] represented a repeated cohort of patients, the 2021 study was considered for the collection of clinical data, however the 2018 study reported microbiological data and was therefore considered for those outcomes. Morales et al. of 2021 was included in Tables 1–3 and Morales et al. of 2018 was included in Table 5.

In the 25 studies included, a total of 894 patients (451 test group, 443 control group) were analyzed. *Lactobacillus reuteri* was the most commonly used probiotic in 16 studies, alone in 13 studies or in combination with other bacteria in 3 studies.

There was not enough data to perform any sub-analyzes for long-term outcomes and for medium-term outcomes when probiotics were administered in capsule form, in all other cases sub-analyzes could be performed as planned.

The basic characteristics of the studies, the type and dosage of probiotics used and the outcomes are illustrated in Tables 1, 2 and 3.

The results of all analyzes and sub-analyzes for PD change and CAL gain, and the related funnel plots, are shown in Figs. 3 to 34.

3.1. Risk of bias

Most of the 25 studies analyzed had a low risk of bias, only 7 had a moderate risk and two had a high risk due to allocation concealment in both. Most of the doubts of bias in the moderate-risk studies were in the blinding of outcome assessment. (Fig. 2) The only non randomized study analyzed was also found to have a low risk of bias. (Table 4).

3.2. PD change

The results in terms of PD change are summarized in the forest plots (Figs. 6–8). Twenty-five studies reported short-term, 12 medium-term and 4 long-term results. In summary, the difference between the groups was always statistically significant in favour of the test group in the short, medium and long term. The sub-analyses showed that the difference was statistically significant, in favour of the test group, when the probiotics were administered in the form of

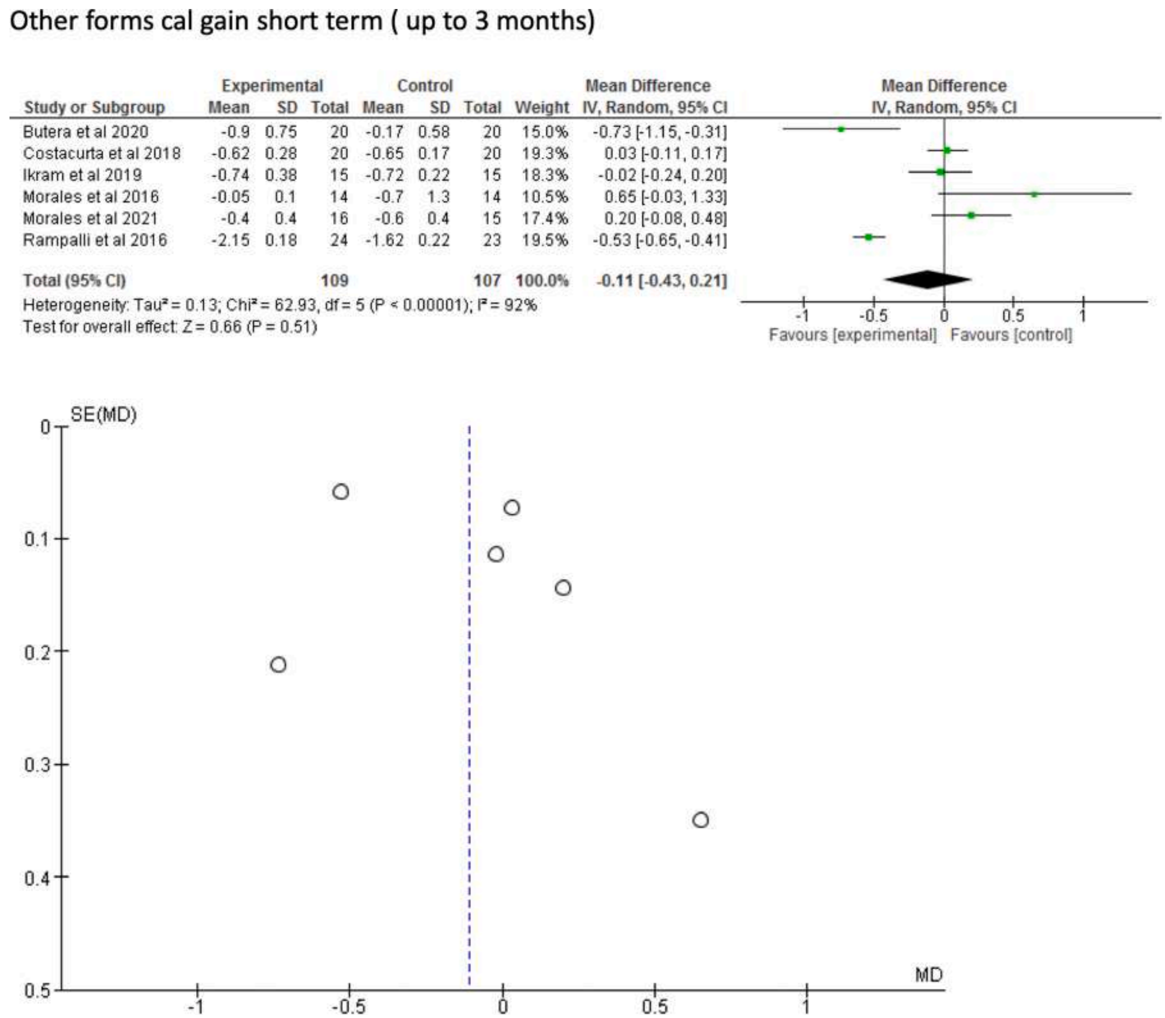


Fig. 11. Forest plot and funnel plot for CAL gain (other forms) short term (up to 3 months).

lozenges, when given twice a day and when the probiotic is *L. reuteri*. In all other cases of sub-analysis (capsules, other forms, once daily, other probiotics strains) the difference was never statistically significant.

3.3. CAL gain

Concerning the CAL gain (Figs. 3–5) 23 studies reported short-term, 12 medium-term and 4 long-term results. The difference between the two groups was significant only in the short and medium term, but not in the long term (P = 0.12; mean difference −0.47 mm, 95% CI, - 1.06, 0.13 mm; I^2 = 99%), moreover, as for the PD change, the sub-analyzes showed that the difference was significant for the lozenge forms, for the twice daily dosage and for the *L. reuteri* but, in this case, also for the forms other than lozenges and capsules in the short term (P = 0.001; mean difference −0.18, 95% CI, −0.28, −0.07 mm; I^2 = 92%).

3.4. Microbiological findings (Table 5)

Only 10 studies reported results regarding the microbiological component of the outcomes [10,12,14,18,20,22,27,28,33,35]. However, the results were not always comparable, sometimes because they were reported as total pathogenic bacterial species count, sometimes because the results were only provided in graphical form, other times because only detection rates or number of subjects detected with pathogenic bacterial species were provided instead of log10 CFU/ml. Additionally, time points of analysis were very variable between studies. Only two studies were comparable trough a meta-analysis, but this analysis has already been reported by an older systematic review [36]. Six out of 10 studies reported a significant difference between test and control groups in favour of the test group [10,12,14,18,20,28], however, one of them considered the difference between the total counts at 4-month follow-up rather than the difference between the reductions obtained [18] and

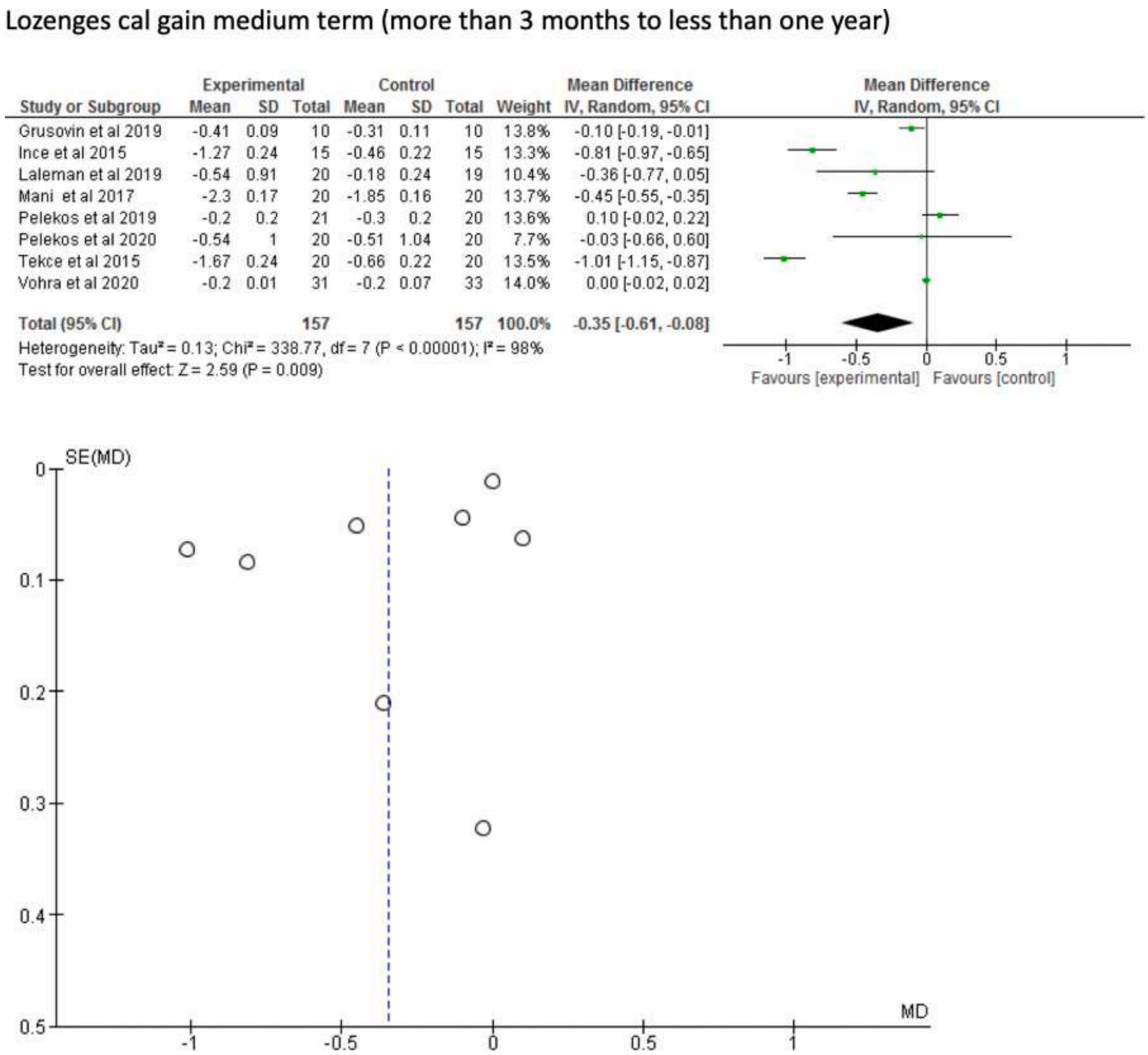


Fig. 12. Forest plot and funnel plot for CAL gain (lozenge form) medium term (more than 3 months, less than 1 year).

another considered the difference between the total viable cell counts (significant at 21 days and 3 months but not at 6 months and one year) and the obligate anaerobes counts (significant at 21 days, 3 and 6 months but not at one year) [14].

The intra-group difference in microbiological parameters was reported by 6 out of 10 studies [14,18,27,28,33,35], in 3 of these the difference in bacterial counts (total or by species depending on the study) was statistically significant in both the test and control groups [14,18,28]. In one study, the intra-group difference in total cultivable species count was not statistically significant in both groups at follow up, however there was a significant difference in the prevalence and proportion of *Porphyromonas gingivalis* and *Tannerella forsythia*, but only in the control group [27]. In another study the intra-group difference was statistically significant only in the test group and only at 6 months, not at 3 and 9 months, in the same study the difference in the number of subjects detected with *Porphyromonas gingivalis* at 9 months compared to baseline was also statistically significant but only in the control group [35].

3.5. Immunological findings (Table 6)

(Table 6)Four studies reporting results regarding immunological parameters were included in this review [15,20,29,31]. The results of the four studies, however, were not included in a quantitative analysis, because they reported results that were not comparable to each other. Two studies reported results regarding MMP8 levels, however one gave outcomes at 21 days and 3, 6 and 12 months [15], the other only at 1 month [31]. Two studies reported results on the inflammatory marker IL − 1ß [20,29], however one of the two did not provide the values but only graphs [20].

