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Review article

Probiotics for managing caries and periodontitis: Systematic review and meta-analysis

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ABSTRACT

Objectives: Probiotics might be beneficial to prevent or treat caries, gingivitis or periodontitis. We aimed to appraise trials assessing probiotics for managing caries and periodontal disease.
Data: We included randomized controlled trials comparing the efficacy of probiotics versus (placebo) control with regards to Streptococcus mutans [SM], lactobacilli [LB], periodontal pathogens numbers, gingivitis, oral hygiene, caries incidence/experience increment, or periodontitis. Meta-analysis and trial-sequential-analysis were performed.
Sources: Three electronic databases (Medline, Embase, Central) were screened.
Study selection: 50 studies (3247 participants) were included. Studies were mainly performed in children and used lactobacilli (45); bifidobacteria (12) or other genus (3). Probiotics significantly increased the chance of reducing SM (OR: 2.20, 95% CI: 1.23/3.92) or LB (OR: 2.84; 1.34/6.03) $<10^4$ CFU/ml. Such reduction was confirmed for SM counts (standardized mean differences: −1.18, 95% CI: −1.64/-0.72), but not LB (SMD: 0.33; 0.15/0.52). For periodontal pathogens, no significant difference was found. Probiotics significantly reduced bleeding-on-probing (SMD: −1.15; −1.68/-0.62) and gingival index (SMD: −0.86; −1.52/-0.20), but not plaque index (SMD: −0.34; −0.89/0.21). Caries incidence was not significantly reduced (OR: 0.60; 0.35/1.04), neither was caries experience (SMD: −0.26; −0.55/0.03) or CAL (SMD: −0.46; −0.84/0.08). In contrast, probing-pocket depths (SMD: −0.86; −1.55/-0.17) were significantly reduced. Data was quantitatively insufficient for conclusive findings, and risk of bias was high.
Conclusion: Current evidence is insufficient for recommending probiotics for managing dental caries, but supportive towards managing gingivitis or periodontitis. Future studies should only record bacterial numbers alongside accepted disease markers or indicators.
Clinical significance: Probiotic therapy could be used for managing periodontal diseases. For caries, further studies should ascertain both efficacy and safety.

1. Introduction

Probiotics are defined as “live microorganisms which, when administered in adequate amounts, confer a health benefit on the host” [1]. Currently, antibacterial effects (e.g. via co-aggregation, toxic by-products or competition for substrates), stabilization of the flora and modulation of the host’s immune system are thought to provide these benefits. A range of bacteria (most of them being acidogenic like lactobacilli, streptococci or bifidobacteria) have been suggested to exert one or more of these effects [2–4].

Probiotics might be beneficial to prevent or treat oral diseases like caries, gingivitis or periodontitis, which are associated with a shift in the bacterial biofilm composition and activity as well as subsequent host reactions. Potential effects of probiotic species on cariogenic or periodontal pathogens have been abundantly demonstrated in vitro [5–10]. Clinically, alterations of surrogate markers like bacterial numbers have been used to argue for the benefits of probiotic therapy. Only few studies, however, have used indicators of the diseases themselves (increment of newly developed caries lesions or caries experience; probing-pocket depths or clinical attachment loss) to prove the efficacy of probiotics for preventing or treating caries or periodontitis [11–15]. Moreover, some studies even claim probiotics to not have beneficial, but potentially harmful effects [9,16,17].

Recent systematic reviews in the field have either qualitatively summarized selected studies on either caries [3] or periodontitis [18], or meta-analyzed available surrogate markers [19]. No study so far has attempted to comprehensively display the available evidence from randomized controlled trials on effects and efficacy

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of probiotics on both caries and periodontal disease using both qualitative and quantitative synthesis. Moreover, no study investigated potential causes for heterogeneity between studies, i.e. assessed the role of potential effect modifying variables. The present study aimed to systematically review and synthesize available randomized controlled studies investigating effects of probiotics on oral caries or periodontal disease (gingivitis and periodontitis). The results of this review should be useful to guide clinical decision-making and further research in the field.

2. Materials and methods

This review follows international guidelines for performing and reporting systematic reviews [20]. The study protocol was registered after the screening stage (PROSPERO CRD42015026138). We deviated from this original protocol by only assessing outcomes at the last recorded visit, not separately after the intervention and after conclusion of follow-up. This was done, as not at all studies had a follow-up period. Moreover, periodontal pathogen numbers were analysed separately for each species (not pooled as planned). The following review question was addressed: In humans, what effects do probiotics exert on caries or periodontal disease, assessed via bacterial numbers, gingival or periodontal health, or dental caries incidence and increment, compared with placebo or alternative treatments?

2.1. Eligibility criteria

We included randomized controlled trials (RCTs) published in 1967 or later, without any language restrictions, which reported on dentate humans who consumed oral probiotics, regardless of the way of consumption or the probiotic species. Inclusion criteria were chosen that broad, as researchers and clinicians will be interested in the (comparative) efficacy of all available bacteria, not only a specific species or strain. The control intervention could have been placebo or alternative treatments (chlorhexidine, xylitol). No further specification was used to be as sensitive as possible. However, only studies allowing to determine the additional effect of probiotics were included (e.g. studies comparing probiotics plus xylitol against only xylitol were included, while those comparing probiotics plus xylitol against placebo were not). One of the following outcomes needed to be assessed: Bacterial numbers (*Streptococcus mutans* [SM], lactobacilli [LB], *Aggregatibacter actinomycemcomitans* [AA], *Porphyromonas gingivalis* [PG], *Prevotella intermedia* [PrI]); oral hygiene and gingival health (Gingiva Index [GI], Plaque Index [PI], Bleeding on Probing [BOP]), caries (caries or caries experience prevalence, i.e. DMFT/dmft > 0 or DT/dt > 0; caries experience or its increment); periodontitis (probing pocket depths [PPD], clinical attachment loss [CAL]).

