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Review Article

Methodological issues in randomized trials assessing probiotics for periodontal treatment

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Background and Objective: Probiotics traditionally used in medicine field are now being used in an attempt to control and treat periodontal disease. However, the trials used to analyze the effects of probiotics have been subject to methodological criticism. The aim of this review was to assess the methodological deficiencies in randomized controlled trials evaluating the efficacy and safety of oral administration of probiotics for the treatment of periodontal disease.

Material and Methods: A manual and electronic literature search (of MEDLINE and The Cochrane Library) was made, to March 2011, for randomized controlled trials presenting clinical, microbiological, immunological and patient-centered data for the efficacy of probiotics compared with a placebo/standard periodontal therapy for the treatment of periodontal disease.

Results: The literature search yielded only four randomized double-blind, placebo-controlled studies that evaluated the efficacy of probiotics (using *Lactobacillus reuteri* and *Lactobacillus salivarius* probiotic strains) in patients with gingivitis. The studies were too methodologically flawed (of mediocre quality) with a high risk of bias for any meaningful conclusions to be reached. These studies lacked adequate descriptions of appropriate randomization, allocation concealment, blinding, formulation and dosage of probiotic and placebo, extent and severity of periodontal disease in patient populations, patient-centered outcomes, results data and potential confounding factors.

Conclusion: The existing randomized controlled trials have important methodological limitations; consequently, there is insufficient evidence to support the efficacy of probiotics in treating periodontal disease. More rigorous scientific research, in accordance with existing guidelines and research recommendations of the present review, is required to examine the safety and efficacy of probiotics before they are embraced in periodontal therapy.

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Periodontal disease is a chronic microbial infection characterized by persistent inflammation, connective tissue breakdown and alveolar bone destruction. Periodontal diseases, along with dental caries, represent a major part of the global burden of oral diseases. Based on World Health Organization (WHO) surveys, most children have signs of gingivitis and, among adults, the initial stages of periodontal disease are highly prevalent (1). Periodontal disease has also been suggested as a risk factor for coronary heart disease, chronic kidney

disease, atherosclerosis and spontaneous preterm births (1).

In a contemporary review on future treatment strategies for periodontal diseases, Chapple (2) noted that although the mechanical approaches (surgical or nonsurgical) to periodontal therapy remain the cornerstone of successful periodontal therapy, novel adjunctive antimicrobial approaches such as probiotics and prebiotics, and photodynamic therapy, as well as one-stage full-mouth disinfection, have emerged within the scientific and clinical literature in recent years. By definition, probiotics are live microorganisms that, when administered in adequate amounts, confer a health benefit on the host (3). For controlling periodontal disease, probiotics may function by preventing the adherence of pathogenic bacteria and their establishment, multiplication and integration within the biofilm structure. Alternatively, they may induce beneficial modulatory effects upon the inflammatory-immune response that negate certain virulence strategies employed by periodontal pathogens (2). A plethora of recent reviews (1,4–10) have also stressed the future role of probiotics in periodontal therapy based on the encouraging results of clinical trials conducted to assess the efficacy of probiotics in treating periodontal patients. It is also stated that if the cascade of harmful immuno-inflammatory reactions could be reduced by probiotic intervention, then the consequences for human health in general could be substantial (1).

Regardless of how a probiotic is currently marketed, when it is intended to prevent or treat a disease or abnormal condition, it becomes a 'drug' (11). There have been calls for regulations in the clinical research and manufacturing of probiotics in the form of drugs and, consequently, few research guidelines (12–14) have been formulated recently for clinical trials of probiotics as 'drugs', in contrast to an earlier guideline for the evaluation of probiotics in food (by the joint working group of the Food and Agriculture Organization of the United Nations and the WHO) (15). Although randomized controlled trials (RCTs) remain the gold standard for evaluating the safety and efficacy of probiotics (12), methodological issues may affect the reliability of such studies. A recent systematic review (16) also concluded that there is an urgent need for properly conducted clinical trials where probiotics are used as adjuncts to standard periodontal care.

In view of the uncertainty regarding the use of probiotics in general and in particular for the treatment of periodontal disease, the objective of this review was to assess the methodological issues in published reports of RCTs that aimed to assess the efficacy and safety of using probiotics for the treatment of periodontal disease. Thus, the review aimed to highlight the deficiencies in the existing research data (in the form of compliance with existing research guidelines) and to provide recommendations for future research, leading to the possibility of evidence-based therapy with probiotics.

Material and methods

Search strategy for identification of studies

The PubMed (MEDLINE) database of the US National Library of Medicine and The Cochrane Library of the Cochrane Collaboration (CENTRAL) were utilized as the electronic databases and a literature search was accomplished on articles published in English from 1990 to March 2011. Articles available online in electronic form before their publication in material form (according to the so-called 'Epub ahead of print' or 'early online articles') were considered eligible for inclusion in this review. The electronic search was carried out by applying the MeSH terms (Table 1). In addition, a hand search (up to March 2011) of the following journals was performed: *Journal of Periodontology*, *Journal of Clinical Periodontology*, *Journal of Periodontal Research*, *Molecular Oral Microbiology*, *Oral Diseases* and *Periodontology 2000*. The reference lists of review articles were scanned and the reference lists of articles selected for inclusion in the present review were screened.