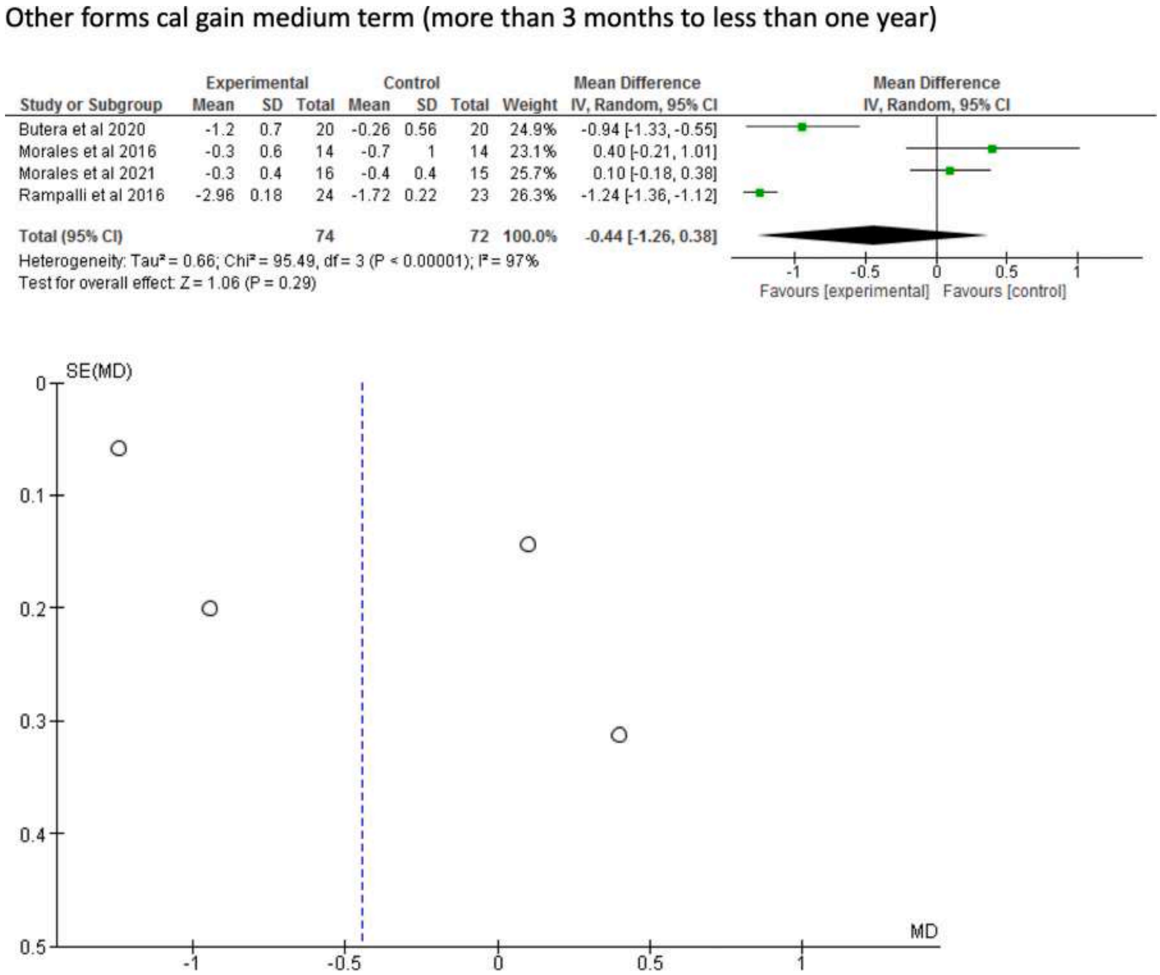


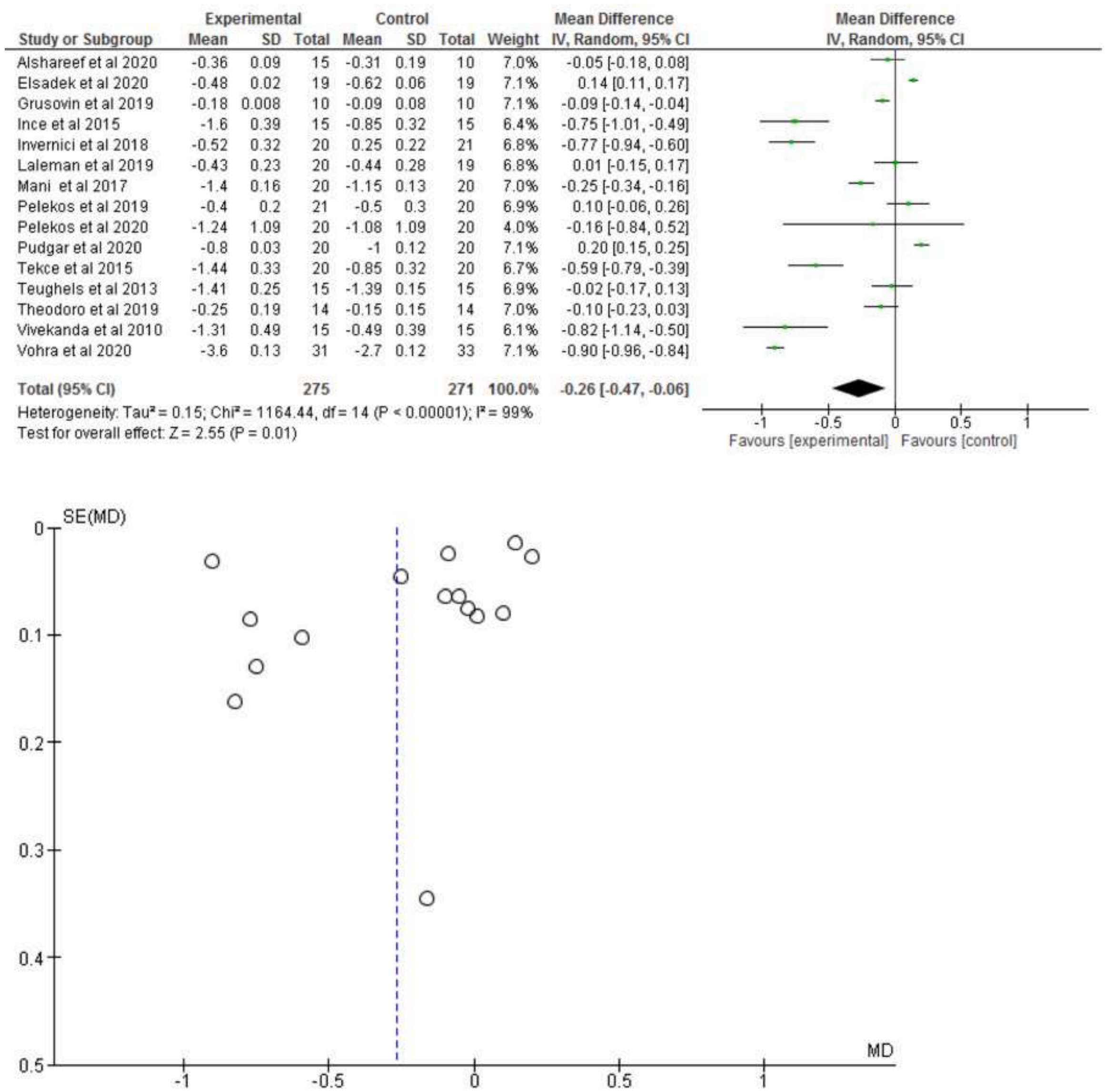
Fig. 13. Forest plot and funnel plot for CAL gain (capsule form) medium term (more than 3 months, less than 1 year).

Specifically, Ince et al. [15] shows a reduction in the volume of gingival-crevicular fluid (GCF) and levels of MMP8, as well as an increase in the levels of tissue inhibitor of matrix metalloprotease (TIMP-1) which are always significant at 21 days and at 3 and 6 months in the intra-group analysis of both the test group and the control group, while the same values returned almost to baseline levels, losing statistical significance, at 12 months. In the same study, the intergroup analysis showed a statistically significant difference, in favour of the test group, at 21 days, 3 and 6 months but not at 12 months. In Alshareef et al. [31], the difference in MMP8 crevicular levels at 30 days compared to baseline was statistically significant in both the control and test groups although much more markedly in the latter ($P = 0.17$ vs. $P < 0.001$), however, the intergroup analysis showed no statistically significant difference between the two groups.

The results provided by Invernici et al. [20] are not easy to interpret, although in the graphs provided they show an increase in levels of IL-1ß, IL-8 and IL-10, in both groups, compared to the baseline, the description of the results in the text reports that only the test group showed higher levels of IL-10 at 30 days compared to baseline, a statement repeated in the discussions of the same article. In general, the control group showed higher ratios of all three markers, at 1 month and 3 months, than the test group, except IL-10 at 3 months which, looking at the bar graph, would appear the same. Even in this case there is a discrepancy with what is reported in the text which states that the ratio of IL-8 was higher only at one month and does not mention the ratios of IL-10.

Finally, Bazyar et al. [29] showed, in the test group at two months, a significant reduction of IL-1ß and MDA (malondialdehyde) and a significant increase in TAC (total antioxidant capacity), SOD

Lozenges PD reduction short term (up to 3 months)



Study or Subgroup	Experimental Mean	Experimental SD	Experimental Total	Control Mean	Control SD	Control Total	Weight	Mean Difference IV, Random, 95% CI
Alshareef et al 2020	-0.36	0.09	15	-0.31	0.19	10	7.0%	-0.05 [-0.18, 0.08]
Elsadek et al 2020	-0.48	0.02	19	-0.62	0.06	19	7.1%	0.14 [0.11, 0.17]
Grusovin et al 2019	-0.18	0.008	10	-0.09	0.08	10	7.1%	-0.09 [-0.14, -0.04]
Ince et al 2015	-1.6	0.39	15	-0.85	0.32	15	6.4%	-0.75 [-1.01, -0.49]
Invernici et al 2018	-0.52	0.32	20	0.25	0.22	21	6.8%	-0.77 [-0.94, -0.60]
Laleman et al 2019	-0.43	0.23	20	-0.44	0.28	19	6.8%	0.01 [-0.15, 0.17]
Mani et al 2017	-1.4	0.16	20	-1.15	0.13	20	7.0%	-0.25 [-0.34, -0.16]
Pelekos et al 2019	-0.4	0.2	21	-0.5	0.3	20	6.9%	0.10 [-0.06, 0.26]
Pelekos et al 2020	-1.24	1.09	20	-1.08	1.09	20	4.0%	-0.16 [-0.84, 0.52]
Pudgar et al 2020	-0.8	0.03	20	-1	0.12	20	7.1%	0.20 [0.15, 0.25]
Tekce et al 2015	-1.44	0.33	20	-0.85	0.32	20	6.7%	-0.59 [-0.79, -0.39]
Teughels et al 2013	-1.41	0.25	15	-1.39	0.15	15	6.9%	-0.02 [-0.17, 0.13]
Theodoro et al 2019	-0.25	0.19	14	-0.15	0.15	14	7.0%	-0.10 [-0.23, 0.03]
Vivekanda et al 2010	-1.31	0.49	15	-0.49	0.39	15	6.1%	-0.82 [-1.14, -0.50]
Vohra et al 2020	-3.6	0.13	31	-2.7	0.12	33	7.1%	-0.90 [-0.96, -0.84]
Total (95% CI)			**275**			**271**	**100.0%**	**-0.26 [-0.47, -0.06]**

Heterogeneity: Tau² = 0.15; Chi² = 1164.44, df = 14 (P < 0.00001); I² = 99%
Test for overall effect: Z = 2.55 (P = 0.01)

Fig. 14. Forest plot and funnel plot for PD reduction (lozenge form) short term (up to 3 months).

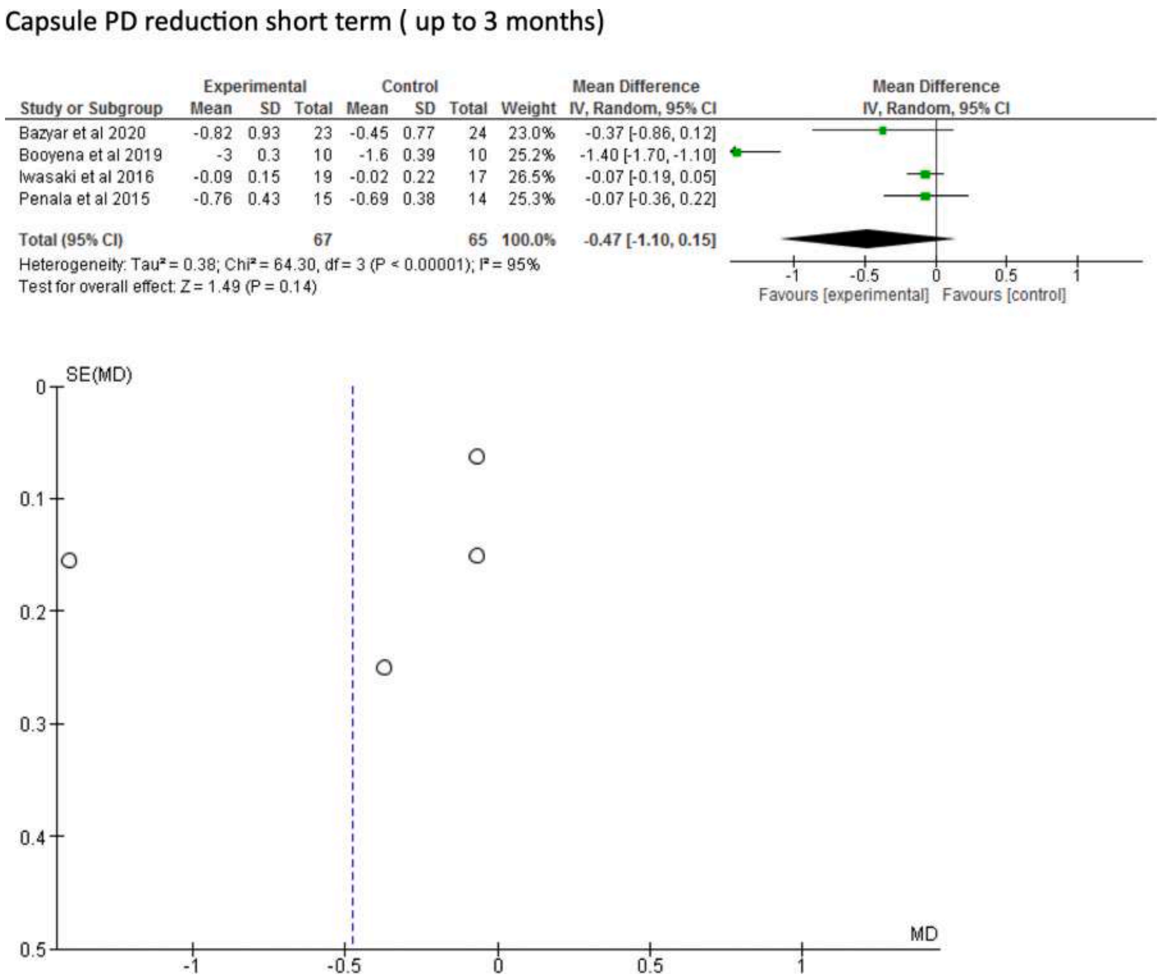


Fig. 15. Forest plot and funnel plot for PD reduction (capsule form) short term (up to 3 months).

(superoxide dismutase) and GPx (glutathione peroxidase), while in the control group these differences were never significant.

4. Discussions

The present systematic review was aimed at analyzing the effect of probiotics, used as an adjunct, to non-surgical periodontal therapy in patients with periodontitis. The results show encouraging and significant data in terms of clinical parameters. These results tend to improve from the short to medium term with statistically significant differences between test and control. The change in PD remains statistically significant also in the long term.

In the long term the difference in terms of CAL loses statistical significance, however it always remains in favour of the test group. The scarcity of studies reporting long-term results does not allow a more complex analysis and sub-analysis, even where the difference is statistically significant, as in the case of PD, the latter is based on only 4 studies. Probably the limited number of studies analyzing the effects of probiotics at one year or more is due to the very nature of probiotic therapy in terms of duration. In fact, the administration of probiotics generally varies between a duration of 3 and 12 weeks, with only two studies having a duration of administration of 16 and 24 weeks [18,33], in some studies probiotics are administered only at the baseline [11,26]. Probably the duration of the administration of

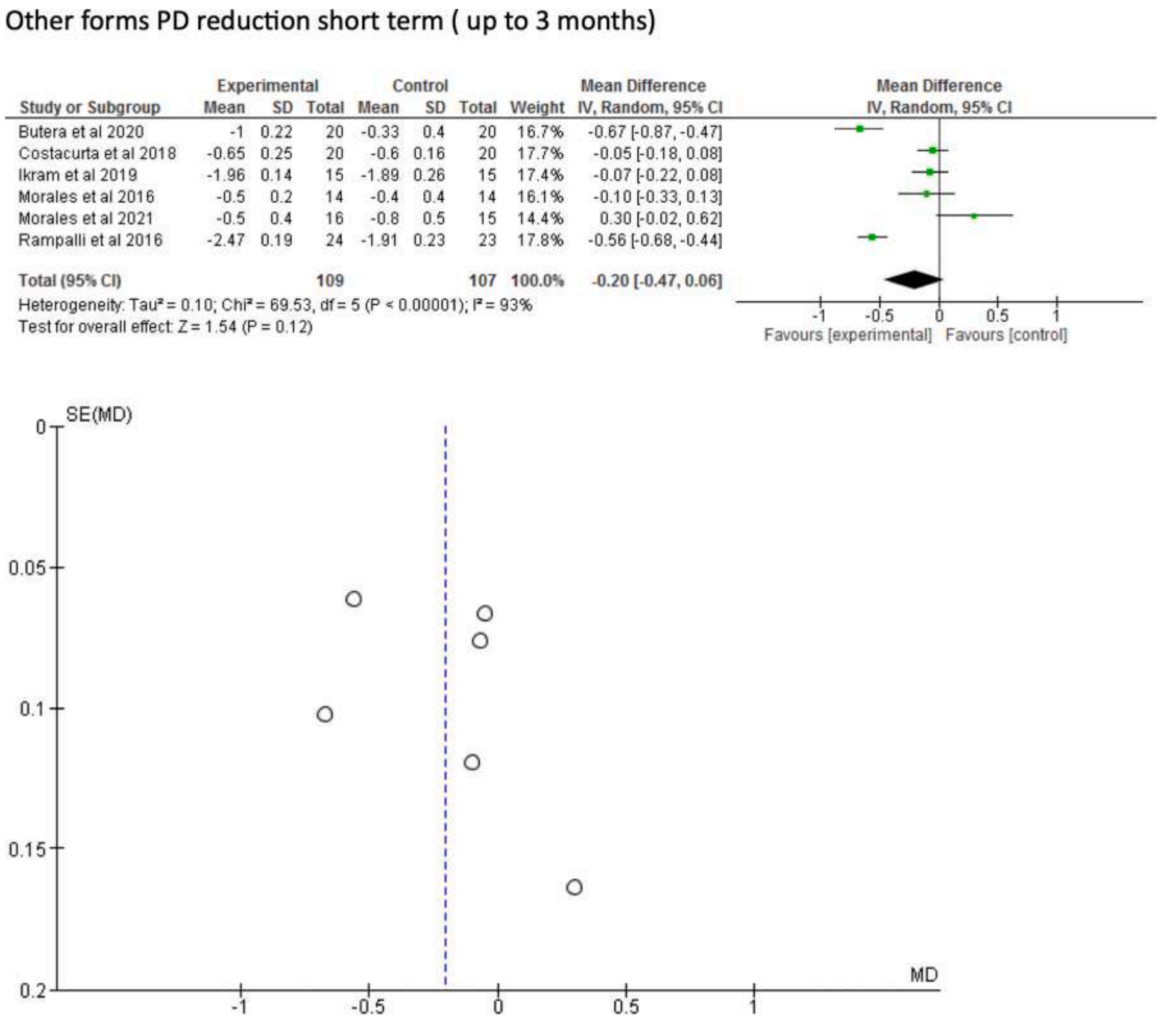


Fig. 16. Forest plot and funnel plot for PD reduction (other forms) short term (up to 3 months).

the probiotic influences the design of the research at the time of its conception.

In the sub-analyzes it was found that, in terms of PD change, the difference was statistically significant only when probiotics were administered twice daily in the form of lozenges and when the bacterial species was *L. reuteri*. However, one should not conclude that these are the modalities and dosages indicated for probiotic therapy as adjuvant to non-surgical periodontal therapy, in fact the statistical significance was always reached in those subgroups that included a higher number of subjects. The form of lozenges was used in 15 out of 25 studies, only 4 utilized capsules and 6 used other forms. In 17 studies the probiotic was administered in a double daily dose, in 6 it was administered in a single daily dose and in two studies it was administered in a single dose only at baseline [11,26]. We should wonder how significant the contribution of probiotics was in the studies where it was administered only once at baseline, given that the results are reported at 12 and 24 weeks after therapy, after a single intake of the probiotic. Finally, the bacterial species used was L reuterii in 16 out of 25 studies. The representativeness of all the other forms, dosages and bacterial species in the subanalyses was therefore low, but despite this, both for PD and CAL, even in all subanalyses that did not reach statistical significance, the trend in mean difference was always in favour of the test group.