2.2. Search strategy and study selection

Identification of studies was based on a search strategy for each electronic database (Cochrane Central Register of Controlled Trials, Medline via PubMed, Embase); the search was carried out on September 29th 2014 and last updated June 1st 2015. Screening procedures used a three-pronged approach without controlled vocabularies (MeSH) being used (Fig. 1). Cross-referencing from retrieved full-text studies was used to identify further articles. Neither authors nor journals were blinded to reviewers. Title and abstract of identified studies were screened independently by two calibrated reviewers (FS, DG). Calibration with regards to possible eligibility and inclusion of studies was performed on a subset of 20 studies prior to searching all databases. Consensus was obtained by discussion or consulting a third reviewer (SP).

2.3. Data collection

Data from eligible studies was independently extracted by two reviewers (FS, DG) using piloted electronic spreadsheets. Data was recorded according to guidelines outlined by the Cochrane Collaboration [21]. If data was missing, we contacted authors via e-mails, and sent reminders after 2 weeks.

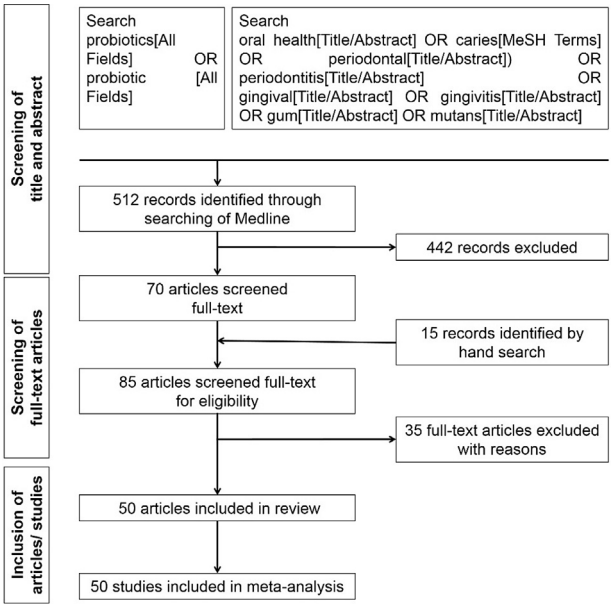

Fig. 1. Flow of the search.

2.4. Data items

The following items were recorded: Year, type and setting of study; age, size and recruitment of sample; test and control interventions including probiotic species and any pre-treatment (mechanical or chemical disinfection), vehicle, total dose (daily dose multiplied with consumption time in colony-forming units [CFU]), frequency and length of consumption; possible wash-out period in cross-over trials; follow-up; drop-out and sample size at follow-up. Outcomes were recorded as follow: bacterial numbers on ordinal or continuous scale, periodontal health and caries (experience)-increment on continuous scale, caries (experience) prevalence (e.g. DT > 0, DMFT > 0) dichotomously. Outcome data was extracted from the last follow-up visit (i.e. either after the intervention if patients were not followed further, or after conclusion of follow-up).

For ordinal records of bacterial numbers, we assessed how many patients experienced a reduction below the threshold of 10^4 CFU/ml. The latter was varied in sensitivity analyses, as we wanted to avoid to arbitrarily set a threshold. Moreover, such analyses all served to assess the effect of threshold choice. Note that none of these thresholds has a specific clinical relevance. For all other outcomes, post-intervention parameters in test and control group were compared.

2.5. Risk of bias in individual studies

Selection bias (sequence generation, allocation concealment), performance and detection bias (blinding of participants, operators, examiners), attrition bias (loss-to-follow-up and missing values or participants) and reporting bias (selective reporting, unclear withdrawals, missing outcomes) were recorded, assessed and classified according to Cochrane guidelines [21]. For cross-over studies, separate aspects (e.g. wash-out period and risk of carry-over) were additionally recorded.

2.6. Summary measures and synthesis

The unit of analysis for meta-analysis was the patient. Comparisons were only made between groups measuring the additional effect of probiotics. In studies reporting on more than two interventions, groups were combined if possible to avoid unit-of-analysis conflicts. If studies employed a factorial design (e.g. probiotic with or without fluoride, placebo with or without fluoride), comparisons between comparable groups were separately entered into meta-analysis as study subgroups. If required, control groups were divided for meta-analysis (this was only possible for count outcomes, not continuous data). Alternatively, only those groups with the largest difference between test and control were entered. Individuals in cross-over trials were treated as independent, i.e. results from the first and second period were treated as if they came from different groups of patients. Note that this ignores any within-patient correlation, for which paired analyses are recommended. This approach was chosen as paired data were not reported by most trials, and insufficient detail available to reconstruct paired estimates. The chosen approach does not introduce bias, but is overly conservative, i.e. leads to under-weighting of studies and confidence intervals being wider than when paired data were used [22,23]. Given the limited number of cross-over per all trials and the relatively large numbers of recorded outcomes, we avoided separate reporting of parallel and cross-over trials.

Meta-analysis was performed using random-effect model via Comprehensive Meta-Analysis 2.2.64 (Biostat, Englewood, NJ, USA), with Odds Ratios (OR) or Standardized Mean Differences (SMD) and 95% confidence intervals (95% CI) being calculated as effect estimates. Meta-regression was performed for comparisons with min. 10 studies included to assess the impact of pre-treatment disinfection (yes/no), total dose (in CFU), mono versus mixed species probiotic therapy, and total intervention and follow-up period (in months) on effect estimates. Missing co-variables for meta-regression were imputed by means, the effect of which was tested by sensitivity analysis. For meta-regression, the unrestricted maximum-likelihood method was used, and Bonferroni adjustments applied to correct for multiple testing [21,24]. For each outcome variable, subgroups of different bacterial genus were additionally displayed.