Table 1. Search terms for MEDLINE and CENTRAL databases

#1 Search 'Probiotics'[MeSH]
#2 Search 'Periodontal diseases' [MeSH]
#3 Search 'Gingivitis' [MeSH]
#4 Search 'Chronic periodontitis' [MeSH]
#5 Search 'Aggressive periodontitis' [MeSH]
#6 Search 'Periodontitis' [MeSH]
#7 Search 'Randomized controlled trials' [MeSH]
#8 Search 'Therapy' [MeSH]
#9 Search 'Dental scaling' [MeSH]
#10 Search 'Root planing' [MeSH]
#11 Search 'Tablets' [MeSH]
#12 Search 'Placebos' [MeSH]
#13 Search '*Lactobacillus*' [MeSH]
#14 Search '*Bifidobacterium*' [MeSH]
#15 Search '*Streptococcus*' [MeSH]
#16 Search '*Bacillus*' [MeSH]
#17 Search #2 OR #3 OR #4 OR #5 OR #6
#18 Search #1 AND #7 AND #8 AND #17
#19 Search #9 AND #10
#20 Search #11 OR #12
#21 Search #13 OR #14 OR #15 OR #16
#22 Search #1 AND #17 AND #20 AND #21
#23 Search #8 AND #19 AND #22
Data search date: 31 March 2011

Selection criteria for studies

Reports were included only if the study design was identified as an RCT, if it was published as a full-text article, if blinded evaluation was implemented and if the effect (clinical, microbiological, immunological and patient-centered outcomes) of oral administration of probiotic was compared with administration of a placebo or standard periodontal therapy in the treatment of gingivitis and chronic and aggressive periodontitis. Case series, uncontrolled studies and articles published as abstract only, editorials, news and correspondence sections were excluded.

Quality and risk of bias assessment

In order to improve the scientific evidence for usage of probiotics as drugs for the treatment of various diseases, a few research guidelines (12–14) have recently been formulated. According to Tamayo (12), trials of probiotics for the treatment of disease need to be standardized with regard to their methodological quality, protocol design, selection of population and product

characterization. Also, clinical trials seldom report adverse effects and may lack the power or duration to identify them.

In addition, Hoffman *et al.* (13) suggested that validated methods in preclinical studies, such as the use of *in vitro* and *in vivo* animal models of disease, may provide important new data and surrogate markers of both the safety and efficacy of probiotics. These may translate into better designed phase 1 studies of healthy subjects and patients, as well as increasing the success rate of phase 2 and phase 3 clinical studies (13). Phase 3 clinical trials should compare the efficacy of the investigational product against that of a placebo, the best available treatment, or both (12). There is also an important need to assess how the probiotic properties of adherence, agglutination, and up- and down-regulation of cytokines relate to and/or affect the maintenance of health or therapeutic effects (13). One key issue that needs to be addressed is the establishment of standards for recognizing and reporting adverse events, particularly the detection of invasive infection caused by probiotics (13). Other factors that may affect the outcomes of probiotic use, including comorbid conditions, composition of the diet and concomitant use of medications, also need to be explored (13).

More recently, Rijkers *et al.* (14) presented guidelines for conducting and evaluating research on probiotics at the workshop 'Guidance for assessing probiotics beneficial effects: how to fill the gap,' held in Switzerland in 2008 and organized by the International Life Sciences Institute (Europe) in association with the International Dairy Federation. These guidelines included the following.

(i) Identification of the tested strain with description of the food matrix or probiotic carrier as well as an indication of manufacturing process of the probiotic product being tested in clinical trials.
(ii) Conducting human intervention studies according to good clinical practice, which include monitoring and reporting on confounding factors, for example, ingestion of other potentially active microorganisms, dietary components or drugs, or a lifestyle that may interfere with the explored benefit. A protocol (similar design, number of subjects and duration) should be used that discriminates between an active and an inactive strain for that specific benefit.
(iii) Harmonized criteria and expression of results: meta-analyses should be used to distinguish between (clusters of) active and nonactive strains on a given benefit and in compiling data on a given probiotic for a specific benefit.
(iv) A defined target population, to detect the benefit efficiently and to allow extrapolation to the general population.