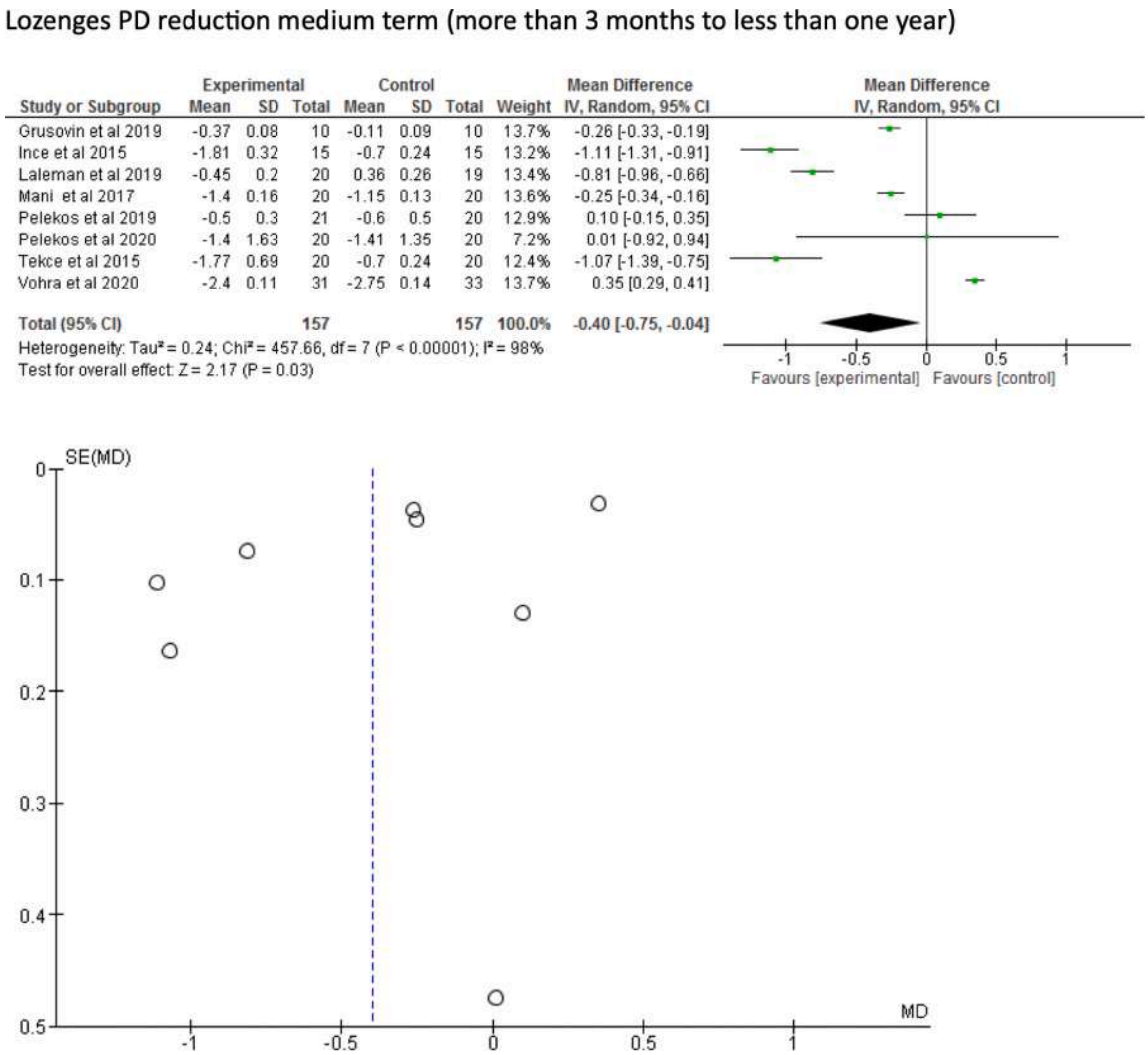


Fig. 17. Forest plot and funnel plot for PD reduction (lozenge form) medium term (more than 3 months, less than 1 year).

The analysis of clinical findings showed a reduction of PD and a gain in CAL in favour of the test groups over time. One may wonder if the use of probiotics as an adjunct to NSPT can reduce the need for further surgical intervention. As much as this is a valid question it is very difficult to give an answer because of the subjective nature of the clinical decision of whether to proceed with surgery or not. However, considering that in periodontology a need for surgical intervention is found in the presence of deeper pockets [37,38] one can speculate that when administering probiotics as an adjunct to NSPT this need may be somewhat reduced. NSPT is the part of periodontal treatment that aims at reducing inflammation, controlling the infection and changing the microbiota, the surgical phase corrects the deformities left behind by active disease. This is why conceptually there seems to be a stronger indication to add probiotics to NSPT rather than to surgical therapy however more studies would be needed to determine the effect of probiotics not only as an adjunct to NSPT but also to surgical therapy.

As for microbiological analysis, all 10 studies considered analyzed subgingival plaque and two of them [12,22] also supragingival plaque and saliva. Nine out of 10 studies performed an intergroup

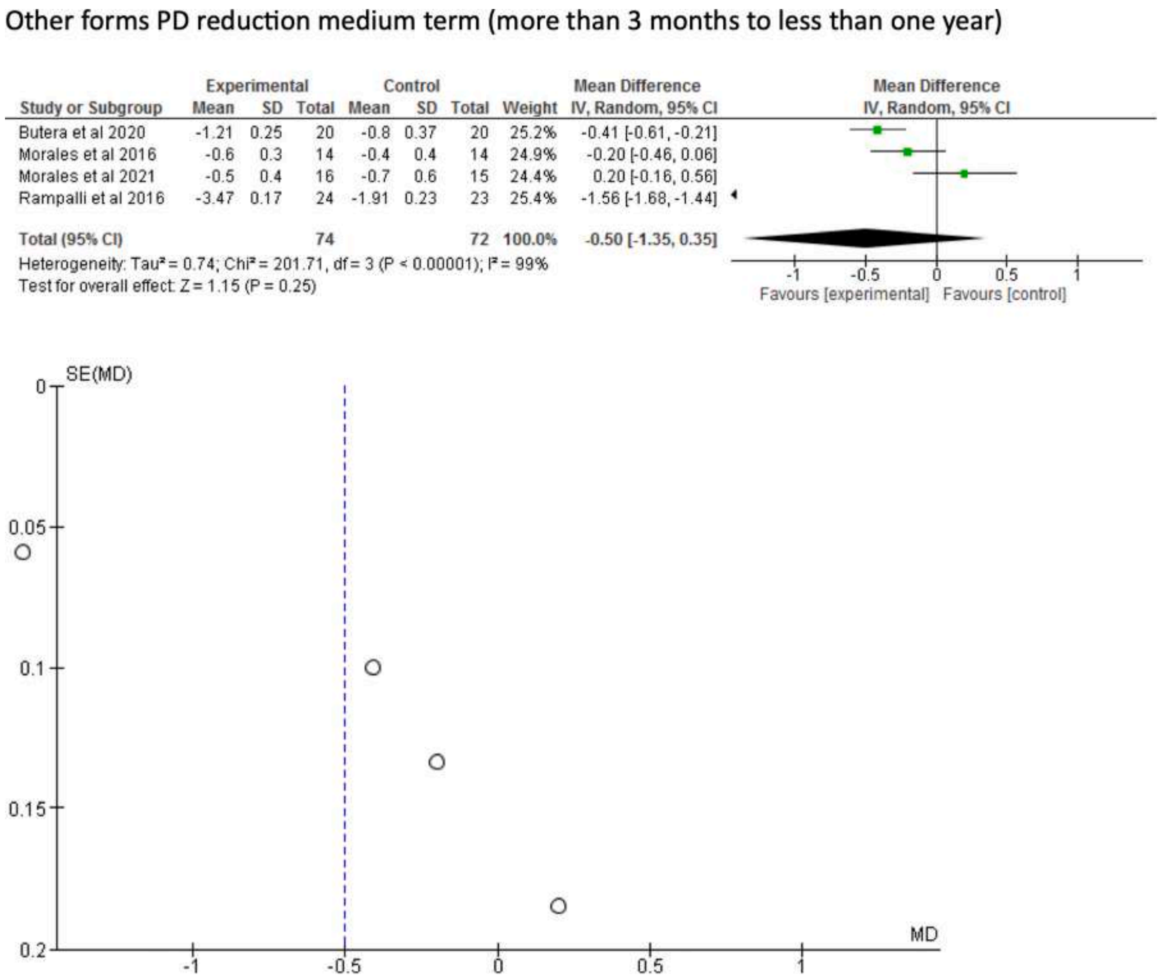


Fig. 18. Forest plot and funnel plot for PD reduction (capsule form) medium term (more than 3 months, less than 1 year).

microbiological analysis and only one [33] performed an intra-group analysis exclusively. Of these 9 studies only 6 [10,12,14,18,20,28] found a statistically significant difference between the groups in favour of the test group, one of these 6 studies [18] only considers the total count of bacteria and not the individual species including at least one species belonging to the red complex or *A actinomycetemcomitans*. This latest study, however, compares the total counts of the two groups at 4 months and not, as one would expect, the reductions.

Six out of 10 studies [14,18,27,28,33,35] analyzed intra-group changes from a microbiological standpoint by comparing reductions at different time points. Of these, only 3 [14,18,28] found statistically significant differences in both the test and control groups. Another study [35] found a significant reduction in the total count only in the test group and non significant in both groups when individual species were analyzed. A different study [27] found no statistically significant difference in both groups for the total count, but only when individual species were considered and, surprisingly, only in the control group. Finally, Butera et al. [33] never found significant differences in the intragroup analysis except for the single species *P intermedia* and *F nucleatum* and only at 6 months in the two test groups.

In the analysis of the immunological parameters both Ince et al. [15] and Alshareef et al. [31] report the same parameter in regards to MMP8, however one reports the values measured at 3, 6, 12 months, the other at one month only, thus making it impossible to compare or meta-analyze this data between the two different studies. Likewise, both Invernici et al. [20] and Bazyar et al. [29] report the data

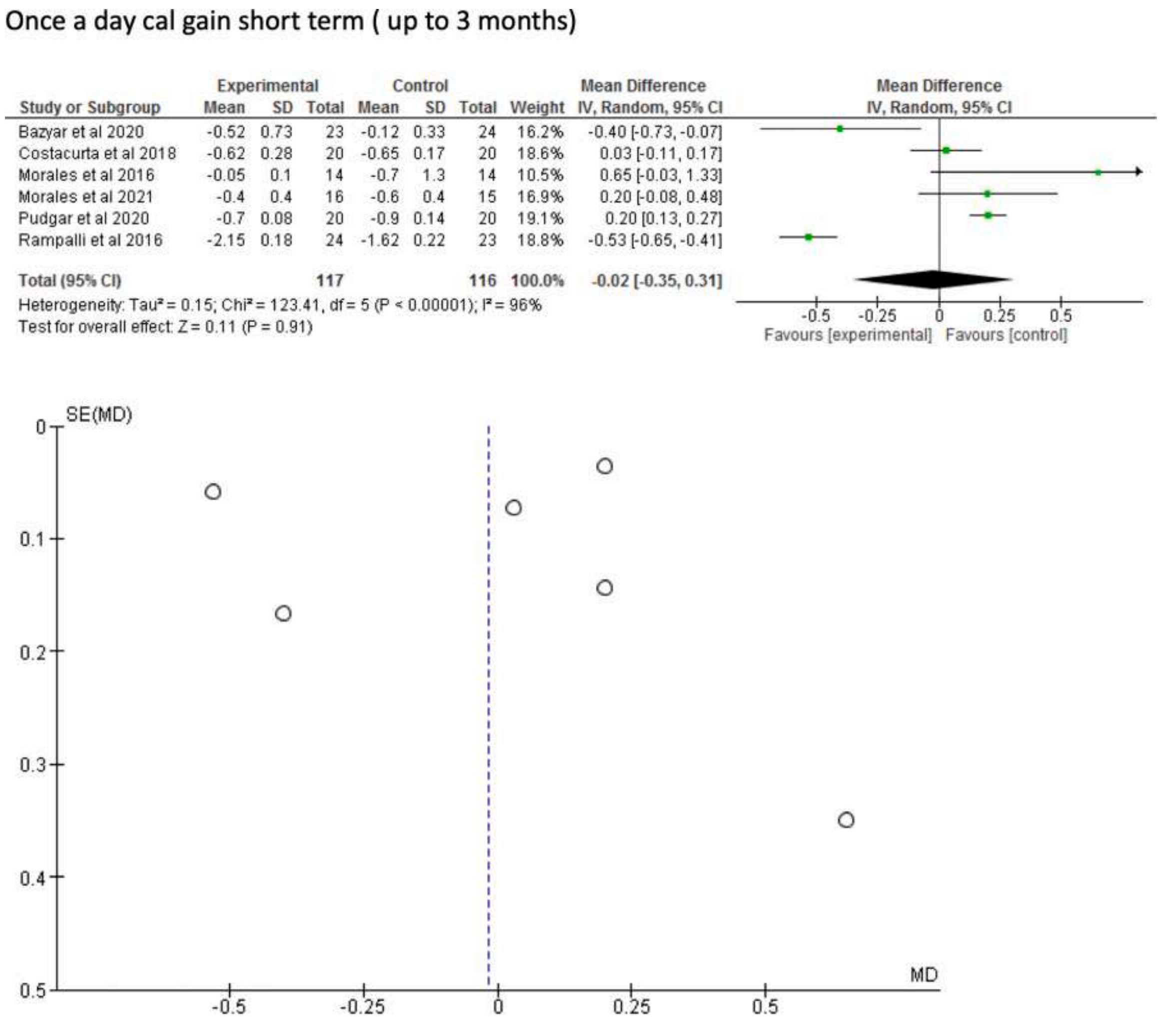
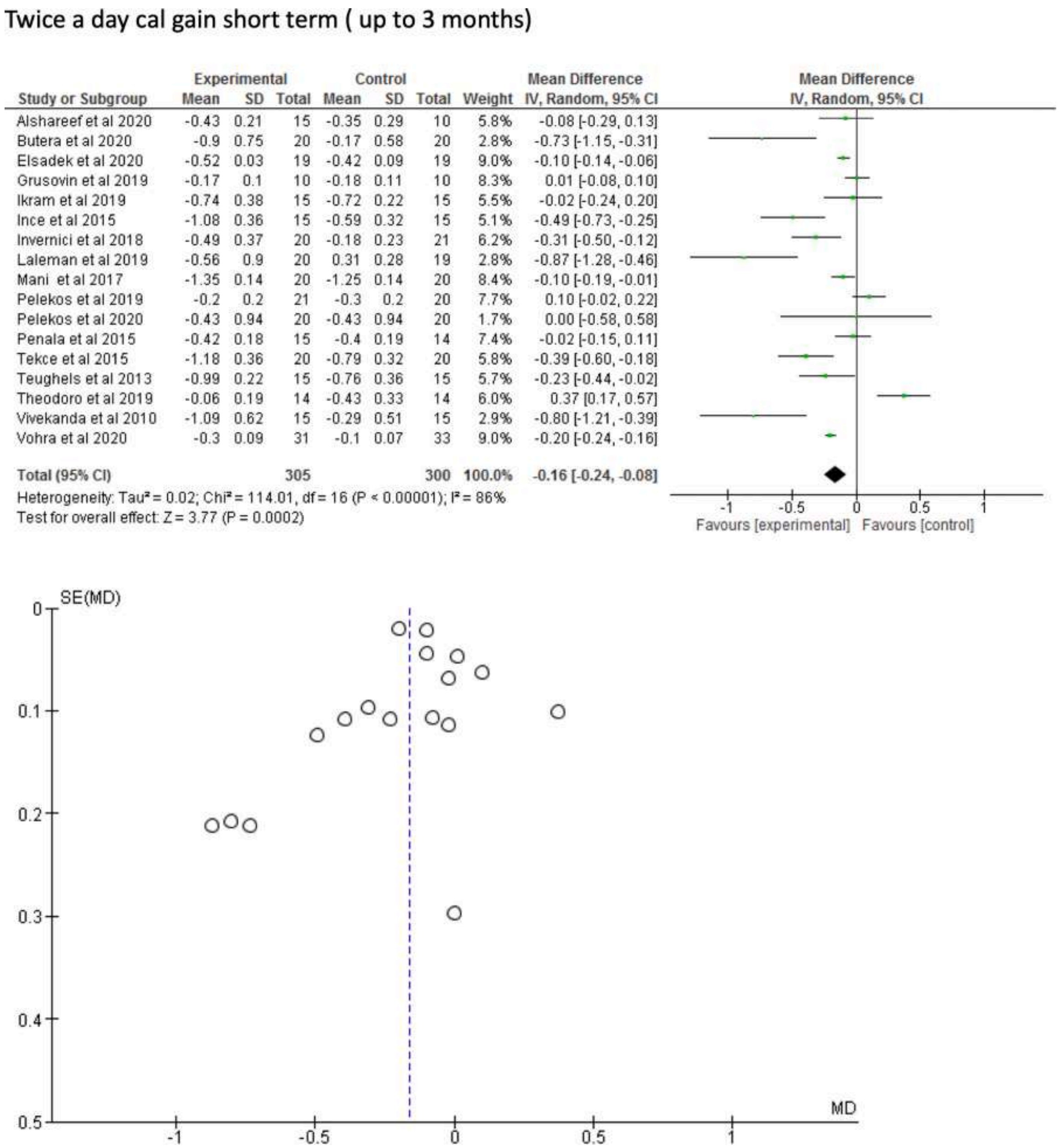


Fig. 19. Forest plot and funnel plot for CAL gain (once a day intake) short term (up to 3 months).

relating to IL-1ß but Invernici does not report numerical values but only graphs. However, despite these inconsistencies in reporting data between one study and another which does not allow direct comparison, all 4 studies considered in this systematic review agree in reporting significantly positive results in the reduction of pro-inflammatory parameters taken into account. Moreover, the reduction of pro-inflammatory cytokines, as well as of MMP8, collagenase most involved in the tissue destruction caused by periodontitis, seem to be one of the main mechanisms of the action of probiotics such as *L reuteri* [15,39]. This effect is also demonstrated in other studies not considered in this review, Ercan et al. [40], in fact, shows a marked reduction of IL-6, IL-8 and IL-10 in patients with gingivitis who were taking probiotics compared to the control group.