2.7. Consistency measures and risk of bias across studies

Heterogeneity was assessed quantitatively using Cochran's Q and I^2-statistics [25]. Funnel plot analysis and Egger test were performed to assess small study effects or publication bias for analyses with five or more trials being present [21,26]. OR or SMD were adjusted (ORa, SMDa) to check the impact of possible publication bias [27].

2.8. Evidence grading and trial sequential analysis

Evidence for estimates was graded using Grade Profiler 3.6 according to GRADE guidelines (Atkins et al., 2004). In addition, trial sequential analysis (TSA) was performed to assess if quantitative findings were robust, and to calculate the required information size (RIS), i.e. the cumulative sample size needed to yield significant differences between probiotic and control therapy [28,29]. RIS was calculated based on type I error risk of α=0.05 and a type II error risk of β = 0.20 (equivalent to a power of 0.80). For dichotomous outcomes, the control event proportion (i.e. event incidence in control group) and the relative risk reduction (RRR) were used to estimate RIS, while empirical estimates of effect and variance were used for continuous outcomes. In this review, RRR was based on an a priori defined worthwhile interventional effect of 20%, while smaller effects might well be relevant, but would increase RIS even further. RIS was adjusted for the diversity in the meta-analysis (DARIS). To assess if differences yielded by conventional meta-analysis are robust, TSA additionally estimates trial sequential monitoring boundaries (TSMB), i.e. statistical thresholds for significance which are adapted depending on the so far reached sample size. Effect estimates supported by only few small trials are thus handled stricter than those supported by large samples. Further details regarding the applied method to calculate TSMB have been reported elsewhere [30]. TSA was performed with TSA 0.9 (Copenhagen Trial Unit, Copenhagen, Denmark) [29].

3. Results

3.1. Study selection

From 512 identified studies, 85 were assessed full-text. Eventually, 50 studies, were included (Fig. 1), while 35 reports were excluded. Excluded studies and reasons for exclusion can be found in Table S1.

3.2. Study characteristics

Included studies (Table S2) were published between 2001 and 2015, and used parallel group (40), cross-over (9) or split-mouth design (1). Studies were performed in children (aged <18: 20 studies) or adults (18–65 years: 30 studies). Samples sizes ranged between 18 and 524 participants (mean: 65), overall sample size was 3247. The majority of studies used probiotic

lactobacilli [45]; other genus used were bifidobacteria (12), streptococci (2) or bacilli (1). The total dose provided range between 6.3×10^7 CFU/ml and 5.6×10^{13} CFU/ml (mean: 1.26×10^{12} CFU/ml). Most studies used mono species therapies, 20 employed mixed species. Typical vehicles were milk (7) and milk products (curd, ice cream, cheese, yoghurts: 15 studies), tablets/lozenges/candies/gum (20), or non-milk drinks or liquids (4), powders (3), straws (1), or cereals (1), Interventions lasted 2 days to 84 weeks (mean: 8 weeks); total trial duration (intervention plus follow-up) ranged between 2 days and 9 years (mean: 30 weeks).

3.3. LB and SM numbers

Most studies assessed bacterial numbers in probiotic and control group either directly after therapy or a follow-up period. Bacterial numbers for SM and LB were provided both as ordinal counts (e.g. number of patients with 10^4–10^5 CFU/ml before and after therapy) or on continuous scale (e.g. CFU/ml in test and control group after therapy). For ordinal data, the number of patients which experienced a reduction in SM or LB numbers below certain thresholds (10^4, 10^5, 10^6 CFU/ml) were calculated, and the chances of such reduction in probiotic versus control group estimated. The threshold of 10^4 CFU/ml was used as primary analysis for this outcome, as it showed greatest differences of SM and LB between test and control (Fig. 2a, b). Probiotic therapy significantly increased the chance of reducing SM numbers below 10^4 CFU/ml (OR: 2.20; 95% CI: 1.23/3.92). If analysed in subgroups of probiotic genus, only bifidobacteria seemed to significantly contribute to this beneficial effect. Heterogeneity was low, and neither graphical nor statistical assessment indicated publication bias. All but one study supporting this finding were of unclear or high risk (Fig. 2a). If the threshold was altered to patients with SM $<10^5$ or $<10^6$ CFU/ml, effects were reduced and no significant benefit detected (Fig. S1).

For LB, probiotic therapy also significantly increased the chances of reduction below 10^4 CFU/ml (OR: 2.84; 95% CI: 1.34/6.03). Here, only lactobacilli seemed to confer such significant benefit. Heterogeneity was moderate, but publication bias was suspected. Adjusting OR for this reduced the effects below levels of significance (ORa: 2.03; 95% CI: 0.88/4.70). All studies supporting this finding were of unclear or high risk (Fig. 2b). If the threshold was altered to patients with LB $<10^5$ or $<10^6$ CFU/ml, effects were reduced and, for the threshold of LB $< 10^6$ CFU/ml, no significant benefit was detected (Fig. S2).

We further assessed bacterial counts after probiotic or control therapy. SM numbers were significantly decreased in probiotic compared with control group (SMD: −1.18, 95% CI: −1.64/-0.72). If analysed separately for probiotic genus, both bifidobacteria and lactobacilli conferred such significant benefit. Overall heterogeneity was high, and publication bias suspected. All but three studies supporting these findings were of unclear or high risk of bias (Fig. 2c).

In contrast with ordinal outcomes, probiotic therapy significantly increased LB numbers compared with control (SMD: 0.33; 95% CI: 0.15/0.52). This increase was found for both bifidobacteria and lactobacilli. Across studies, there was moderate heterogeneity; publication bias was not suspected. Four studies supporting these findings had low risk of bias (Fig. 2d).