In accordance with the aforementioned research guidelines (12–14) and revised recommendations of the Consolidated Standards of Reporting Trials (CONSORT) (17) statement for evaluation of RCTs, a 17-item quality assessment checklist was formulated to target the methodological issues in RCTs assessing probiotics for treating periodontal disease. This checklist was further subdivided into primary quality criteria (including internal and external validity study characteristics) and secondary quality criteria, as follows:

(i) Primary quality criteria:
1 clear objectives and methodology of the proposed study (12);
2 appropriate study population with clearly defined extent and severity of periodontal disease (12,14);
3 randomization to treatment or control (including description of the method of random allocation/allocation concealment) (17);
4 masked assessment of outcome (including measures for success of masking) (17);
5 diet, nutrition and other confounding factors accounted for in the study population (13,14);
6 clearly stated background studies of probiotic strain used (*in vitro* and animal experiments, and safety-assessment studies) (13,14);
7 details of the formulation and dosage of the probiotic and placebo administered (12,14);
8 adequate duration of the study and of the follow-up period (14);
9 evaluation of patient-centered outcomes and patient satisfaction regarding the treatment in terms of compliance and comfort;
10 evaluation of adverse effects (12,13);
11 sample-size calculation (12,17);
12 completeness of follow up (with reasons for any withdrawals and dropouts in each study group) (17);
13 intention-to-treat analysis (17);
14 retrievable result data (14).
(ii) Secondary quality criteria:
15 publication in a peer-reviewed journal;
16 statement of compliance with regulatory authorities;
17 statement regarding possible conflicts of interest.

Each quality criteria carried a score of 1 point (yes)/0 point (inadequate or unclear or no) for a possible total of 17 points. Studies were graded as:

(i) high quality: all primary and secondary criteria were met;
(ii) high moderate quality: > 7 and < 14 primary criteria and all secondary criteria were met;
(iii) low moderate quality: > 7 primary criteria were met and ≥ 1 secondary criteria were not met;
(iv) poor quality: ≤ 7 primary criteria and all secondary criteria were met;
(v) very poor quality: ≤ 7 primary criteria were met and ≥ 1 secondary criteria were not met.

After quality assessment according to The Cochrane Handbook for Systematic Reviews of Interventions (18), an overall estimate of plausible risk of bias (low, uncertain or high) was performed within each study and across all selected studies based on the six domains (i.e. sequence generation; allocation concealment; blinding of participants, personnel and outcome assessors; incomplete outcome data; selective outcome reporting; and other potential threats to validity) with ratings of 'Yes' (low risk of bias), 'No' (high risk of bias) and 'Unclear' (uncertain risk of bias). A 'low' risk of bias (plausible bias unlikely to seriously alter the results) was estimated within a study when there was a low risk of bias for all key domains and

was estimated across all studies when most information was from studies at low risk of bias. An 'unclear' risk of bias (plausible bias that raises some doubt about the results) was considered within a study when there was unclear risk of bias for one or more key domains and was considered across all studies when most information was from studies at low or unclear risk of bias. A 'high' risk of bias (plausible bias that seriously weakens confidence in the results) was estimated within a study when there was a high risk of bias for one or more key domains and was estimated across all studies when the proportion of information from studies at high risk of bias was sufficient to affect the interpretation of the results.

Results

Study selection and description

The MEDLINE and CENTRAL search provided 48 and 0 hits, respectively. The hand search revealed three articles, which were added to this step. Thus, the initial literature search resulted in a total of 51 articles. Seventeen articles appeared to be double publications and were therefore excluded. The remaining 34 articles were screened based on their title and abstract. Five (19–23) of these were excluded because they were not in English. One *in vitro* study (24), three animal studies (25–27) and two clinical studies (28,29) without placebo groups were excluded. In addition, two pilot studies (30,31), a nonrandomized trial (32) and an open-label pilot trial (33) were also excluded. Finally, 15 literature review articles were excluded.

After the first step of title and abstract screening of 34 articles, only four randomized, double-blind, placebo-controlled studies (34–37) remained. These four RCTs fulfilled the inclusion criteria and were thus selected for inclusion in the present review. The study data were extracted from these trials, including specific details about the interventions, populations, study methods and outcomes of significance to the review question and specific objectives (Tables 2 and 3).

Quality and risk of bias assessment of selected studies

The results of quality assessment of all the selected studies (34–37) are presented in Table 4. The two studies by Shimauchi *et al*. (35) and Mayanagi *et al*. (37), conducted at the same center and on the same group of patients, received a quality score of 11 (high moderate quality). There was a high risk of bias within these studies because there was no re-inclusion of missing data in the analyses and incomplete reporting of one or more outcomes of interest for conducting meta-analyses (Table 5).

The third study, by Krasse *et al*. (34), received a quality score of 7 (very poor quality). This study had an estimated high risk of bias as there was incomplete reporting of one or more outcomes of interest for meta-analysis and no disclosure of possible conflicts of interest (Table 5).

The fourth study, by Twetman *et al*. (36), received a quality score of 12 (high moderate quality). There was an estimated high risk of bias in this study as attrition in each intervention group was not reported (Table 5).

The overall risk of bias was estimated to be 'high' for all four studies (Table 5) because the proportion of information from studies at high risk of bias was sufficient to affect the interpretation of the results.