The limitation of this review is the high heterogeneity between the included studies. The different follow-up period can also represent a limit, although in an attempt to reduce this bias the results were grouped into short, medium and long term. Even in this way, however, the lack of studies that report long-term data is highlighted, for this reason, the long-term results should be interpreted with caution. The different duration of probiotic therapy, the use of different strains, sometimes combined, sometimes single, the different forms of administration and the different daily frequency of



Study or Subgroup	Experimental Mean	Experimental SD	Experimental Total	Control Mean	Control SD	Control Total	Weight	Mean Difference IV, Random, 95% CI
Alshareef et al 2020	-0.43	0.21	15	-0.35	0.29	10	5.8%	-0.08 [-0.29, 0.13]
Butera et al 2020	-0.9	0.75	20	-0.17	0.58	20	2.8%	-0.73 [-1.15, -0.31]
Elsadek et al 2020	-0.52	0.03	19	-0.42	0.09	19	9.0%	-0.10 [-0.14, -0.06]
Grusovin et al 2019	-0.17	0.1	10	-0.18	0.11	10	8.3%	0.01 [-0.08, 0.10]
Ikram et al 2019	-0.74	0.38	15	-0.72	0.22	15	5.5%	-0.02 [-0.24, 0.20]
Ince et al 2015	-1.08	0.36	15	-0.59	0.32	15	5.1%	-0.49 [-0.73, -0.25]
Invernici et al 2018	-0.49	0.37	20	-0.18	0.23	21	6.2%	-0.31 [-0.50, -0.12]
Laleman et al 2019	-0.56	0.9	20	0.31	0.28	19	2.8%	-0.87 [-1.28, -0.46]
Mani et al 2017	-1.35	0.14	20	-1.25	0.14	20	8.4%	-0.10 [-0.19, -0.01]
Pelekos et al 2019	-0.2	0.2	21	-0.3	0.2	20	7.7%	0.10 [-0.02, 0.22]
Pelekos et al 2020	-0.43	0.94	20	-0.43	0.94	20	1.7%	0.00 [-0.58, 0.58]
Penala et al 2015	-0.42	0.18	15	-0.4	0.19	14	7.4%	-0.02 [-0.15, 0.11]
Tekce et al 2015	-1.18	0.36	20	-0.79	0.32	20	5.8%	-0.39 [-0.60, -0.18]
Teughels et al 2013	-0.99	0.22	15	-0.76	0.36	15	5.7%	-0.23 [-0.44, -0.02]
Theodoro et al 2019	-0.06	0.19	14	-0.43	0.33	14	6.0%	0.37 [0.17, 0.57]
Vivekanda et al 2010	-1.09	0.62	15	-0.29	0.51	15	2.9%	-0.80 [-1.21, -0.39]
Vohra et al 2020	-0.3	0.09	31	-0.1	0.07	33	9.0%	-0.20 [-0.24, -0.16]
Total (95% CI)			**305**			**300**	**100.0%**	**-0.16 [-0.24, -0.08]**

Fig. 20. Forest plot and funnel plot for CAL gain (twice a day intake) short term (up to 3 months).

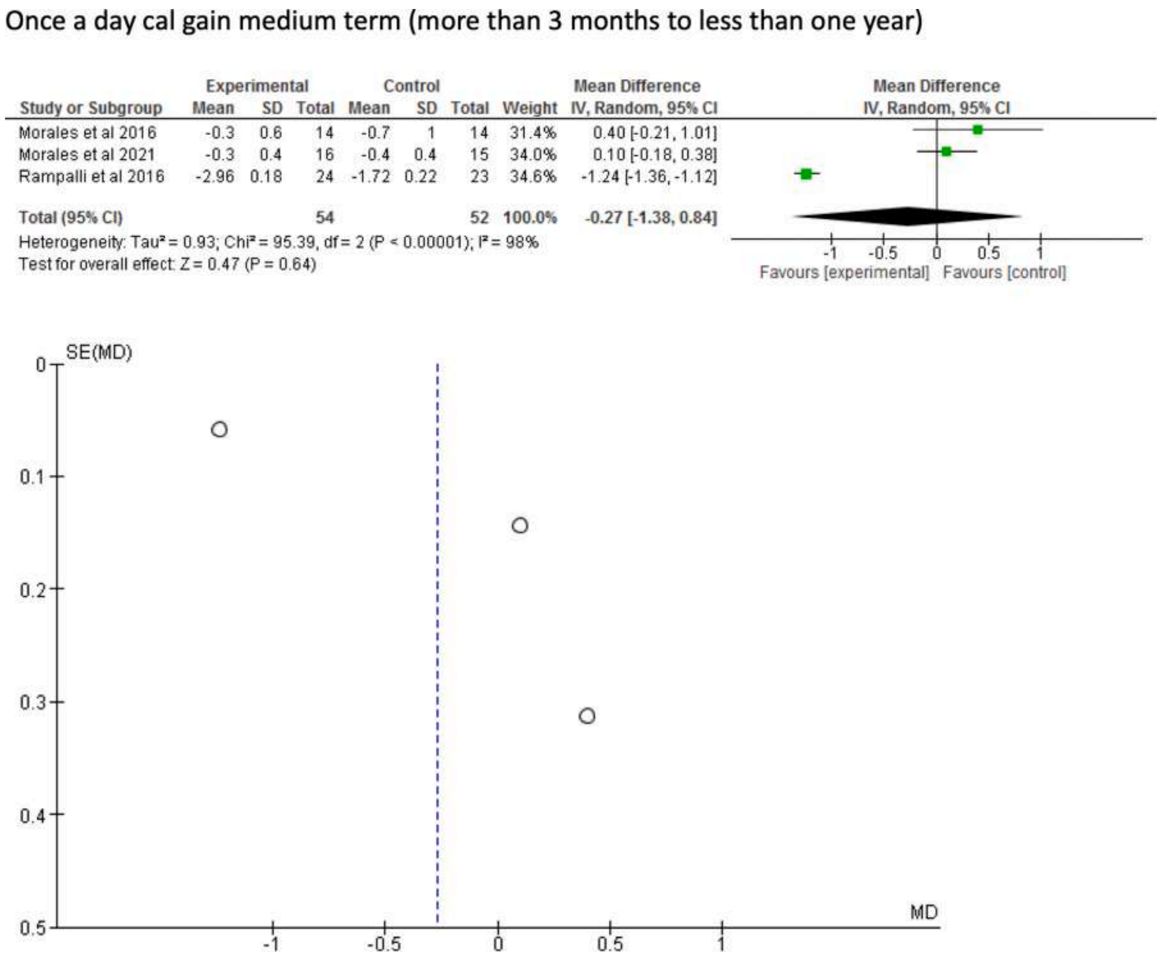


Fig. 21. Forest plot and funnel plot for CAL gain (once a day intake) medium term (more than 3 months, less than 1 year).

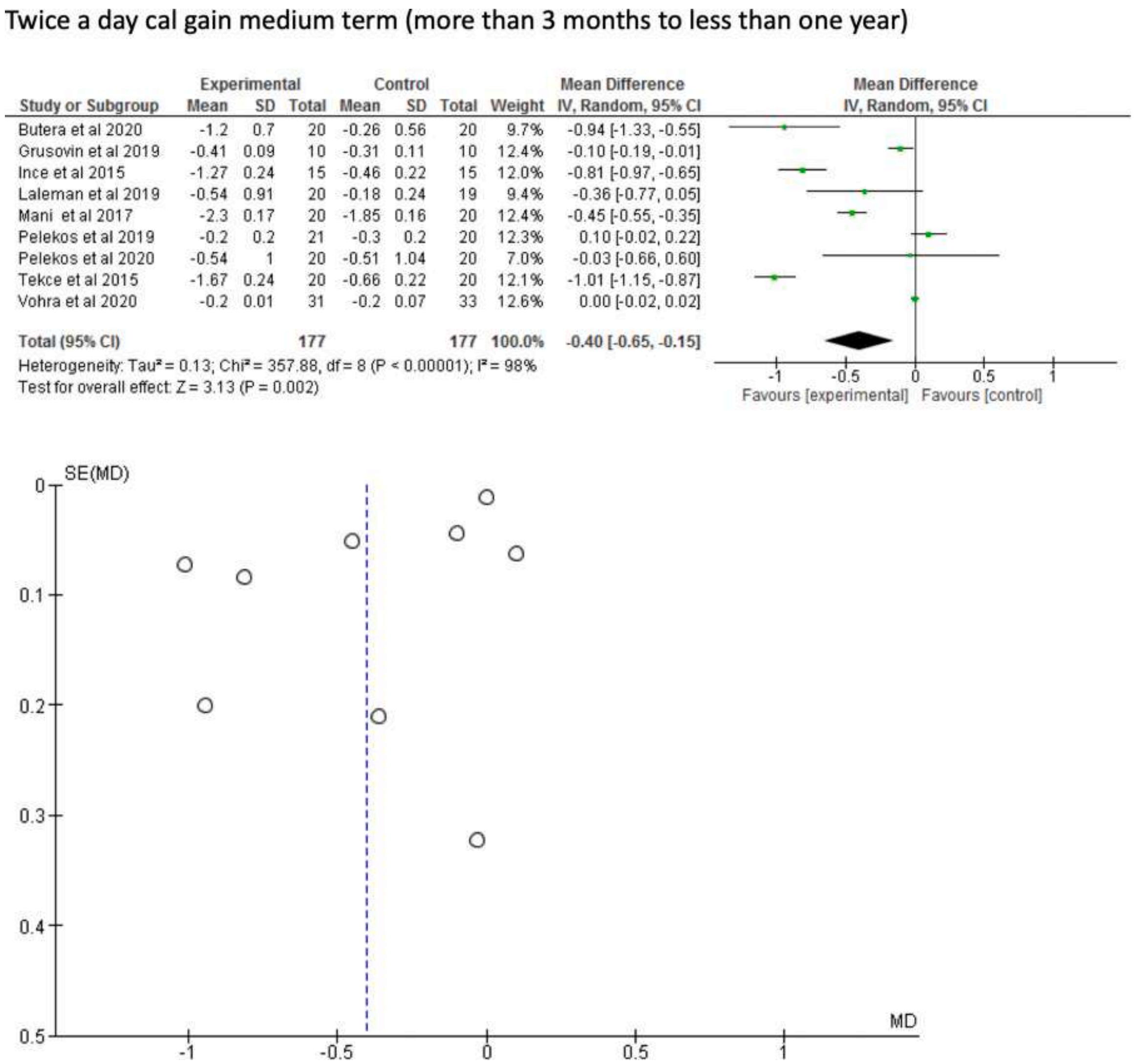
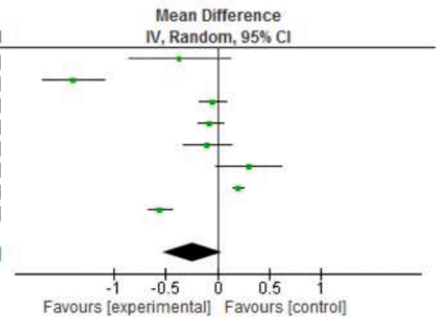


Fig. 22. Forest plot and funnel plot for CAL gain (twice a day intake) medium term (more than 3 months, less than 1 year).

Once a day PD reduction short term (up to 3 months)

	Experimental			Control				Mean Difference
Study or Subgroup	Mean	SD	Total	Mean	SD	Total	Weight	IV, Random, 95% CI
Bazyar et al 2020	-0.82	0.93	23	-0.45	0.77	24	9.9%	-0.37 [-0.86, 0.12]
Booyena et al 2019	-3	0.3	10	-1.6	0.39	10	11.9%	-1.40 [-1.70, -1.10]
Costacurta et al 2018	-0.65	0.25	20	-0.6	0.16	20	13.4%	-0.05 [-0.18, 0.08]
Iwasaki et al 2016	-0.09	0.15	19	-0.02	0.22	17	13.4%	-0.07 [-0.19, 0.05]
Morales et al 2016	-0.5	0.2	14	-0.4	0.4	14	12.6%	-0.10 [-0.33, 0.13]
Morales et al 2021	-0.5	0.4	16	-0.8	0.5	15	11.8%	0.30 [-0.02, 0.62]
Pudgar et al 2020	-0.8	0.03	20	-1	0.12	20	13.7%	0.20 [0.15, 0.25]
Rampalli et al 2016	-2.47	0.19	24	-1.91	0.23	23	13.4%	-0.56 [-0.68, -0.44]
Total (95% CI)			**146**			**143**	**100.0%**	**-0.24 [-0.54, 0.05]**

Heterogeneity: Tau² = 0.16; Chi² = 223.43, df = 7 (P < 0.00001); I² = 97%
Test for overall effect: Z = 1.65 (P = 0.10)



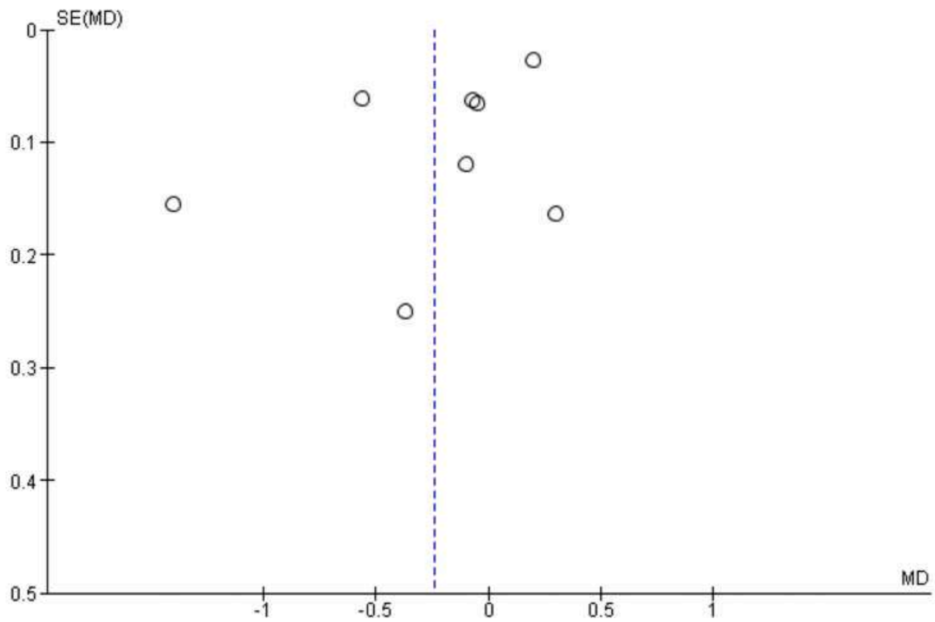
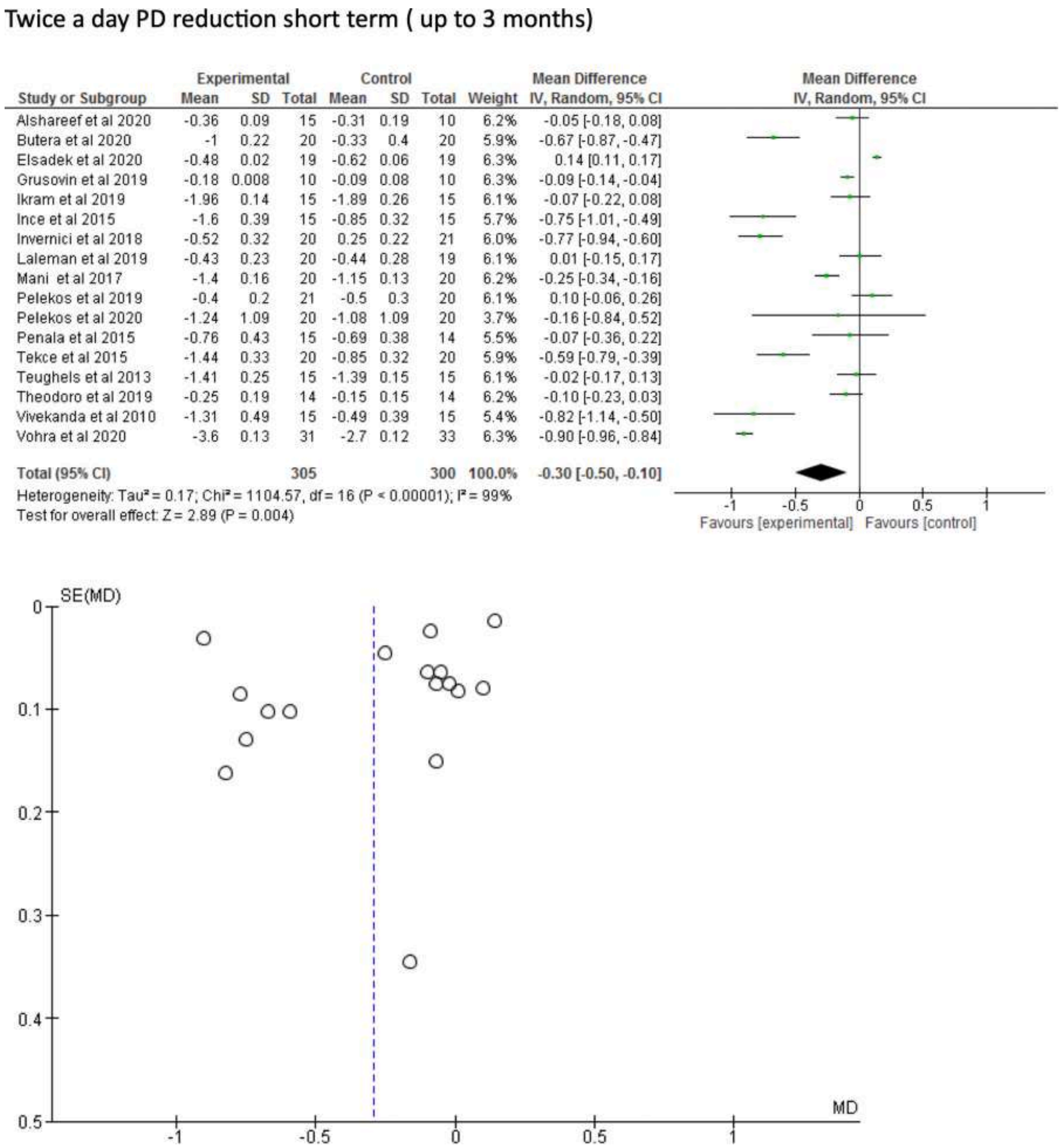


Fig. 23. Forest plot and funnel plot for PD reduction (once a day intake) short term (up to 3 months).