3.4. Periodontal pathogen numbers

Three studies assessed if periodontal pathogen numbers (AA, PG, PrI) differed between probiotic and control group after therapy (Fig. 3). All studies employed lactobacilli. Pooled estimates did not show any significant difference between groups for all three pathogens. Heterogeneity varied between outcomes; publication bias was not assessed given the limited number of studies. One of the three studies had low risk of bias.

3.5. Oral hygiene and gingivitis

Three measures for oral hygiene and gingivitis (BOP, PI, GI) had been compared after probiotic versus control therapy (Fig. 4). For BOP (SMD: −1.15; 95% CI: −1.68/-0.62) and GI (SMD: −0.86; 95% CI: −1.52/-0.20), significant benefits of probiotic therapy were found. For PI (SMD: −0.34; −0.89/0.21) estimates remained non-significant. Heterogeneity was high and publication bias suspected for all outcomes either statically or graphically. Adjusting the effect estimate for BOP for this possible bias did not eliminate probiotics yielding significantly reduced BOP. All except 3 studies (4 study groups) supporting these estimates showed high or unclear risk of bias.

3.6. Caries and periodontitis

Two studies compared the number of new or non-arrested lesions in probiotic and control group (Fig. 5a), with one study reporting on two subgroups which were treated as independent units. Studies showed low and unclear risk of bias, respectively. There was a homogeneous effect of benefit by probiotic therapy; this did not reach statistical significance (OR: 0.60; 95% CI: 0.35/1.04). Adjusting the effect estimated for suspected publication bias further reduced the OR.

Three, five and four studies reported on caries experience, PPD and CAL, respectively, after probiotic compared with control therapy (Fig. 5b). There was no significant benefit of probiotic therapy regarding caries experience (SMD: −0.26; 95% CI: −0.55/0.03) or CAL (SMD: −0.46, 95% CI: −0.84/0.08), but PPD (SMD: −0.86; −1.55/-0.17). For all but one outcome (CAL), heterogeneity was high. Five out of eight studies (or study groups) showed low risk of bias.

3.7. Adverse events

Overall, 25 studies stated to have monitored adverse events. Three of them did not report on such events; one reported mild gastrointestinal irritations, none reported serious adverse events.

3.8. Meta-regression

For four independent variables (total probiotic dose, disinfection prior treatment, duration of intervention plus follow-up, mixed versus mono species probiotic therapy), meta-regression analyses were performed to assess effect modification (Table S3). Only for one analysis, a significant effect after correction for multiple testing was found: mixed species therapies significantly decreased PI compared with mono species therapies ($p < 0.001$). For this variable, subgroup analyses were performed to display effects on all outcomes with 10 or more studies/study subgroups (Fig. S3). Findings were highly ambiguous: For three outcomes, mono species therapy was found to possibly provide advantages over mixed species, while for three other outcomes the opposite was the case.

3.9. Risk of bias

From all studies, 8 had low, 34 unclear and 8 high risk of bias (Table S4). Most often, allocation concealment (37), sequence generation (26) and blinding of personnel (13) were not sufficiently described or not satisfyingly performed. Cross-over