Discussion

Probiotics or health-beneficial bacteria have recently been introduced in dentistry and oral medicine after years of successful use in mainly gastrointestinal disorders (8). Studies reporting the anticariogenic effects of probiotics, and their use in the treatment of periodontal disease, halitosis and *Candida albicans* infection have been identified in the literature (9). Although the currently available data indicate an effect of probiotics on the oral microbiota and a more limited effect on clinical periodontal outcome measures, one needs to be careful in the interpretation of this data owing to the low quality of the probiotic trials (16). The present review attempted to highlight the methodological issues affecting probiotic trials in periodontics. For this, a manual and electronic search was performed, up to a defined period of March 2011, to explore the randomized, blinded clinical trials that evaluated the clinical, microbiological, immunological, adverse and patient-centered outcomes following probiotic administration (compared with placebo/standard periodontal therapy) in either gingivitis or periodontitis patients.

Using the above-mentioned search strategy and inclusion criteria, only four randomized, double-blind, placebo-controlled studies (34–37) were retrieved for inclusion in the present review. These RCTs assessed the clinical, microbiological and immunological effects of oral probiotic administration to patients with gingivitis. Among these, Krasse *et al*. (34) observed a significant reduction in the gingival index and the plaque index in patients treated with *Lactobacillus reuteri* chewing gums compared with the placebo group and concluded that *L. reuteri* was efficacious in reducing both gingival inflammation and plaque in patients with moderate to severe gingivitis. Next, Shimauchi *et al*. (35) found that oral administration of tablets containing *Lactobacillus salivarius* WB21 resulted in significantly greater improvement of the plaque index and probing pocket depths and in a significant reduction of salivary lactoferrin levels in smoker subjects. Using the same probiotic tablets, Mayanagi *et al*. (37) observed a significant reduction in periodontopathic bacteria in subgingival plaque samples. Furthermore, Twetman *et al*. (36), using *L. reuteri*-containing chewing gum, found a significant reduction in crevicular fluid volume, cytokine levels (tumor necrosis factor-α and interleukin-8) and bleeding on probing. In these trials, no adverse effects were noted, except in one study (34), which reported increased bowel movements in one of the study patients.

The four RCTs included were subjected to quality and risk of bias assessments in the present review. The assessment of the methodological quality of a trial is essential because

Table 2. Experimental characteristics of randomized controlled trials of probiotics ($n = 4$) included in the present review

Author and year	Study design	Population	Inclusion criteria	Study duration (in wk)	Probiotic administered	Comparator (drug/therapy) used
Krasse *et al.* 2006 (34)	Parallel, double-blind, placebo-controlled prospective randomized	Total 59 patients LR-1 group: 20 (10 men, 10 women) mean age 55.9 years; LR-2 group: 21 (9 men, 12 women) mean age 51.7 years; Placebo group: 18 (10 men, 8 women) mean age 50.7 years	Moderate or severe gingivitis; gingival index score of 2–3; no ongoing antibiotic treatment	2	*L. reuteri* (LR) formulations (LR-1 and LR-2) of human origin, each containing 100 million CFU of live bacteria; formulated as chewing gum; given twice daily for 14 d	Placebo (chewing gum), identical in shape, texture and taste to probiotic; given twice daily
Shimauchi *et al.* 2008 (35)	Parallel, double-blind, randomized, placebo-controlled	66 healthy volunteers Test group: 29 men and 5 women (mean age 45 years) Placebo group: 28 men and 4 women (mean age 44.8 years)	Smokers and nonsmokers; no current dental treatment; not using probiotic supplements; free from adverse reactions to lactose or fermented milk products; antibiotics not taken within last month; probing pocket depth < 6 mm and absence of excess tooth mobility and/or abscess formation (i.e. without severe periodontitis)	8	Three tablets containing *L. salivarius* WB21 with xylitol (2.01×10^9 CFU/d and 840 mg/d, respectively); to be placed in the mouth and allowed to dissolve without chewing, daily, for 8 wk	Three placebo tablets containing xylitol only (840 mg/d); to be placed in the mouth and allowed to dissolve without chewing, daily, for 8 wk
Twetman *et al.* 2009 (36)	Parallel, double-blind placebo-controlled	42 healthy adults (16 women and 26 men; mean age 24 years)	Presence of at least two buccal marginal sites with moderate chronic gingival inflammation; probing depth < 4 mm; no habitual use of probiotic food; nonsmoker; nonpregnant women; no history of antibiotics and/or anti-inflammatory drugs in past mo; no low stimulated saliva secretion (< 0.8 mL/min) and rampant caries	4	Chewing gums containing two strains of *L. reuteri*: ATCC 55730 and ATCC PTA 5289 (1×10^8 CFU/gum, respectively). Group A/P – one active and one placebo gum, Group A/A – two active chewing gums, Group P/P – two placebo gums. Probiotic gums to be chewed for 10 min daily for 2 wk	Placebo gum identical in size and composition but without addition of probiotic strains; to be chewed for 10 min daily for 2 wk
Mayanagi *et al.* 2009 (37)	Parallel, randomized, double-blinded, placebo-controlled	66 healthy volunteers Test group: 29 men and 5 women (mean age 45 years) Placebo group: 28 men and 4 women (mean age 44.8 years)	Smokers and nonsmokers; no current dental treatment; not using probiotic supplements; free from adverse reactions to lactose or fermented milk products; antibiotics not taken within last month; probing pocket depth < 6 mm and absence of excess tooth mobility and/or abscess formation (i.e. without severe periodontitis)	8	Tablets containing *L. salivarius* WB21 (6.7×10^8 CFU/tablet) and xylitol (280 mg/tablet) Subjects instructed to place one tablet in the mouth and allow it to dissolve without chewing, three times a day for 8 wk	Placebo: xylitol 280 mg tablets. Subjects instructed to place one tablet in the mouth and allow it to dissolve without chewing, three times a day for 8 wk