Study or Subgroup	Experimental Mean	SD	Total	Control Mean	SD	Total	Weight	Mean Difference IV, Random, 95% CI
Alshareef et al 2020	-0.36	0.09	15	-0.31	0.19	10	6.2%	-0.05 [-0.18, 0.08]
Butera et al 2020	-1	0.22	20	-0.33	0.4	20	5.9%	-0.67 [-0.87, -0.47]
Elsadek et al 2020	-0.48	0.02	19	-0.62	0.06	19	6.3%	0.14 [0.11, 0.17]
Grusovin et al 2019	-0.18	0.008	10	-0.09	0.08	10	6.3%	-0.09 [-0.14, -0.04]
Ikram et al 2019	-1.96	0.14	15	-1.89	0.26	15	6.1%	-0.07 [-0.22, 0.08]
Ince et al 2015	-1.6	0.39	15	-0.85	0.32	15	5.7%	-0.75 [-1.01, -0.49]
Invernici et al 2018	-0.52	0.32	20	0.25	0.22	21	6.0%	-0.77 [-0.94, -0.60]
Laleman et al 2019	-0.43	0.23	20	-0.44	0.28	19	6.1%	0.01 [-0.15, 0.17]
Mani et al 2017	-1.4	0.16	20	-1.15	0.13	20	6.2%	-0.25 [-0.34, -0.16]
Pelekos et al 2019	-0.4	0.2	21	-0.5	0.3	20	6.1%	0.10 [-0.06, 0.26]
Pelekos et al 2020	-1.24	1.09	20	-1.08	1.09	20	3.7%	-0.16 [-0.84, 0.52]
Penala et al 2015	-0.76	0.43	15	-0.69	0.38	14	5.5%	-0.07 [-0.36, 0.22]
Tekce et al 2015	-1.44	0.33	20	-0.85	0.32	20	5.9%	-0.59 [-0.79, -0.39]
Teughels et al 2013	-1.41	0.25	15	-1.39	0.15	15	6.1%	-0.02 [-0.17, 0.13]
Theodoro et al 2019	-0.25	0.19	14	-0.15	0.15	14	6.2%	-0.10 [-0.23, 0.03]
Vivekanda et al 2010	-1.31	0.49	15	-0.49	0.39	15	5.4%	-0.82 [-1.14, -0.50]
Vohra et al 2020	-3.6	0.13	31	-2.7	0.12	33	6.3%	-0.90 [-0.96, -0.84]
Total (95% CI)			**305**			**300**	**100.0%**	**-0.30 [-0.50, -0.10]**

Heterogeneity: Tau² = 0.17; Chi² = 1104.57, df = 16 (P < 0.00001); I² = 99%
Test for overall effect: Z = 2.89 (P = 0.004)

Fig. 24. Forest plot and funnel plot for PD reduction (twice a day intake) short term (up to 3 months).

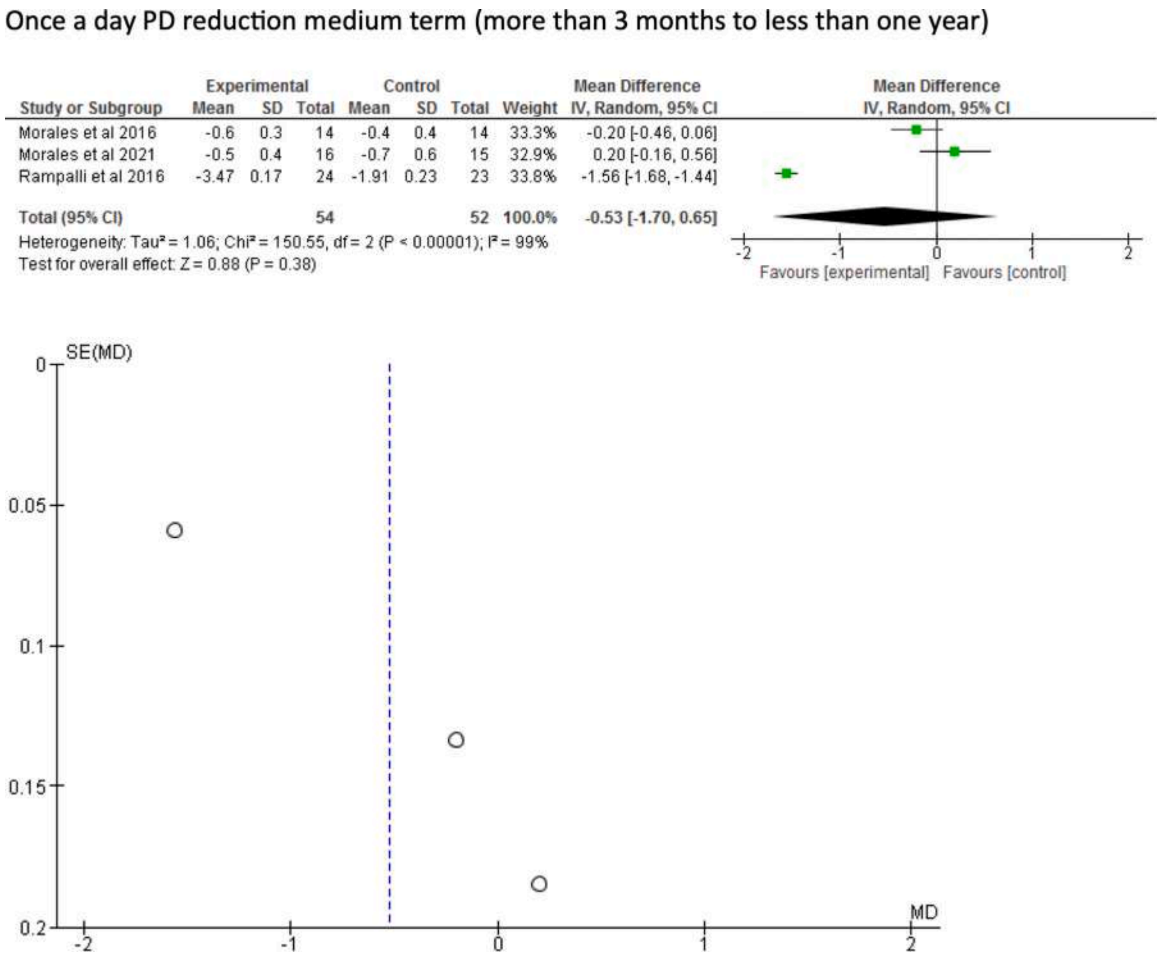


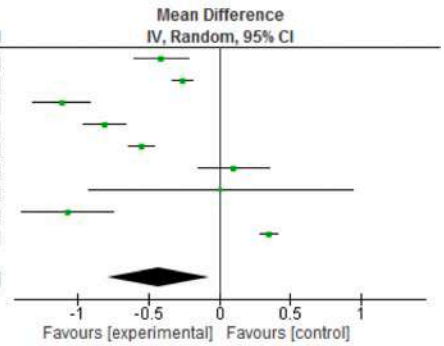
Fig. 25. Forest plot and funnel plot for PD reduction (once a day intake) medium term (more than 3 months, less than 1 year).

Twice a day PD reduction medium term (more than 3 months to less than one year)

	Experimental			Control				Mean Difference
Study or Subgroup	Mean	SD	Total	Mean	SD	Total	Weight	IV, Random, 95% CI
Butera et al 2020	-1.21	0.25	20	-0.8	0.37	20	11.6%	-0.41 [-0.61, -0.21]
Grusovin et al 2019	-0.37	0.08	10	-0.11	0.09	10	12.0%	-0.26 [-0.33, -0.19]
Ince et al 2015	-1.81	0.32	15	-0.7	0.24	15	11.6%	-1.11 [-1.31, -0.91]
Laleman et al 2019	-0.45	0.2	20	0.36	0.26	19	11.8%	-0.81 [-0.96, -0.66]
Mani et al 2017	-2.55	0.18	20	-2	0.12	20	12.0%	-0.55 [-0.64, -0.46]
Pelekos et al 2019	-0.5	0.3	21	-0.6	0.5	20	11.4%	0.10 [-0.15, 0.35]
Pelekos et al 2020	-1.4	1.63	20	-1.41	1.35	20	6.6%	0.01 [-0.92, 0.94]
Tekce et al 2015	-1.77	0.69	20	-0.7	0.24	20	11.0%	-1.07 [-1.39, -0.75]
Vohra et al 2020	-2.4	0.11	31	-2.75	0.14	33	12.0%	0.35 [0.29, 0.41]
Total (95% CI)			**177**			**177**	**100.0%**	**-0.43 [-0.79, -0.08]**

Heterogeneity: Tau² = 0.27; Chi² = 537.81, df = 8 (P < 0.00001); I² = 99%

Test for overall effect: Z = 2.38 (P = 0.02)



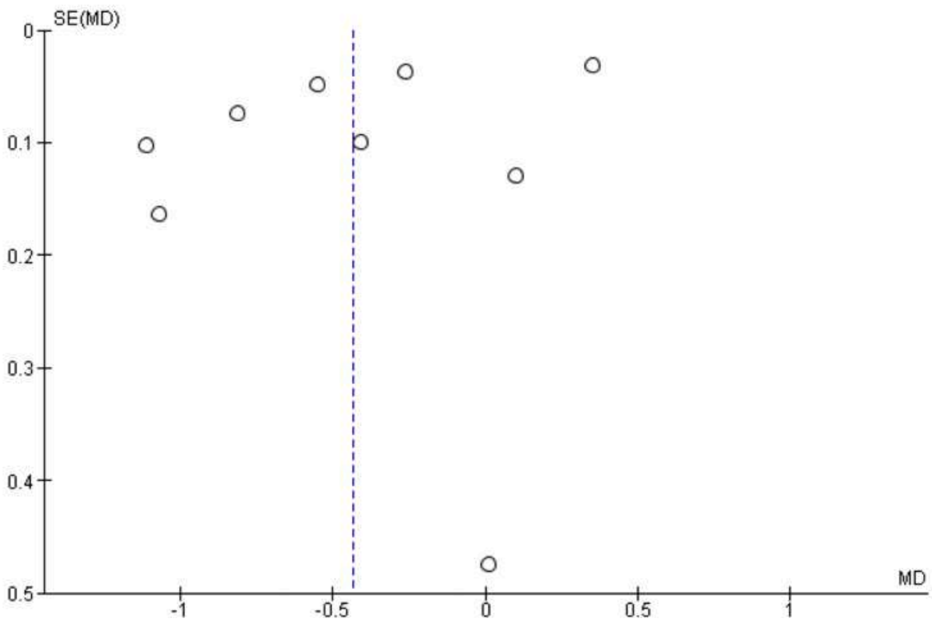


Fig. 26. Forest plot and funnel plot for PD reduction (twice a day intake) medium term (more than 3 months, less than 1 year).

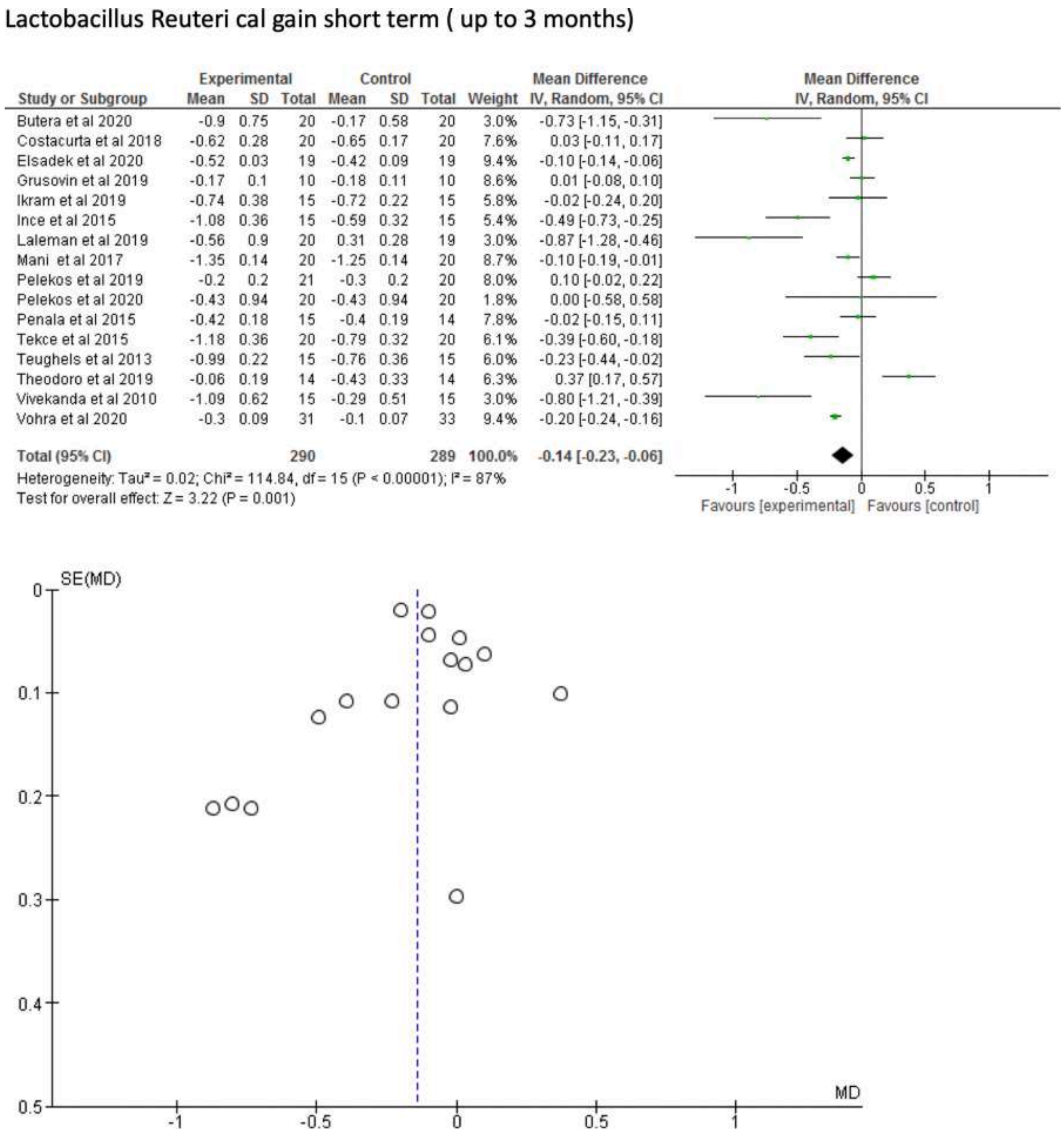
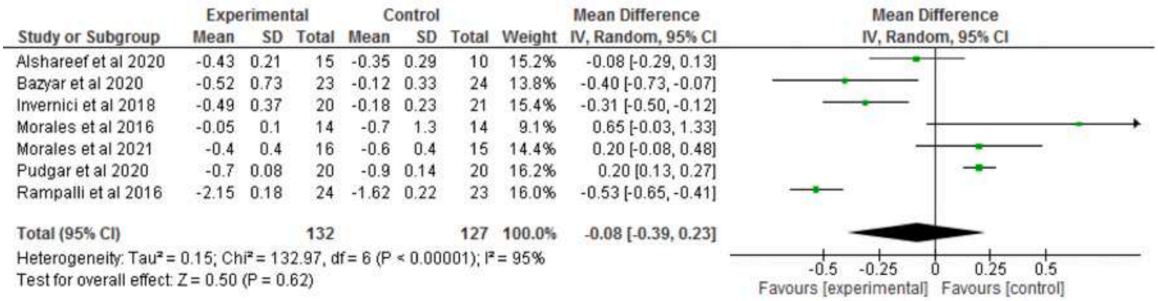


Fig. 27. Forest plot and funnel plot for CAL gain (Lactobacillus reuteri) short term (up to 3 months).