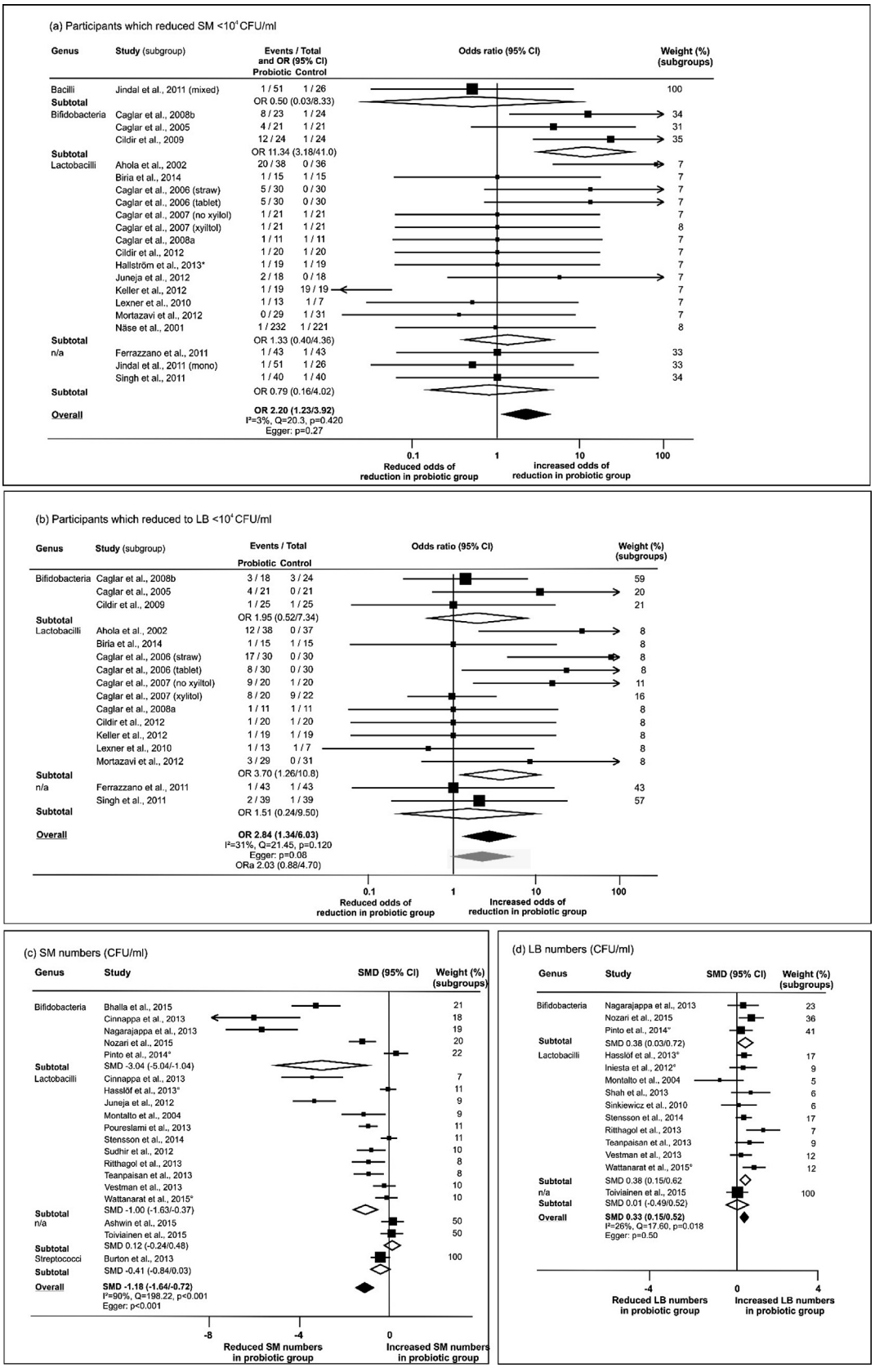

Fig. 2. Effects of probiotic therapy on SM and LB counts. (a), (b) The odds of patients reducing their SM/LB counts below 10^4 CFU/ml in probiotic and control groups are given. Events (patients with reduction $<10^4$ CFU/ml) per all patients are given. Certain studies reported subgroups of treatment, which were handled as independent studies for meta-analysis as long as unit-of-analysis issues were avoided. Results are separately shown according to probiotic genus are shown. Estimates and weights for subgroups as well as overall estimates are given (Odds ratios, 95% confidence intervals). Higher odds (>1) indicate a higher chance of reduction in SM/LB counts in probiotic versus control group. Heterogeneity across studies is indicated by I^2 and Q statistics. Egger-test was used to statistically assess publication bias. OR and 95% CI adjusted for such bias using trim-and-fill are additionally shown in grey (ORa). (c), (d) Comparative SM and LB counts (CFU/ml) as given on a continuous scale after probiotic or control therapy (at latest

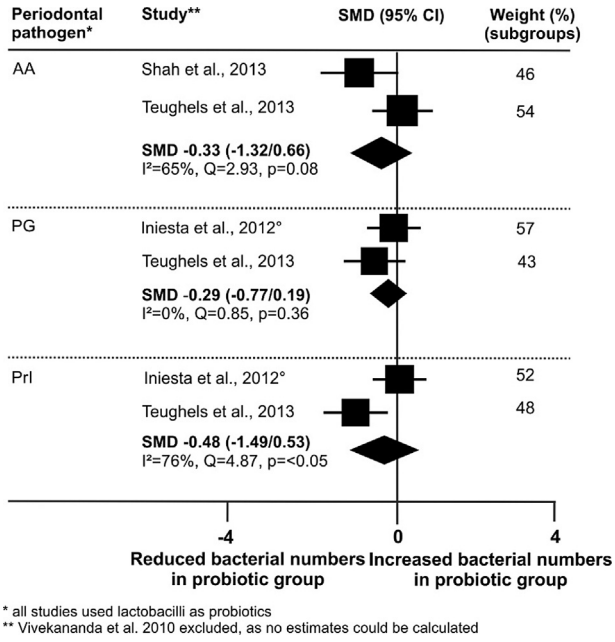

Fig. 3. Effects of probiotic therapy on numbers of periodontal pathogens. Comparative *Aggregatibacter actinomycetemcomitans* (AA), *Porphyromonas gingivalis* (PG) and *Prevotella intermedia* (PrI) counts (CFU/ml) as given on a continuous scale after probiotic or control therapy (at latest follow-up) were synthesized as standardized mean differences (SM) and 95% CI. Values below or above 0 indicate lower or higher bacterial numbers in probiotic versus control group. ◦ studies with low risk of bias.

studies used wash-out periods between 2 and 6 weeks, one study did not report such periods at all (31). The effects of risk of bias were considered serious.

3.10. Evidence grading

Calculated diversity-adjusted required information sizes (Table 1) were reached for SM numbers, and in reach for PPD, BOP, and CAL. However, our findings for caries experience and periodontal disease (PPD, CAL) were inconsistent, as were findings for numbers of SM, LB, periodontal pathogens (AA, PG, PrI), and PI. This inconsistency was considered serious. Only for SM, BOP, GI and the numbers of new/non-arrested lesions, consistency was confirmed. For all outcomes, serious imprecision was found. Publication bias was suspected for several outcomes, too. Overall, the evidence supporting probiotic therapy was graded as very low for all outcomes; the confidence in any of the calculated effect estimates is thus very limited.

4. Discussion

4.1. Review findings

This review investigated the effects of probiotic therapy on several outcomes associated with caries or periodontal disease. The most frequently assessed parameter was bacterial numbers. We found probiotics, most of all bifidobacteria, to significantly reduce SM numbers regardless if dichotomous or continuous variables were used. On the other hand, the numbers of LB after probiotic therapy were found to be increased when reported on a continuous scale. Such increase is not surprising given that most probiotics were in fact lactobacilli, and is in line with a previous meta-analysis [19]. Given the possible cariogenicity stemming from LB, their use in anti-caries therapies has been questioned, with some studies finding such treatment possibly harmful [9]. Future studies should employ more strain-specific analytic methods to evaluate if non-probiotic LB or probiotic LB were in fact increased. That studies which reported LB numbers on an ordinal scale reported the opposite, i.e. fewer individuals with LB $\geq 10^4$ CFU/ml after probiotic therapy, highlights the difficulties in many probiotic trials. First, the persistent use of surrogate markers might not add value with regards to decision-making for patient benefit, as it is unable to provide sufficiently strong evidence for any such conclusions, especially given its limited correlation with hard clinical endpoints (see below). Second, it underlines the difficulties of setting relevant thresholds for deciding which reduction (10^3, 10^4 etc.) is worthwhile or not, and the associated loss of information with any such reporting. In future studies, bacterial numbers should be reported on a continuous scale. Moreover, the validity of this surrogate parameter should be established, or they should only be measured alongside relevant outcomes.