ATCC, American Type Culture Collection; CFU, colony-forming units; LR, *Lactobacillus reuteri*; *L. reuteri*, *Lactobacillus reuteri*; *L. salivarius*, *Lactobacillus salivarius*; PTA, patent deposit accession number.

Table 3. Changes in outcomes assessed following probiotic administration in the randomized controlled trials ($n = 4$) included in the present review

Author and year	Clinical results	Microbiological results	Immunological results	Patient-centered outcomes	Adverse events
Krasse *et al.* 2006 (34)	▲GI LR-1: ? LR-2: ? Placebo: ? ▲PI LR-1: ? LR-2: ? Placebo: ?	–	–	–	Only one patient in the LR-2 group reported increased bowel movements
Shimauchi *et al.* 2008 (35)	▲PI Test group (smokers and nonsmokers): ? Placebo group (smokers and nonsmokers): ? ▲GI Test group (smokers and nonsmokers): ? Placebo group (smokers and nonsmokers): ? ▲BOP Test group (smokers and nonsmokers): ? Placebo group (smokers and nonsmokers): ? ▲PPD Test group (smokers and nonsmokers): ? Placebo group (smokers and nonsmokers): ?	–	▲Salivary lactoferrin levels Test group (smokers and nonsmokers): ? Placebo group (smokers and nonsmokers): ?	–	No adverse events
Twetman *et al.* 2009 (36)	▲BOP (percentage of positive sites) A/P: 1 wk 31; 2 wk 46*; 4 wk 31 A/A: 1 wk 35; 2 wk 50*; 4 wk 43 P/P: 1 wk 10; 2 wk 23; 4 wk 04	–	Mean values (pg/mL) ▲IL-1β ▲TNF-α ▲IL-6 ▲IL-8 ▲IL-10 1 wk A/P 0.4 0.09 0.25 14 0.4 A/A 4.3 0.27* 0.96 17.1 0.05 P/P 3.4 0.02 0.07 24.4 0.4 2 wk A/P 1.4 0.18 0.16 21 0.4 A/A 4.6 0.17 0.95 25.9* 0.02 P/P 1.1 0.06 0.38 21.5 0.08 4 wk A/P 0.3 0.01 0.07 18.5 0.08 A/A 0.8 0.08 1.16* 2.5 0.05 P/P 0.5 0.05 0.5 0.6 0.12	–	No adverse events
Mayanagi *et al.* 2009 (37)	–	Levels of individual and sum of five bacteria: *Aggregatibacter actinomycetemcomitans*: ? *Prevotella intermedia*: ? *Porphyromonas gingivalis*: ? *Treponema denticola*: ? *Tannerella forsythia*: ?	–	–	No adverse events

▲, change in parameter; ?, results could not be deciphered because they were depicted in graphs, not tables; *, statistically significant difference compared with baseline ($p < 0.05$).
A/A, two active chewing gums; A/P, one active and one placebo gum; BOP, bleeding on probing; GI, gingival index; IL, interleukin; LR, *Lactobacillus reuteri* formulation; PI, plaque index; P/P, two placebo gums; PPD, probing pocket depth; TNF, tumor necrosis factor.

Table 4. Quality assessment of included studies ($n = 4$)[a]

Author and year	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	Quality score and rating
Krasse *et al*. 2006 (34)	X	X								X		X	X		X	X		7 (very poor quality)
Shimauchi *et al*. 2008 (35)	X		X	X	X		X	X		X		X			X	X	X	11 (high moderate quality)
Twetman *et al*. 2009 (36)	X	X	X	X	X					X	X	X		X	X	X	X	12 (high moderate quality)
Mayanagi *et al*. 2009 (37)	X		X	X	X		X	X		X		X			X	X	X	11 (high moderate quality)

X, criteria fulfilled.