Other probiotic cal gain short term (up to 3 months)



Study or Subgroup	Experimental Mean	Experimental SD	Experimental Total	Control Mean	Control SD	Control Total	Weight	Mean Difference IV, Random, 95% CI
Alshareef et al 2020	-0.43	0.21	15	-0.35	0.29	10	15.2%	-0.08 [-0.29, 0.13]
Bazyar et al 2020	-0.52	0.73	23	-0.12	0.33	24	13.8%	-0.40 [-0.73, -0.07]
Invernici et al 2018	-0.49	0.37	20	-0.18	0.23	21	15.4%	-0.31 [-0.50, -0.12]
Morales et al 2016	-0.05	0.1	14	-0.7	1.3	14	9.1%	0.65 [-0.03, 1.33]
Morales et al 2021	-0.4	0.4	16	-0.6	0.4	15	14.4%	0.20 [-0.08, 0.48]
Pudgar et al 2020	-0.7	0.08	20	-0.9	0.14	20	16.2%	0.20 [0.13, 0.27]
Rampalli et al 2016	-2.15	0.18	24	-1.62	0.22	23	16.0%	-0.53 [-0.65, -0.41]
Total (95% CI)			**132**			**127**	**100.0%**	**-0.08 [-0.39, 0.23]**

Heterogeneity: Tau² = 0.15; Chi² = 132.97, df = 6 (P < 0.00001); I² = 95%
Test for overall effect: Z = 0.50 (P = 0.62)

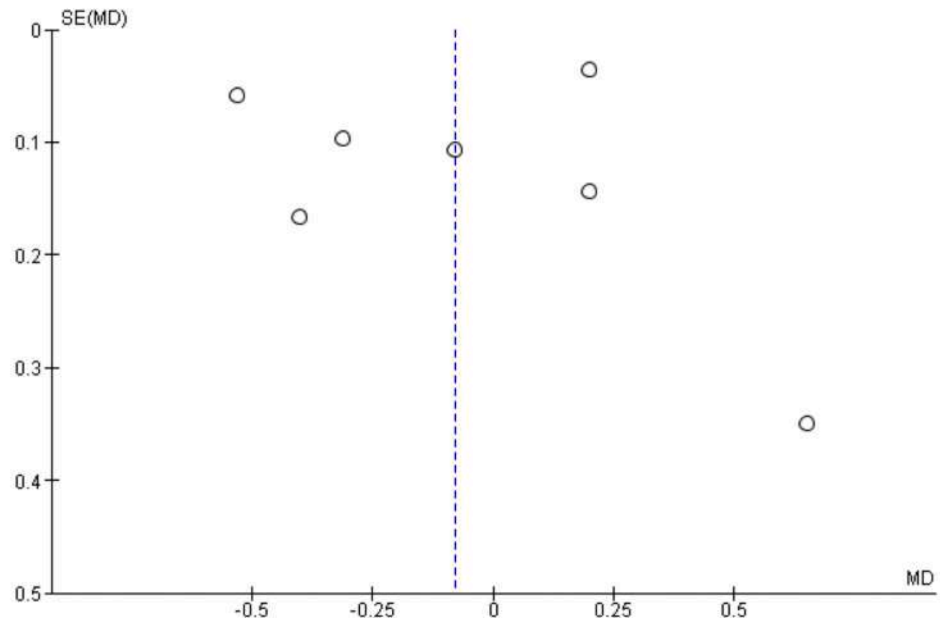
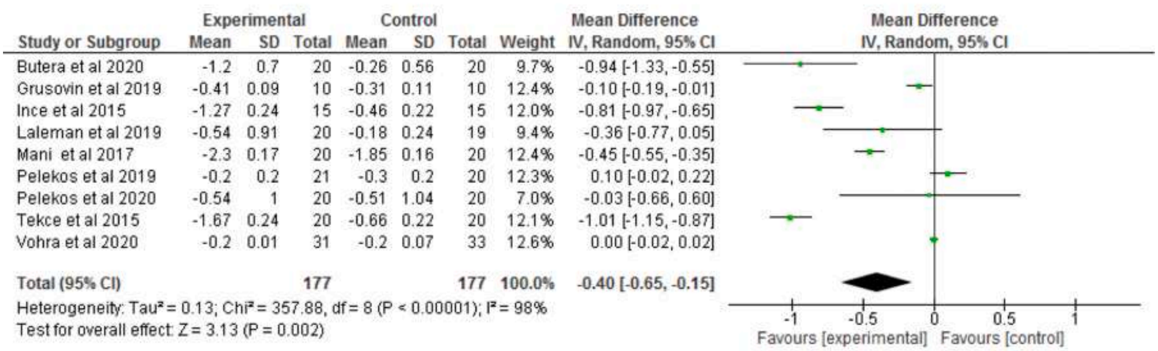


Fig. 28. Forest plot and funnel plot for CAL gain (other probiobitcs) short term (up to 3 months).

Lactobacillus Reuteri cal gain medium term (more than 3 months to less than one year)



Study or Subgroup	Experimental Mean	Experimental SD	Experimental Total	Control Mean	Control SD	Control Total	Weight	Mean Difference IV, Random, 95% CI
Butera et al 2020	-1.2	0.7	20	-0.26	0.56	20	9.7%	-0.94 [-1.33, -0.55]
Grusovin et al 2019	-0.41	0.09	10	-0.31	0.11	10	12.4%	-0.10 [-0.19, -0.01]
Ince et al 2015	-1.27	0.24	15	-0.46	0.22	15	12.0%	-0.81 [-0.97, -0.65]
Laleman et al 2019	-0.54	0.91	20	-0.18	0.24	19	9.4%	-0.36 [-0.77, 0.05]
Mani et al 2017	-2.3	0.17	20	-1.85	0.16	20	12.4%	-0.45 [-0.55, -0.35]
Pelekos et al 2019	-0.2	0.2	21	-0.3	0.2	20	12.3%	0.10 [-0.02, 0.22]
Pelekos et al 2020	-0.54	1	20	-0.51	1.04	20	7.0%	-0.03 [-0.66, 0.60]
Tekce et al 2015	-1.67	0.24	20	-0.66	0.22	20	12.1%	-1.01 [-1.15, -0.87]
Vohra et al 2020	-0.2	0.01	31	-0.2	0.07	33	12.6%	0.00 [-0.02, 0.02]
Total (95% CI)			**177**			**177**	**100.0%**	**-0.40 [-0.65, -0.15]**

Heterogeneity: $Tau^2 = 0.13$; $Chi^2 = 357.88$, $df = 8$ ($P < 0.00001$); $I^2 = 98\%$

Test for overall effect: $Z = 3.13$ ($P = 0.002$)

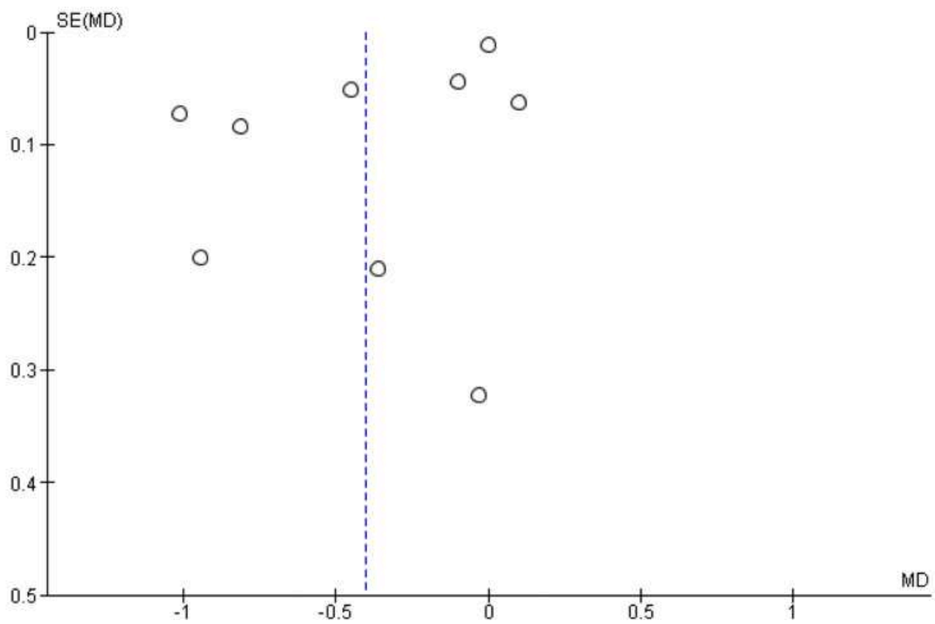
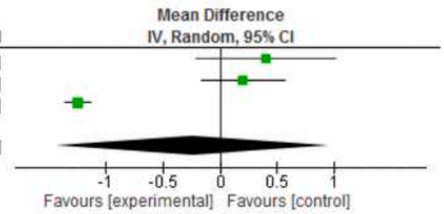


Fig. 29. Forest plot and funnel plot for CAL gain (Lactobacillus reuteri) medium term (more than 3 months, less than 1 year).

Other probiotic cal gain medium term (more than 3 months to less than one year)

Study or Subgroup	Experimental Mean	Experimental SD	Experimental Total	Control Mean	Control SD	Control Total	Weight	Mean Difference IV, Random, 95% CI
Morales et al 2016	-0.3	0.6	14	-0.7	1	14	31.8%	0.40 [-0.21, 1.01]
Morales et al 2021	-0.5	0.4	16	-0.7	0.6	15	33.6%	0.20 [-0.16, 0.56]
Rampalli et al 2016	-2.96	0.18	24	-1.72	0.22	23	34.6%	-1.24 [-1.36, -1.12]
Total (95% CI)			**54**			**52**	**100.0%**	**-0.23 [-1.42, 0.95]**

Heterogeneity: $Tau^2 = 1.06$; $Chi^2 = 78.02$, df = 2 ($P < 0.00001$); $I^2 = 97\%$
Test for overall effect: $Z = 0.39$ ($P = 0.70$)



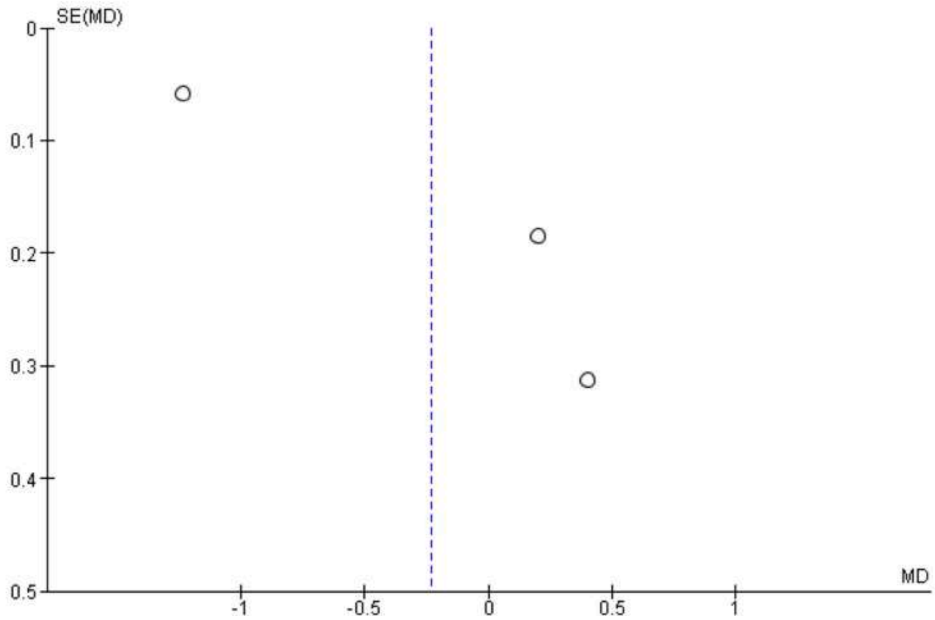
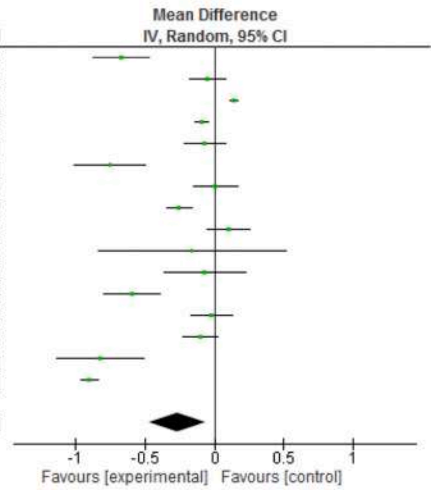


Fig. 30. Forest plot and funnel plot for CAL gain (other probiobitcs) medium term (more than 3 months, less than 1 year).

Lactobacillus Reuteri PD reduction short term (up to 3 months)

Study or Subgroup	Experimental Mean	Experimental SD	Experimental Total	Control Mean	Control SD	Control Total	Weight	Mean Difference IV, Random, 95% CI
Butera et al 2020	-1	0.22	20	-0.33	0.4	20	6.3%	-0.67 [-0.87, -0.47]
Costacurta et al 2018	-0.65	0.25	20	-0.6	0.16	20	6.5%	-0.05 [-0.18, 0.08]
Elsadek et al 2020	-0.48	0.02	19	-0.62	0.06	19	6.7%	0.14 [0.11, 0.17]
Grusovin et al 2019	-0.18	0.008	10	-0.09	0.08	10	6.7%	-0.09 [-0.14, -0.04]
Ikram et al 2019	-1.96	0.14	15	-1.89	0.26	15	6.5%	-0.07 [-0.22, 0.08]
Ince et al 2015	-1.6	0.39	15	-0.85	0.32	15	6.1%	-0.75 [-1.01, -0.49]
Laleman et al 2019	-0.43	0.23	20	-0.44	0.28	19	6.5%	0.01 [-0.15, 0.17]
Mani et al 2017	-1.4	0.16	20	-1.15	0.13	20	6.6%	-0.25 [-0.34, -0.16]
Pelekos et al 2019	-0.4	0.2	21	-0.5	0.3	20	6.5%	0.10 [-0.06, 0.26]
Pelekos et al 2020	-1.24	1.09	20	-1.08	1.09	20	3.9%	-0.16 [-0.84, 0.52]
Penala et al 2015	-0.76	0.43	15	-0.69	0.38	14	5.9%	-0.07 [-0.36, 0.22]
Tekce et al 2015	-1.44	0.33	20	-0.85	0.32	20	6.3%	-0.59 [-0.79, -0.39]
Teughels et al 2013	-1.41	0.25	15	-1.39	0.15	15	6.5%	-0.02 [-0.17, 0.13]
Theodoro et al 2019	-0.25	0.19	14	-0.15	0.15	14	6.6%	-0.10 [-0.23, 0.03]
Vivekanda et al 2010	-1.31	0.49	15	-0.49	0.39	15	5.8%	-0.82 [-1.14, -0.50]
Vohra et al 2020	-3.6	0.13	31	-2.7	0.12	33	6.7%	-0.90 [-0.96, -0.84]
Total (95% CI)			**290**			**289**	**100.0%**	**-0.26 [-0.47, -0.06]**

Heterogeneity: Tau² = 0.16; Chi² = 1041.40, df = 15 (P < 0.00001); I² = 99%
Test for overall effect: Z = 2.55 (P = 0.01)