Bacterial numbers have traditionally been measured to act as surrogate for caries, mostly based on an understanding of caries as infectious disease. For caries, all included studies reported (non-significant) beneficial effects, i.e. tended to find reduced caries or caries experience increment. This was true both in children and older populations. However, studies supporting this outcome were found prone for publication bias and of limited validity. The resulting evidence was neither qualitatively nor quantitatively sufficient for strong recommendations.

More recent studies also employed bacterial number measurements as surrogate for periodontal disease, mainly determining AA, PG and PrI. These studies also measured plaque or gingiva indices or bleeding-on-probing as parameters for gingivitis. Pooled estimates for bacterial numbers or PI (which are potentially linked to each other) did not show benefits of probiotic therapy, which has been reported before [18]. In contrast, GI and BOP, both markers of inflammation, were significantly reduced, indicating a possible effect of probiotics on host response rather than on the

follow-up) were synthesized as standardized mean differences (SM) and 95% CI. Again, subgroups and overall estimates are shown. Negative values (<0) indicate lower bacterial counts in probiotic versus control group, while positive estimates indicate higher counts in the probiotic than the test group. ◦ studies with low risk of bias. n/a no genus reported.

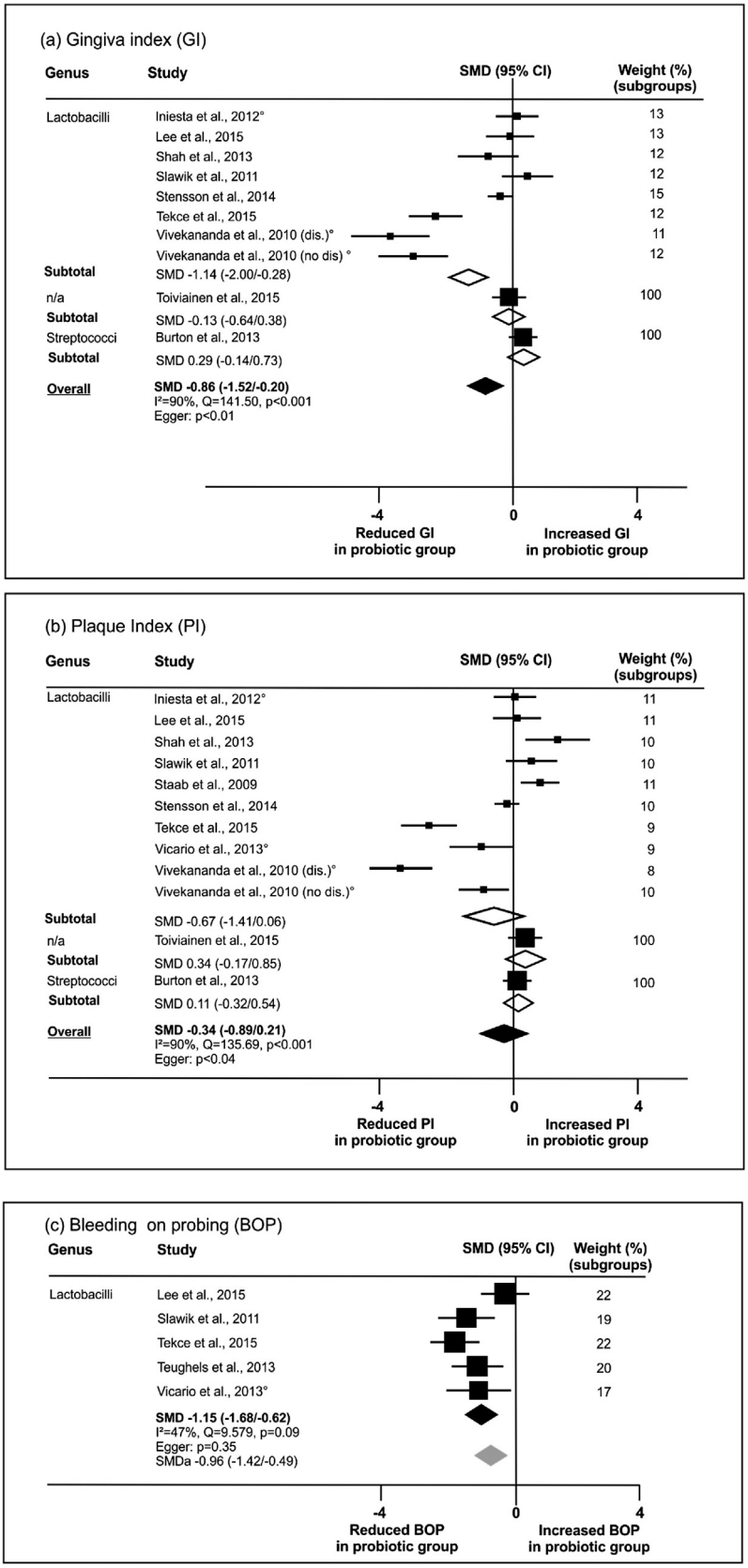

Fig. 4. Gingiva Index (GI), Plaque Index (PI) and Bleeding on Probing (BOP) after probiotic and control therapy. Standardized mean differences (SMD) and 95% CI are given; values below or above 0 indicate reduced or increased values in probiotics versus control group. One study with two subgroups (with or without disinfection prior treatment) was treated as two independent units. ° studies with low risk of bias. For PI and GI, subgroups of probiotic genus are shown. Grey diamond: effect estimate adjusted for publication bias.