[a]Studies that fulfilled the criteria of: (1) clear objectives and methodology of the proposed study; (2) appropriate study population with clearly defined extent and severity of periodontal disease; (3) randomization to treatment or control (including description of method of random allocation/allocation concealment); (4) masked assessment of outcome (including measures for success of masking); (5) diet, nutrition and other confounding factors accounted for in the study population; (6) clearly stated background studies of probiotic strain used (*in vitro* and animal experiments and safety-assessment studies); (7) details of formulation and dosage of probiotic and placebo administered; (8) adequate duration of the study and follow-up period; (9) evaluation of patient-centered outcomes and patient satisfaction regarding the treatment in terms of compliance and comfort; (10) evaluation of adverse effects; (11) sample-size calculation; (12) completeness of follow-up (with reasons for any withdrawals and dropouts in each study group); (13) intention-to-treat analysis; (14) retrievable result data; (15) publication in a peer-reviewed journal; (16) statement of compliance with regulatory authorities; (17) statement regarding possible conflicts of interest.

quality can considerably influence the scientific outcomes and clinical interpretation of the research (38). During quality assessment, two different issues are considered viz., methodological quality of the trial (which relates to its internal and external validity) and the reporting quality (which concerns the reporting of the research design, conduct and data analysis) (38). Opinions differ among researchers regarding combining the reporting quality and the methodological quality of the trials for their overall quality assessment. Some argue that well-conducted trials may be reported badly and discrepancies may occur between the study protocol and the resulting publication (39–41). More appropriately, others state that because the only instrument available to readers for assessing the quality of a trial is the published manuscript, RCT reports must provide an accurate information about the trial design, how it was conducted and data analysis (38,42). In accordance with the latter views, the quality checklist in this review included assessment of both reporting and methodological quality of the RCTs.

The results of quality and risk of bias assessment showed that the included RCTs had 'very poor' (34) to 'high moderate' (35–37) quality and were often poorly reported. Sources of bias and variation were present in all the studies, and important criteria for determining the presence of bias were often either not mentioned or unclearly reported. Thus, although the results were statistically significant, the inherent methodological limitations of these studies warrant their conclusions to be interpreted with great caution. The inconclusive studies also did not allow conclusions to be reached regarding whether any particular probiotic is more effective than another.

The components of the quality checklist and Cochrane Collaboration's tool (18) for risk of bias assessment, which were not addressed in these studies, are as follows:

(i) Appropriate study population with clearly defined extent and severity of periodontal disease. This was not fulfilled by two studies (35,37) because they incorporated patients with the diagnosis of 'without severe periodontitis'. The mean periodontal probing depth was 2.5 ± 0.1 mm in the subjects (probiotic group) and 2.4 ± 0.2 mm in the placebo group, while the gingival index was 0.8 ± 0.1 in the probiotic group and 0.7 ± 0.1 in the placebo group at baseline. Thus, from these clinical parameters, it is apparent that the subjects had mild to moderate gingivitis, which should have been clearly described in these studies.

(ii) Randomization to treatment or control. Although the study by Krasse *et al*. (34) was a 'randomized' clinical trial, the randomization process was not described in detail (i.e. sequence generation and allocation concealment).

(iii) Masked assessment of outcome. Even though the study by Krasse *et al*. (34) was a 'double-blinded' clinical trial, the blinding process was not described in terms of blinding of participants and key study personnel and evaluation of the success of blinding (the randomization code was not broken until data analysis was complete).

(iv) Accounting for diet, nutrition and other confounding factors in the study population. Factors such as history/current use of antibiotic and anti-inflammatory drugs in the previous month, consumption of probiotic supplements, adverse reactions to lactose or fermented milk products and smoking habit may affect the outcomes of probiotic use. However, the study by Krasse *et al*. (34) did not include these factors when recruiting subjects.

(v) Clearly stated background studies of the probiotic strain used (*in vitro* and animal experiments and safety-assessment studies). None of the selected RCTs included a description of the background studies of the probiotic strain used in the study. Mentioning the background studies and method of formulation of the probiotic strain being tested lends more transparency to clinical research, as studies involving the administration of probiotics are often supported by the manufacturers, with the possibility that background data (with positive or negative results) may not be

Table 5. Risk of bias assessment of included studies ($n = 4$) determined using Cochrane Collaboration's tool (18)