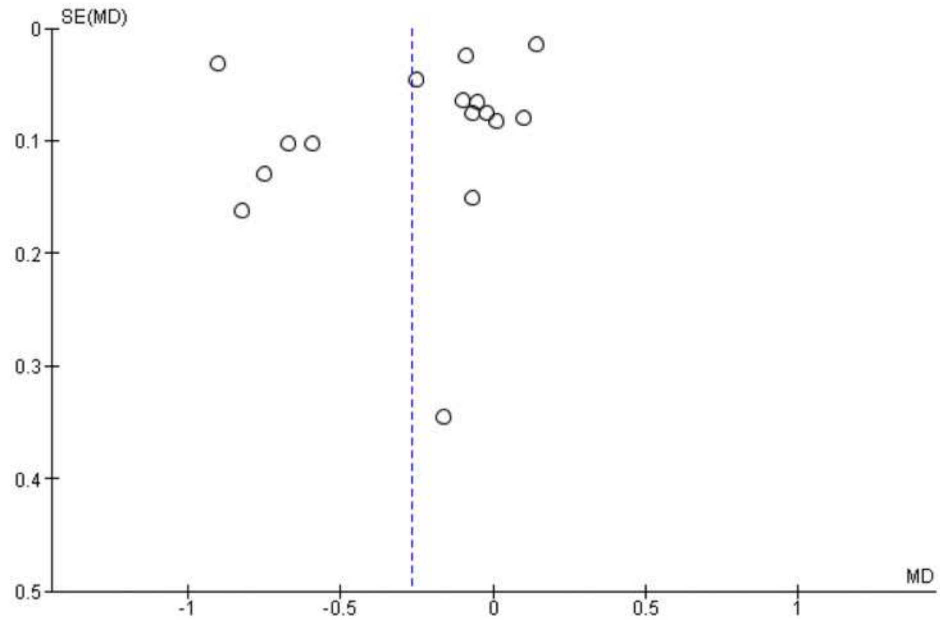
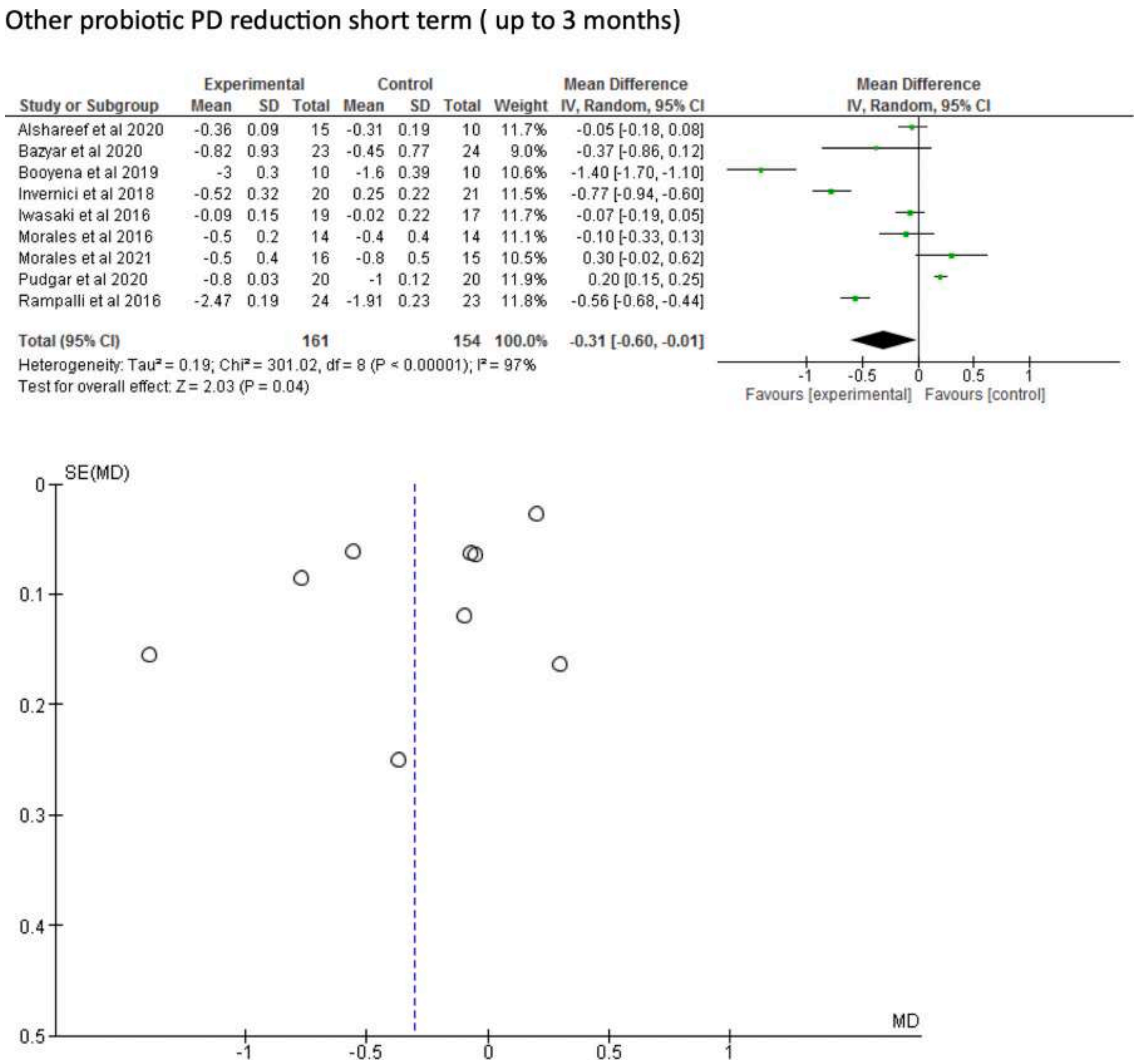


Fig. 31. Forest plot and funnel plot for PD reduction (Lactobacillus reuteri) short term (up to 3 months).


Other probiotic PD reduction short term (up to 3 months)

Study or Subgroup	Experimental Mean	Experimental SD	Experimental Total	Control Mean	Control SD	Control Total	Weight	Mean Difference IV, Random, 95% CI
Alshareef et al 2020	-0.36	0.09	15	-0.31	0.19	10	11.7%	-0.05 [-0.18, 0.08]
Bazyar et al 2020	-0.82	0.93	23	-0.45	0.77	24	9.0%	-0.37 [-0.86, 0.12]
Booyena et al 2019	-3	0.3	10	-1.6	0.39	10	10.6%	-1.40 [-1.70, -1.10]
Invernici et al 2018	-0.52	0.32	20	0.25	0.22	21	11.5%	-0.77 [-0.94, -0.60]
Iwasaki et al 2016	-0.09	0.15	19	-0.02	0.22	17	11.7%	-0.07 [-0.19, 0.05]
Morales et al 2016	-0.5	0.2	14	-0.4	0.4	14	11.1%	-0.10 [-0.33, 0.13]
Morales et al 2021	-0.5	0.4	16	-0.8	0.5	15	10.5%	0.30 [-0.02, 0.62]
Pudgar et al 2020	-0.8	0.03	20	-1	0.12	20	11.9%	0.20 [0.15, 0.25]
Rampalli et al 2016	-2.47	0.19	24	-1.91	0.23	23	11.8%	-0.56 [-0.68, -0.44]
Total (95% CI)			**161**			**154**	**100.0%**	**-0.31 [-0.60, -0.01]**

Heterogeneity: Tau² = 0.19; Chi² = 301.02, df = 8 (P < 0.00001); I² = 97%
Test for overall effect: Z = 2.03 (P = 0.04)

Fig. 32. Forest plot and funnel plot for PD reduction (other probiobitcs) short term (up to 3 months).

Lactobacillus Reuteri PD reduction medium term (more than 3 months to less than one year)

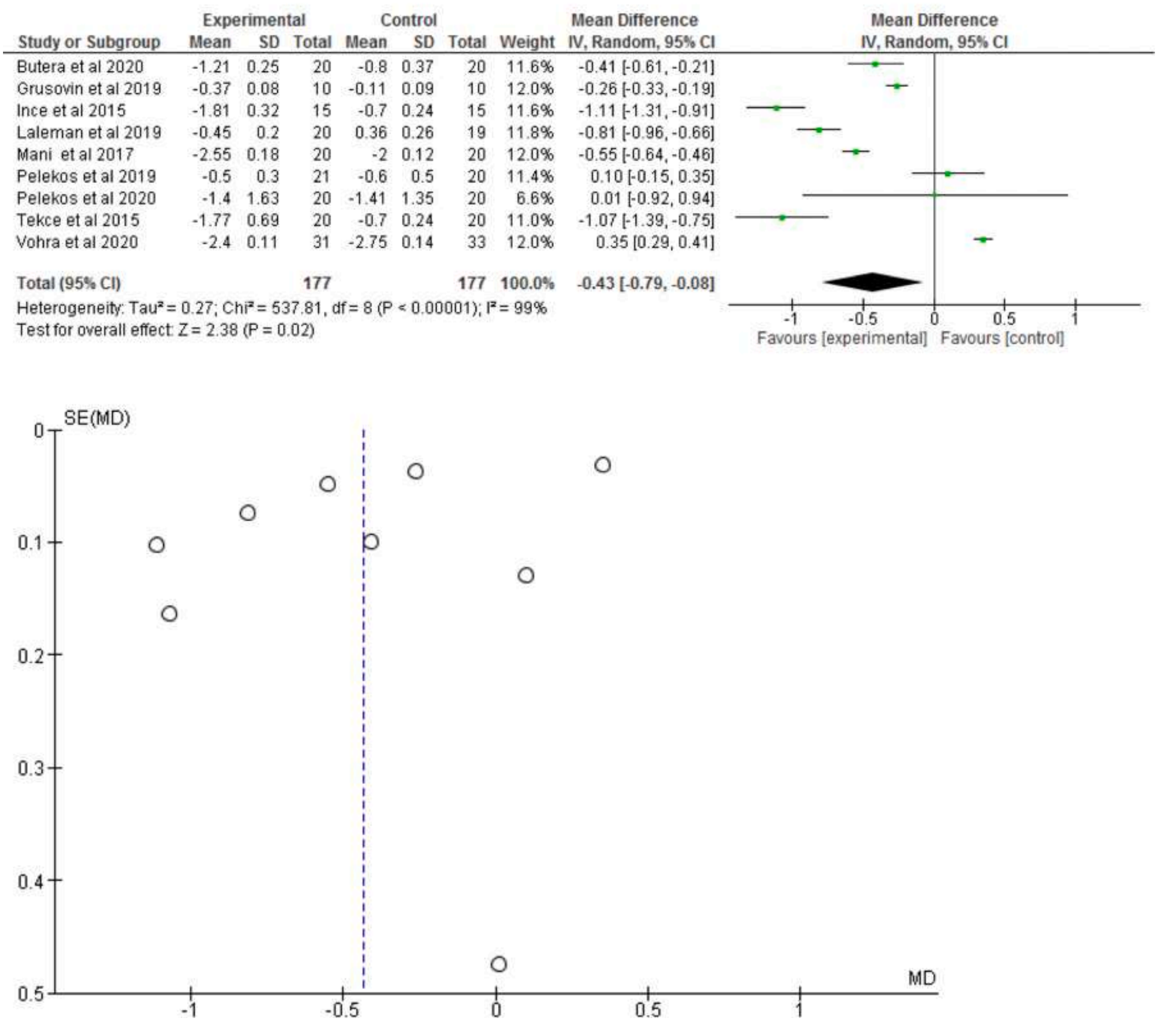


Fig. 33. Forest plot and funnel plot for PD reduction (Lactobacillus reuteri) medium term (more than 3 months, less than 1 year).

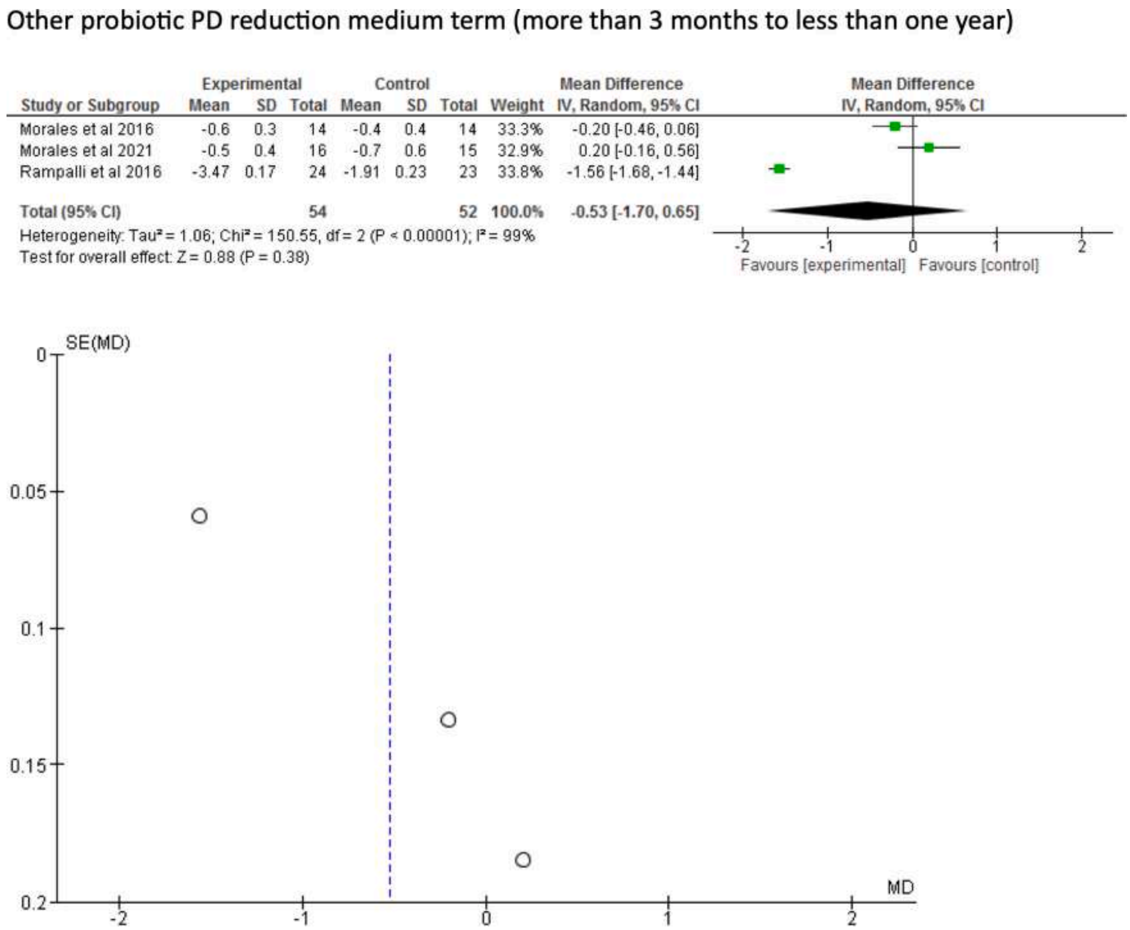


Fig. 34. Forest plot and funnel plot for PD reduction (other probiobitcs) medium term (more than 3 months, less than 1 year).

Table 1
Basic characteristics of the studies.

First Author	Year	Study design	Planned no. of pat. (all Pat.)	Actual no. of pat at the end of study (Test/Control)	Drop-out %	Drop-out number	Mean age	Age range	Gender M/F	Smokers (Y/N)	Smokers (number)	Diabetics (Y/N)	Diabetics (number)
Vivekanda et al.	2010	RCT	30	30 (15/15)	0	0	41.45	35–50	19/11	N	0	N	0
Teughels et al.	2013	RCT	30	30 (15/15)	0	0	46.16	38–50	15/15	N	0	N	0
Penala et al.	2015	RCT	32	29 (15/14)	10.66	3	36.25	25–59	NR	N	0	N	0
Tekce et al.	2015	RCT	55	40 (20/20)	36	15	42.2	35–50	18/22	N	0	N	0
Ince et al.	2015	RCT	30	30 (15/15)	0	0	41.6	35–50	17/13	N	0	N	0
Morales et al.	2016	RCT	28	28 (14/14)	0	0	49.8	35–68	14/14	Y	6 (4 test, 2 control)	N	0
Iwasaki et al.	2016	RCT	39	36 (19/17)	13	3	67.6	NR	13/23	NR		NR	NR
Rampalli et al.	2016	RCT	30	30 (15/15)	10	3	NR	25–50	NR	N	0	N	0
Mani et al.	2017	RCT	40	40 (20/20)	0	0	39.85	18–55	20/20	N	0	N	0
Costacurta et al.	2018	RCT	40	40 (20/20)	0	0	46.55	18–70	20/20	NR		NR	NR
Invernici et al.	2018	RCT	41	41 (20/21)	0	0	NR	NR	NR	N	0	N	0
Grusovin et al.	2019	RCT	20	20 (10/10)	0	0	49.65	31–70	8/12	Y	8	N	0
Laleman et al.	2019	RCT	44	39 (20/19)	8.8	5	58	34–83	27/12	N	0	N	0
Theodoro et al.	2019	RCT	34	28 (14/14)	17.65	6	46.16	30–56	15/13	Y	28	N	0
Pelekos et al.	2019	RCT	59	41 (21/20)	32.7	18	53.3	NR	15/26	N	0	N	0
Ikram et al.	2019	RCT	30	30 (15/15)	0	0	40.85	NR	17/13	N	0	N	0
Booyena et al.	2019	RCT	30	20 (10/10)	0	0	NR	20–50	NR	N	0	N	0
Pudgar et al.	2020	RCT	40	40 (20/20)	0	0	46.3	25–80	18/22	Y	13 (5 test, 8 control)	N	0
Elsadek et al.	2020	Prospective	40	38 (19/19)	5	2	52.37	35–75	26/17	N	0	Y	38
Bazyar et al.	2020	RCT	50	47 (23/24)	6	3	49.35	30–60	14/33	N	0	Y	47
Pelekos et al.	2020	RCT	40	40 (20/20)	0	0	51.95	NR	14/26	N	0	N	0
Alshareef et al.	2020	RCT	40	25 (15/10)	37.5	15	29	25–58	NR	NR		NR	NR
Vohra et al.	2020	RCT	64*	64 (31/33)	0	0	52.67	NR	127/0	Y	0	N	0
Butera et al.	2021	RCT	40	60 (20/20)	0	0	53	18–70	32/28	NR		NR	NR
Morales et al.	2021	RCT	47	31 (16/15)	0	0	49.43	NR	26/21	Y	16 (7 test, 6 control)	N	0

NR: not reported
*Shamma chewers were excluded from the meta-analysis

Table 2
Diagnosis and treatment type.