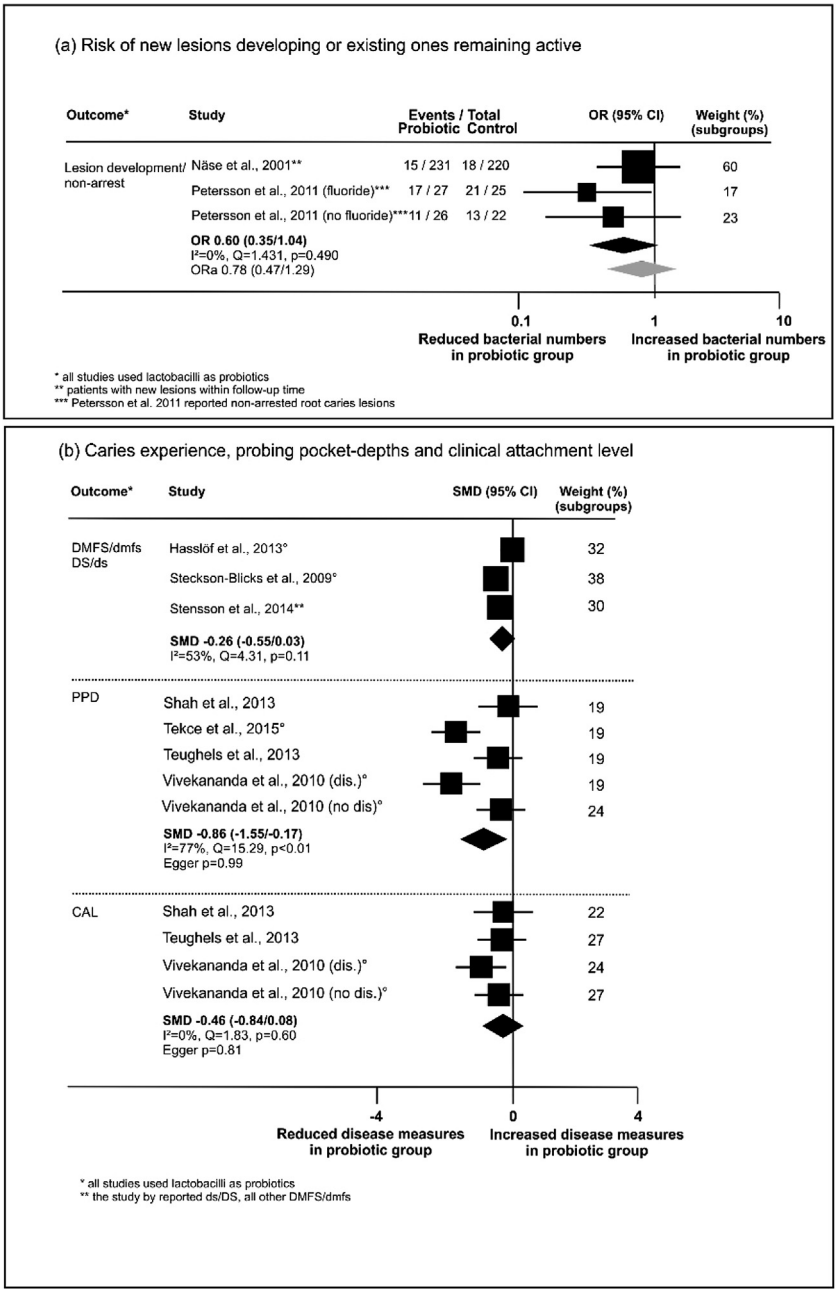

Fig. 5. Caries and periodontitis after probiotic and control therapy. (a) Caries was reported as counts by two studies, with either patients with newly developed lesions per all patients or the number of non-arrested (active) per all root caries lesions being used as estimates. One study reported different subgroups of treatments, which were handled as independent statistical units. The odds ratio (OR, with 95% confidence interval) indicates that probiotic therapy possibly reduced the risk of new lesions occurring or root lesions remaining active compared with control. Heterogeneity is presented by I^2 and Q statistics. Funnel plot indicated publication bias; adjusting the OR for this effect using trim-and-fill (ORa) reduced the beneficial effect by probiotics. (b) Caries experience, probing-pocket depths (PPD) and clinical attachment loss (CAL) after probiotic compared with control therapy were compared. Standardized mean differences (SMD) were used to compare outcomes, with SMD above and below 0 indicating in- and decreased risks of caries experience, PPD or CAL, respectively. For outcomes with $\geq$5 studies, Egger-test was additionally used to detect publication bias. Adjusting the SMD for this effect (SMDa) did not greatly affect the effect estimate for CAL: ° studies with low risk of bias; *** subgroups of the same study.

periodontal pathogens themselves. In agreement with this, PPD was also significantly reduced, as PPB are largely a function of periodontal tissue inflammation. In contrast, we found probiotic therapy to not significantly improve CAL. It might be that available studies on CAL had too limited follow-up periods to show any significant effect on this parameter, which is not as sensitive as PPD, but rather helps to record periodontal tissue loss long-term. In general, given that only few data were available to support our findings, the evidence was nevertheless graded as very low.