Author and year	Sequence generation	Allocation concealment	Blinding	Incomplete outcome data	Selective outcome reporting	Other potential threats to validity	Estimated risk of bias within study	Estimated risk of bias across all studies
Krasse *et al.* 2006 (34)	Unclear (insufficient information to permit judgment of 'Yes'/'No')	Unclear (insufficient information to permit judgment of 'Yes'/'No')	Unclear (insufficient information to permit judgment of 'Yes'/"No")	Yes (no missing data)	No (one or more outcomes of interest were reported incompletely so they cannot be entered in a meta-analysis)	No (no disclosure of possible conflicts of interest which may lead to biased data interpretation)	High (high risk of bias for one or more key domains)	High (proportion of information from studies at high risk of bias is sufficient to affect the interpretation of the results)
Shimauchi *et al.* 2008 (35)	Yes (random allocation using a randomization table)	Yes (allocation concealment using sequentially numbered drug containers of identical appearance)	Yes (blinding of participants and key study personnel ensured, and not broken until completion of data analysis)	No (no re-inclusion of missing data in analyses)	No (one or more outcomes of interest were reported incompletely so they cannot be entered in a meta-analysis)	Yes (study appears to be free of other sources of bias)	High (high risk of bias for one or more key domains)	
Twetman *et al.* 2009 (36)	Yes (random allocation using computer-generated random numbers)	Yes (allocation concealment using sequentially numbered drug containers of identical appearance)	Yes (blinding of participants and key study personnel ensured, and not broken until completion of data analysis)	No (attrition in each intervention group not reported)	Yes (study protocol is available and all of the study's prespecified primary and secondary outcomes of interest have been reported in a prespecified way)	Yes (study appears to be free of other sources of bias)	High (high risk of bias for one or more key domains)	
Mayanagi *et al.* 2009 (37)	Yes (random allocation using a randomization table)	Yes (allocation concealment using sequentially numbered drug containers of identical appearance)	Yes (blinding of participants and key study personnel ensured, and not broken until completion of data analysis)	No (no re-inclusion of missing data in analyses)	No (one or more outcomes of interest were reported incompletely so they cannot be entered in a meta-analysis)	Yes (study appears to be free of other sources of bias)	High (high risk of bias for one or more key domains)	

provided to the researchers before testing probiotic products in clinical trials. This may create a potential conflict of interest, which needs to be checked. Also, the probiotic strain used in clinical trials needs to be supported by the results of efficacy in background studies, as the effects of the probiotics are considered to be strain specific (15). Thus, mentioning the results of the clinical studies with different strains or species of probiotic bacteria as a background/motivation for the new probiotic trial seems to be inappropriate and unjustified.

(vi) Details of formulation and dosage of probiotic and placebo administered. These are important to provide transparency to clinical research. The probiotic strain used was not mentioned in the study by Krasse *et al*. (34), whereas Twetman *et al*. (36) did not provide the composition of the placebo used in their study.

(vii) Adequate duration of the study and the follow-up period. The studies of Krasse *et al*. (34) and Twetman *et al*. (36) were of short-term duration (2 and 4 wk, respectively).

(viii) Evaluation of patient-centered outcomes and patient satisfaction regarding the treatment in terms of compliance and comfort. Patient-centered outcomes are 'true endpoints' (43) that measure tangible benefits of the intervention to the periodontal patients in terms of a reduction in common subjective symptoms such as discomfort/pain (44) and bleeding on brushing (43). None of the included RCTs assessed the patient-centered outcomes. Assessment of compliance and comfort following an intervention constitutes patient-centered care and measures patient satisfaction regarding the intervention. Compliance of the patients with the administered probiotic was reported in only one study – that of Twetman *et al*. (36).

(ix) Sample-size calculation. Large samples are necessary to detect small differences between the groups in terms of outcomes assessed (17) and to identify any adverse effects (12). Sample-size calculation was reported in only one study – that of Twetman *et al*. (36).

(x) Intention-to-treat analysis. This involves including and analyzing all randomized patients according to their original treatment allocation, irrespective of whether they actually received that treatment (17). Except for the study by Krasse *et al*. (34) (all patients were analyzed with no reported exclusions), none of the other studies analyzed the outcomes in the patients excluded/attrited after the randomization process.

(xi) Retrievable result data. Mentioning the results of the outcomes assessed should ideally be depicted in tables with actual figures of changes of parameters compared with baseline values. Except for the study by Twetman *et al*. (36), other studies (34,35,37) depicted the results of the outcomes assessed in the form of graphs, making it difficult to decipher the changes in the clinical, microbiological and immunological parameters. Consequently, it is not possible to statistically pool their results to conduct a meta-analysis. Inadequate reporting of the results of outcomes also resulted in an estimated 'high' risk of bias being introduced in these studies (34,35,37).

(xii) Statement regarding possible conflicts of interest. This holds value because the financial interests of authors or sponsors may lead to biased data interpretation. The study by Krasse *et al*. (34) was the only study that did not report any conflicts of interest and consequently there was an estimated 'high' risk of bias in this study.

(xiii) Incomplete outcome data. The studies by Shimauchi *et al*. (35) and Mayanagi *et al*. (37) did not re-include the missing data in the analyses, while the study by Twetman *et al*. (36) did not report attrition in each intervention group, thus leading to the introduction of attrition bias in these three studies.

Apart from the above shortcomings, most studies included did not address the issue of the 'Hawthorne effect'. The improvement seen in clinical parameters may also be attributed to the subject's improved oral hygiene as a response to the oral hygiene instructions and the anticipation of forthcoming oral examination at intervals during the study (i.e. the Hawthorne effect) (45). Even though oral hygiene instructions were given before starting the probiotic intervention in the studies of Krasse *et al*. (34) and Twetman *et al*. (36), the influence of the Hawthorne effect on the improvement of clinical parameters was not described. On the other hand, the study by Shimauchi *et al*. (35) accounted for the influence of the Hawthorne effect on clinical parameters because of altered oral hygiene regimens of the subjects as a result of observation.