First Author	Year	Case definition	Probiotic strain	Frequency	Form	Duration of probiotic (weeks)	Intervention	Time points (weeks)
Vivekanda et al.	2010	Moderate-severe CP	L. reuteri	Twice/day	Lozenges	3	SRP	3,6
Teughels et al.	2013	Moderate-severe CP	L. reuteri	Twice/day	Lozenges	12	SRP	3, 6, 9, 12
Penala et al.	2015	Moderate-severe CP	L. reuteri, L. salivarius	Twice/day	Capsules	4	SRP	1,2,4
Tekce et al.	2015	Moderate-severe CP	L. reuteri	Twice/day	Lozenges	3	SRP	3,12, 24, 52
Ince et al.	2015	Moderate-severe CP	L. reuteri	Twice/day	Lozenges	3	SRP	3,12, 24, 52
Morales et al.	2016	CP	L. rhamnosus	Once/day	Sachet	12	SRP	12,24,36,48
Iwasaki et al.	2016	CP	L. plantarum	Once/day	Capsules	12	SRP	4,8,12
Rampalli et al.	2016	Moderate-severe CP	S. boulardii	Single dose	Powder	baseline only	SRP	12,24
Mani et al.	2017	Mild-moderate CP	S. salivarius, L. reuteri, L. paracasei	Twice/day	Lozenges	16	SRP	8,16
Costacurta et al.	2018	CP	L. reuteri	Once/day	Tablets	4	SRP	4
Invernici et al.	2018	CP	B. lactis	Twice/day	Lozenges	4	SRP	4,12
Grusovin et al.	2019	Periodontitis stage III-IV grade C	L. reuteri	Twice/day	Lozenges	12	SRP	12, 24, 36, 52
Laleman et al.	2019	Moderate-severe CP	L. reuteri	Twice/day	Lozenges	12	SRP	12,24
Theodoro et al.	2019	Severe CP	L. reuteri	Twice/day	Lozenges	3	SRP	1,3,12
Pelekos et al.	2019	Moderate-severe CP	L. reuteri	Twice/day	Lozenges	3	SRP	1,3, 12,24
Ikram et al.	2019	CP	L. reuteri	Twice/day	Powder	12	SRP	6,12
Booyena et al.	2019	CP	L. acidophilus, L. rhamnosus, B. bifidus, B. longum	Single dose	Capsules	baseline only	SRP	6
Pudgar et al.	2020	Periodontitis stage III-IV	L. brevis, L. plantarum	Once/day	Lozenges (+ gel after SRP)	12	SRP	12
Elsadek et al.	2020	Diabetes mellitus; Periodontitis stage III grade C	L. reuteri	Twice/day	Lozenges	3	SRP	12
Bazyar et al.	2020	Diabetes mellitus; Mild-moderate CP	L. acidophilus, L. casei, L. rhamnosus, L. bulgaricus, B. breve, B. longum, S. thermophilus	Once/day	Capsules	8	SRP	8
Pelekos et al.	2020	Periodontitis stage III-IV	L. reuteri	Twice/day	Lozenges	3	SRP	12,24
Alshareef et al.	2020	Moderate-severe CP	L. acidophilus, L. casei, B. bifidum, L. rhamnosus, L. salivarius	Twice/day	Lozenges	4	SRP	4
Vohra et al.	2020	Periodontitis stage III grade C	L. reuteri	Twice/day	Lozenges	3	SRP	12,24
Butera et al.	2021	Periodontitis stage II-III	L. reuteri, L. salivarius, L. plantarum	Twice/day	Chewing gum and toothpaste	24	SRP	12,24
Morales et al.	2021	Periodontitis stage III	L. rhamnosus	Once/day	Sachet	12	SRP	12,24,36

NR: not reported; CP: Chronic periodontitis; SRP: scaling and root planing

Table 3
Outcomes.

First Author	Year	Follow up (weeks)	CAL gain test (mm)	CAL gain control (mm)	PD reduction test (mm)	PD reduction control (mm)
Vivekanda et al.	2010	6	1.09 ± 0.62	0.29 ± 0.51	1.31 ± 0.49	0.49 ± 0.39
Teughels et al.	2013	12	0.99 ± 0.22	0.76 ± 0.36	1.41 ± 0.25	1.39 ± 0.15
Penala et al.	2015	12	0.42 ± 0.18	0.40 ± 0.19	0.76 ± 0.43	0.69 ± 0.38
Tekce et al.	2015	12	1.18 ± 0.36	0.79 ± 0.32	1.44 ± 0.33	0.85 ± 0.32
		24	1.67 ± 0.24	0.66 ± 0.22	1.77 ± 0.69	0.70 ± 0.24
		52	1.39 ± 0.26	0.53 ± 0.24	1.74 ± 0.62;	0.57 ± 0.24
Ince et al.	2015	12	1.08 ± 0.36	0.59 ± 0.32	1.60 ± 0.39	0.85 ± 0.32
		24	1.27 ± 0.24	0.46 ± 0.22	1.81 ± 0.32	0.70 ± 0.24
		52	1.39 ± 0.026	0.43 ± 0.24	1.70 ± 0.31	0.55 ± 0.26
Morales et al.	2016	12	0.05 ± 0.1	0.7 ± 1.3	0.5 ± 0.2	0.4 ± 0.4
		24	0.3 ± 0.6	0.7 ± 1	0.6 ± 0.3	0.4 ± 0.4
		48	0.07 ± 0.5	0.09 ± 0.8	0.6 ± 0.3	0.4 ± 0.4
Iwasaki et al.	2016	12	NR	NR	0.09 ± 0.15	0.02 ± 0.22
Rampalli et al.	2016	12	2.15 ± 0.18	1.62 ± 0.22	2.47 ± 0.19	1.76 ± 0.23
		24	2.96 ± 0.18	1.72 ± 0.22	3.47 ± 0.17	1.91 ± 0.23
Mani et al.	2017	8	1.35 ± 0.14	1.25 ± 0.14	1.4 ± 0.16	1.15 ± 0.13
		16	2.3 ± 0.17	1.85 ± 0.16	2.55 ± 0.18	2 ± 0.12
Costacurta et al.	2018	4	0.62 ± 0.28	0.65 ± 0.17	0.65 ± 0.25	0.6 ± 0.16
Invernici et al.	2018	12	0.49 ± 0.37	0.18 ± 0.23	0.52 ± 0.32	0.25 ± 0.22
Grusovin et al.	2019	12	0.17 ± 0.10	0.18 ± 0.11	0.18 ± 0.08	0.09 ± 0.08
		36	0.41 ± 0.09	0.31 ± 0.11	0.37 ± 0.08	0.11 ± 0.09
		52	0.50 ± 0.09	0.50 ± 0.10	0.47 ± 0.07	0.31 ± 0.08
Laleman et al.	2019	12	0.56 ± 0.90	0.31 ± 0.28	0.43 ± 0.23	0.44 ± 0.28
		24	0.54 ± 0.91	0.18 ± 0.24	0.45 ± 0.20	0.36 ± 0.26
Theodoro et al.	2019	12	0.06 ± 0.19	0.43 ± 0.33	0.25 ± 0.19	0.15 ± 0.15
Pelekos et al.	2019	12	0.2 ± 0.2	0.3 ± 0.2	0.4 ± 0.2	0.5 ± 0.3
		24	0.2 ± 0.2	0.3 ± 0.2	0.5 ± 0.3	0.6 ± 0.5
Ikram et al.	2019	12	0.74 ± 0.38	0.72 ± 0.22	1.96 ± 0.14	1.89 ± 0.26
Booyena et al.	2019	6	NR	NR	3 ± 0.3	1.6 ± 0.39
Pudgar et al.	2020	12	0.7 ± 0.08	0.9 ± 0.14	0.8 ± 0.03	1 ± 0.12
Elsadek et al.	2020	12	0.52 ± 0.03	0.42 ± 0.09	0.48 ± 0.02	0.62 ± 0.06
Bazyar et al.	2020	8	0.52 ± 0.73	0.12 ± 0.33	0.82 ± 0.93	0.45 ± 0.77
Pelekos et al.	2020	12	0.43 ± 0.94	0.43 ± 0.94	1.24 ± 1.09	1.08 ± 1.09
		24	0.54 ± 1.00	0.51 ± 1.04	1.40 ± 1.63	1.41 ± 1.35
Alshareef et al.	2020	4	0.43	0.35	0.36	0.31
Vohra et al.	2020	12	0.3 ± 0.09	0.1 ± 0.07	3,6 ± 0.13	2.7 ± 0.12
		24	0.2 ± 0.01	0.2 ± 0.07	2.4 ± 0.11	2.75 ± 0.14
Butera et al.	2021	12	0.9 ± 0.75	0.17 ± 0.58	1 ± 0.22	0.33 ± 0.4
		24	1.2 ± 0.7	0.26 ± 0.56	1.21 ± 0.25	0.08 ± 0.37
Morales et al.	2021	12	0.4 ± 0.4	0.6 ± 0.4	0.5 ± 0.4	0.8 ± 0.5
		36	0.3 ± 0.4	0.6 ± 0.4	0.5 ± 0.4	0.7 ± 0.6

NR: not reported; CP: Chronic periodontitis; SRP: scaling and root planning

Table 4
Newcastle-Ottawa Quality Assessment Scale.

Study	Selection				Comparability	Outcome		
	Representativeness of the exposed cohort	Selection of the non exposed cohort	Ascertainment of exposure	Demonstration that outcome of interest was not present at start of study	Comparability of cohorts on the basis of the design or analysis	Assessment of outcome	Was follow-up long enough for outcomes to occur	Adequacy of follow up of cohorts
Elsadek et al. (2020)	*	*	*	*	*	*	*	*

Table 5
Microbiological findings.

Study	Level of analysis	Main results	Outcome variables	Sampling site	Follow up (weeks)
Vivekanda et al. (2010)	Intergroup	Significant reduction of CFU counts with probiotics use for all species (A actinomycetemcomitans, P gingivalis, P intermedia).	Δ mean log10 cfu/ml for Aa, Pg, Pi.	Subgingival plaque	3, 6
	Intragroup	NR			
Teughels et al. (2013)	Intergroup	Significant reduction of P Gingivalis in sub and supragingival plaque and saliva samples at week 9 and 12 for the test group compared to SRP alone. Significant lower count of Prevotella intermedia in saliva samples of test group compared to control group at week 12	Mean log10 cfu/ml and Δ mean log10 cfu/ml for Aa, Fn, Pg, Pi, Tf and total load	Supra and subgingival plaque, saliva	3, 6, 9, 12
	Intragroup	NR			
Tekce et al. (2015)	Intergroup	The difference between the two groups was always statistically significant in favor of the test group except for total viable cell count at the last time point.	Total viable cell count and proportions of obligate anaerobes	Subgingival plaque	3,12, 24, 52
	Intragroup	Both test and control groups had a significant decrease in total viable cell count and proportions of obligate anaerobes over the follow-up period.			
Mani et al. (2017)	Intergroup	Statistically significant difference of total counts for all species between test and control group.	PCR - Aa, Pi, Pg, Fn	Subgingival plaque	8, 16
	Intragroup	Statistically significant difference of total counts for all species at all time points in both test and control group.			
Invernici et al. (2018)	Intergroup	More pronounced reduction in total count of *P. gingivalis*, *Td*, *Fn*, *Cs*, and *En in test group compared to* Control group ($p < 0.05$) for deep periodontal pockets Significantly lower mean proportions of orange (at 30 days) and red (at 90 days) in test group compared to Control group ($p < 0.05$). Significantly larger proportion of blue complex in the Test group compared to Control group at 90 days ($p < 0.05$)	Total count and proportions - no data is provided, only graphs - 40 species examined with PCR	Subgingival plaque	4, 12
	Intragroup	NR			
Morales et al. (2018)	Intergroup	No significant differences in total cultivable microbiota and percentages of *Pg*, *Aa* and *Tf* at any time point	Total cultivalble microbiota, n. of subjects detected with Pg, Aa and Tf and mean proportions of Pg.	Subgingival plaque	12, 24, 36
	Intragroup	Significant reduction of total cultivable microbiota in the probiotic group at 6-month. No significant difference in subjects detected with Pg, Aa or Tf, and no significant difference in mean proportions of Pg between groups			
Laleman et al. (2019)	Intergroup	No statistically significant inter- or intra-group differences could be found between test and control groups at any time point for all parameters.	Mean log10 cfu/ml and Δ mean log10 cfu/ml for Aa, Fn, Pg, Pi.	Supra and subgingival plaque, saliva, tongue	12, 24
	Intragroup	NR			
Pudgar et al. (2020)	Intergroup	No statistically significant difference in detection frequency, total counts or proportions of all the observed species at 3 months.	Total and Δ CFU/ml for Pi, Pm, Fn, Ec, Cr, Pg, Tf.	Subgingival plaque	12
	Intragroup	Statistically non-significant intragroup differences in total counts of cultivable species were observed both in the test and control group. Statistically significant intragroup reduction of prevalence of P micra and reduction of total counts and proportion of P gingivalis and T forsythia in the control group.			
Elsadek et al. (2020)	Intergroup	Probiotics group showed significantly higher reductions in the detection frequency of all species compared with SRP alone.	Detection frequency of Pg, Tf, Td	Subgingival plaque	12
	Intragroup	All groups showed statistically significant reductions in the detection frequency of all species			
Butera et al. (2021)	Intergroup	NR	Total count and meand proportions of Aa, Pg, Tf, Td, Pi, Fn and different bacterial complexes.	Subgingival plaque	4, 12
	Intragroup	No significant differences were detected compared to baseline values for any group, except in Group 2 and 3 at 6 months only for the percentage of the orange complex pathogens and for the copies/microliter of *Prevotella intermedia* and *Fusobacterium nucleatum*.			

Aa: Aggregatibacter actinomycetemcomitans; Pi: Prevotella intermedia; Pm: Parvimonas micra; Fn: Fusobacterium nucleatum; Ec: Eikenella corrodens; Cr: Campylobacter rectus; Co: Capnocytophaga ochracea; Pg: Porphyromonas gingivalis; Tf: Tannerella forsythia; Td: Treponema denticola; An: Actinomyces naeslundii; Sm: Streptococcus mitisCs: Campylobacter showae; En: Eubacterium nodatum; NR: not reported.

Table 6
Immunological findings.

Study	Level of analysis	Main Results	Outcome variables	Follow up (weeks)
Ince et al. 2015	Intergroup	Decrease of MMP-8 and increase of TIMP-1 was significantly superior in favour of the test group up to 24 weeks	GCF levels of MMP-8 and TIMP-1	3, 12, 24, 38
	Intragroup	Significant decrease of MMP-8 concentration up to 24 weeks in both groups – Significant increase of TIMP-1 concentration un to 24 weeks in both groups		
Invernici et al. 2018	Intergroup	Significant difference of IL-8 and IL-1β levels at 4 and 12 weeks compared to baseline in both groups. - Significant difference of IL-10 levels at 4 weeks compared to baseline in test group only	GCF levels of IL-1β, IL-10, and IL-8	4, 12
	Intragroup	Significantly higher levels of IL-8 at 4 weeks in control group compared to test group. - Significantly higher levels of IL-1β at 4 and 12 weeks in control group compared to test group. - No significant differences in the levels of IL-10 at 4 and 12 weeks between the groups.		
Bazyar et al. 2020	Intergroup	Significantly lower serum levels at 8 weeks and means changes of L-1β and MDA in the test group compared to control group. - Significantly lower mean changes of SOD and GPx in test group compared to control group.	Blood Serum markers of oxida- tive stress (TAC, SOD, CAT, GPx, MDA) and serum IL-1β.	8
	Intragroup	Significant reduction of the serum levels of IL-1β and MDA in the test group only. - Significant increase of the serum levels of TAC, SOD and GPx but not CAT in the test group only		
Alshareef et al. 2020	Intergroup	Significant decrease of MMP-8 levels at 4 weeks compared to baseline in both groups although more marked in test group	GCF levels of MMP-8	4
	Intragroup	No significant differences of MMP-8 levels between the groups		

GCF: gingival crevicular fluid; TAC: total antioxidant capacity; SOD: superoxide dismutase; CAT: catalase; GPx: glutathione peroxidase; MDA: malondialdehyde

administration have represented the most common limitations. To try to overcome these limitations targeted sub-analyzes that allow to interpret the results despite these inhomogeneities were performed.

Furthermore, not all included studies are considered to be at low risk of bias for several reasons, although generally good quality was found.

5. Conclusions

In conclusion, in light of these results, probiotics appear to provide an additional benefit to non-surgical periodontal therapy in patients with periodontitis. The longer the period of administration of the probiotic, the greater the gain of CAL and the reduction of PD, especially if administered in double daily dose, in the form of lozenges and if *L reuteri* is the only strain used.

However, significant and wide heterogeneity coupled with the absence of consistent long-term data precludes any firm conclusions. More research is needed to address these issues and make clinical recommendations regarding their effectiveness in the treatment of periodontitis.

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Conflicts of interest

None.

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