We evaluated how a range of plausible effect modifiers impacted on our estimates. In other disciplines, the duration of probiotic therapy and follow-up has been found such confounder [32], as has the dose [33] or the application of mono versus mixed species therapy [34]. We did not find any of the variables to clearly impact on the pooled estimates, and did not investigate further possible modifiers, like vehicle of probiotics [34] or efficacy in different risk groups [32].

4.2. Limitations

This is the first review which comprehensively assessed controlled trials on probiotics and caries and periodontal disease.

Table 1
Summary of findings.

Comparison	Relative effect (95% CI)	No of participants (studies/subgroups)	DARIS for superiority/ inferiority	Quality of the evidence (GRADE)
SM $<10^4$ CFU/ml	OR 2.20 (1.23/3.92)	1435 (21)	n.c	⊕⊖⊖⊖ very low[1,2,3]
LB $<10^4$ CFU/ml	OR 2.84 (1.34/6.03)	785 (16)	n.c	⊕⊖⊖⊖ very low[1,2,3,4]
SM numbers	SMD −1.18 (−1.64/-0.72)	1030 (19)	182	⊕⊖⊖⊖ very low[1,3,4]
LB numbers	SMD 0.33 (0.15/0.52)	724 (14)	2241	⊕⊖⊖⊖ very low[1,2,3]
AA numbers	SMD −0.33 (−1.32/0.67)	50 (2)	>10000	⊕⊖⊖⊖ very low[1,3]
PG numbers	SMD −0.29 (−0.77/0.19)	50 (2)	>10000	⊕⊖⊖⊖ very low[1,3]
PrI numbers	SMD −0.48 (−1.49/0.53)	50 (2)	>10000	⊕⊖⊖⊖ very low[1,3]
GI	SMD −0.86 (−1.52/-0.20)	421 (10)	2208	⊕⊖⊖⊖ very low[1,3,4]
PI	−0.51 (−1.10/0.07)	491 (13)	>10000	⊕⊖⊖⊖ very low[1,2,3,4]
BOP	SMD −1.15 (−1.68/-0.62)	153 (5)	208	⊕⊖⊖⊖ very low[1,3,4]
CAL	SMD −0.46 (−0.84/0.08)	110 (5)	343	⊕⊖⊖⊖ very low[1,3]
PPD	SMD −0.86 (−1.55/-0.17)	150 (6)	426	⊕⊖⊖⊖ very low[1,3]
New/active caries lesions	OR 0.60 (0.35/1.04)	551 (3)	3493	⊕⊖⊖⊖ very low[1,3,4]
Caries experience	−0.26 (−0.55/0.03)	417 (3)	>10000	⊕⊖⊖⊖ very low[1,3]

n.c. not calculated, as no worthwhile (i.e. clinically relevant) reduction available.
[1] Downgraded serious risk bias.
[2] Downgraded due to inconsistency.
[3] Downgraded due to imprecision.
[4] Downgraded due to publication bias.

To do so, a large range of outcomes and outcome measures was evaluated. For ordinal outcomes (i.e. counts of patients according to groups of pre- and post-therapy bacterial numbers), we calculated the number of patients with a reduction beneath a certain CFU threshold. This allows to account for baseline imbalances between groups, but is problematic, as different thresholds yielded different findings. Transforming CFU numbers into an ordinal variable should thus be avoided, as it increases the risk of losing information and biasing the results by selectively choosing a threshold. For continuous data, different measurement systems had oftentimes been used for the same outcome, yielding a variability in the magnitude of results. We used SMDs as effect estimates to reflect on this, measuring the difference between groups per variance. At this point, it should be noted that SMDs are difficult to interpret, as they are unit-less, and to compare between outcomes. Future studies should thus aim to report on outcomes using an agreed outcome measure, allowing to display mean differences etc. as effect estimates [35]. This is also important, as statistical differences in SMD might not necessarily translate into clinically relevant differences with regards to PPD, CAL or caries incidence, for example. Moreover, included studies were found to have limited validities. While most of them were examiner-blinded, sequence generation and allocation concealment oftentimes remained unclear. The resulting risk of over-estimating the benefits of probiotics might be limited given that most trials maintained triple-blinding. Last, we found a great heterogeneity between trials, and tried to account for this by performing subgroup and meta-regression analysis. As this only limitedly reduced heterogeneity, there might be further unobserved confounders. Future studies should strive for more homogeneous designs, and should report on trials in more detail (for example with regards to caries risk in the treated populations).

4.3. Recommendations and conclusions

Based on this review, there is currently insufficient evidence supporting the use of probiotics to manage (i.e. prevent or treat) caries or periodontal disease. However, as none of the study reported serious adverse events, and current data does not indicate increased risks of caries or periodontitis due to probiotic therapy, there is no strong argument against such treatment either. For periodontitis, sufficient evidence is in reach to upgrade the evidence level with regards to one (very frequently used) parameter, namely PPD. Similarly, indicators of gingival inflammation were found to be positively affected by probiotic therapy. More studies in this direction might thus be desirable. Alongside, the effect of vehicle or dose of probiotic treatment should be investigated more closely to deliver the most efficacious therapy. If bacterial numbers are recorded alongside validated outcomes, these should be reported on a continuous scale to avoid arbitrary choice of cutoffs and information loss. Overall, clinical studies investigating probiotics and oral health should strive for more standardized designs and more complete reporting (also with regards to caries or periodontitis risks etc.).

In conclusion, there is a relatively large and growing number of controlled trials investigating probiotic management of caries or periodontal diseases. Most of them measure surrogate markers with limited validity. Future studies should only record such surrogates alongside accepted markers or indicators of caries (like incidence or experience increment) or periodontitis (like PPD and CAL). Current evidence is insufficient for recommending probiotics for managing dental caries. A growing number of studies support probiotic therapy to prevent or treat gingivitis and periodontitis.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jdent.2016.03.002.

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