The shortcomings, listed above, in probiotic trials conducted in the field of periodontics are consistent with the previous observations made by Tamayo (12), who noted that in general, probiotic trials suffer from numerous shortcomings and deficiencies, such as: small sample size; lack of appropriate randomization, allocation concealment, or blinding; different periods of treatment and different doses; lack of product characterization; ill-defined patient populations; lack of data on the etiology and severity of disease; and potential confounding factors. Moreover, at present, few RCTs have investigated the efficacy of probiotics for treating periodontal disease. This may represent few submissions or a lack of interest in the topic on the part of journals (13). Finally, the lesser quality of these published RCTs could reflect inconsistent review policies for probiotic research among mainstream peer-reviewed journals (13).

The search strategy of the present review did not reveal any randomized, placebo-controlled clinical trial on the

Table 6. Research recommendations in EPICOT format (49) for probiotic trials

	Issues to consider	Recommendations
Core elements		
E (Evidence)	What is the current evidence?	Inadequate evidence owing to low quality of probiotic trials
P (Population)	Diagnosis, disease stage, comorbidity, risk factor, sex, age, ethnic group, specific inclusion or exclusion criteria, clinical setting	Patients with gingivitis, chronic or aggressive periodontitis, with or without systemic diseases, belonging to any sex or ethnic group, with controlled diet, nutrition and other confounding factors
I (Intervention)	Type, frequency, dose, duration, prognostic factor	Probiotic containing bacteria (minimum of 10^6 colony-forming units) from strains of *Lactobacillus*, *Bifidobacterium*, *Streptococcus* and *Bacillus* species, in the form of lozenges/tablets/chewing gums
C (Comparison)	Placebo, routine care, alternative treatment/management	Placebo or scaling and root planing (in a randomized controlled trial with a parallel/split-mouth design)
O (Outcome)	Which clinical or patient-related outcomes will the researcher need to measure, improve, influence or accomplish? Which methods of measurement should be used?	Evaluation (blinded assessment) of clinical parameters, microbiological (subgingival microflora), immunological (biomarkers) and patient-centered outcomes following probiotic administration in multicentered trials with clearly stated background studies of probiotic strain used (*in vitro* and animal experiments and safety-assessment studies), formulations of probiotic and placebo along with their administration protocol and evaluation of adverse effects (if any)
T (Time stamp)	Date of literature search or recommendation	March 2011

administration of probiotics in patients with chronic/aggressive periodontitis. Furthermore, there is an absence of clinical trials comparing the effects (clinical/microbiological/immunological) of probiotic therapy alone or as an adjunct with standard periodontal therapy. Although it has a different mechanism of action (mechanical disruption of biofilm), scaling and root planing can be considered as the standard therapy for comparison with probiotics in future trials, because scaling and root planing remains the 'gold standard' in periodontal therapy as a result of its proven benefits of positive changes in various clinical parameters (probing depth, attachment levels, bleeding on probing and gingival inflammation) and control of subgingival bacterial populations (46). Also, the effect of scaling and root planing has been tested on various biomarkers of periodontal health and disease (47,48).

One of the limitations of this review was the consideration of only English language articles published in peer-reviewed journals. As the number of studies was small it was difficult to draw conclusions on publication bias. It is also possible that trials were missed, although the two databases searched are known to be comprehensive regarding clinical trials. Moreover, in the present review, the electronic literature search was supplemented by reviewing references from a variety of sources to retrieve any missing trials.

Research recommendations

Using the 'EPICOT' format (49), the need for future research would be considered in this review in the context of the research evidence (E), the ideal population (P) of the study, the manner in which the intervention (I) should be administered, the optimal comparison (C) of interest, the outcomes (O) that researchers should measure and the time (T) stamp of their recommendations. Thus, based on identified research gaps in probiotics trials in the present review, recommendations for future research for probiotic trials for treating periodontal disease were formulated based upon the EPICOT format, as described in Table 6.

Concluding remarks

Although many guidelines and a systematic review (16) have been published on this subject, this review is the first to highlight the methodological issues raised by trials assessing the use of probiotics in periodontal therapy. The existing randomized placebo-controlled clinical trials are methodologically deficient with incomplete assessment of relevant outcomes and reporting of data regarding the probiotic strain used. Therefore, the data published to date do not provide sufficient scientific evidence to support a general recommendation on the use of probiotics in the treatment of periodontal disease. Given the critical lack of adequate evidence in probiotic research, rigorous RCTs using clinically relevant outcomes and standardized measures to examine the effectiveness of probiotics vs. placebo control or standard periodontal therapy (i.e. scaling and root planing) are needed to determine whether or not this approach can replace or complement standard periodontal therapy. The existing guidelines for conducting probiotic research and evaluating probiotic products in clinical trials (12–14), the quality checklist of the present review, along with the newly formulated CONSORT 2010 statement for reporting parallel group randomized trials (50), may help in better conductance and reporting of probiotic trials.

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