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The role of probiotic bacteria in managing periodontal disease: a systematic review

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ABSTRACT

Introduction: The frequent recolonization of treated sites by periodontopathogens and the emergence of antibiotic resistance have led to a call for new therapeutic approaches for managing periodontal diseases. As probiotics are considered a new tool for combating infectious diseases, we systematically reviewed the evidences for their effectiveness in the management of periodontitis.

Areas covered: An electronic search was performed in the MEDLINE, SCOPUS and Cochrane Library databases up to March 2016 using the terms 'periodontitis', 'chronic periodontitis', 'probiotic(s)', 'prebiotic(s)', 'symbiotic(s)', '*Bifidobacterium* and '*Lactobacillus*'. Only randomized controlled trials (RCTs) were included in the present study. Analysis of 12 RCTs revealed that in general, oral administration of probiotics improved the recognized clinical signs of chronic and aggressive periodontitis such as probing pocket depth, bleeding on probing, and attachment loss, with a concomitant reduction in the levels of major periodontal pathogens. Continuous probiotic administration, laced mainly with *Lactobacillus* species, was necessary to maintain these benefits.

Expert commentary: Oral administration of probiotics is a safe and effective

adjunct to conventional mechanical treatment (scaling) in the management of periodontitis, specially the chronic disease entity. Their adjunctive use is likely to improve disease indices and reduce the need for antibiotics.

Key words: periodontal diseases, periodontitis, probiotics, *Lactobacillus*, *Streptococcus*, inflammation, dental hygiene.

1. INTRODUCTION

Periodontal disease is perhaps the commonest human affliction. Inflammation of the periodontium, periodontitis, is a chronic inflammatory disease with a complex aetiology, caused mainly by Gram-negative bacteria that destroys supporting structures of the tooth viz, the gingivae, periodontal ligament and alveolar bone [1]. Severe periodontitis can result in tooth mobility caused by bone resorption, and may lead to loss of the affected tooth [2].

Periodontal pathogens, besides causing tooth loss, may have systemic impact through a variety of mechanisms. These include bacteremia caused by the translocation of periodontal pathogens into the systemic circulation, and endotoxaemias due to the lipopolysaccharides of the periodontopathogenic bacteria. Additionally the production of interleukins generated through the chronic inflammatory process has been linked to atherosclerotic vascular disease [3], adverse pregnancy outcomes, diabetes and nosocomial pneumonias [4]. *Porphyromonas gingivalis*, *Treponema denticola*, *Tannerella forsythia* and *Aggregatibacter actinomycetemcomitans* have all been implicated as the major bacterial agents of periodontitis [5]. However, recent metagenomic studies suggest that a yet to be identified, diverse range of periodontitis-associated microbiota may be involved in the disease process [6].

The conventional treatment of periodontitis entails non-surgical management aimed at controlling the pathogenic plaque biofilm and calculus burden from the coronal and root surfaces by mechanical procedures (scaling), together with improved personal hygiene, thus reducing inflammation and pocket depths, and promoting periodontal reattachment [2,7]. In severe cases, antibiotic therapy may be required to facilitate disease resolution [8].

Such therapeutic approaches are not always associated with success and the frequent recolonization of treated sites by periodontopathogens, as well the emergence of antibiotic resistance, have led to a call for new therapeutic approaches for managing periodontal diseases [9]. One such approach that has recently gained universal popularity is probiotic therapy. Probiotics seem to be an alternative biological approach to modify, at least in the short term, the periodontal plaque biofilm and help control periodontitis [10].

Probiotics are defined as 'live microorganisms that, when administered in adequate amounts, confer a health benefit on the host' [11]. The most common probiotics belong to two main genera *Lactobacillus* and *Bifidobacterium* [12]. *L. acidophillus* and *L. rhamnosus* have been associated with antibacterial as well as antifungal effects [13,14], while *L. reuteri* and *L. salivarius* are known to suppress both cariogenic [15] and periodontal pathogens [16-18].

In dentistry, probiotics have been employed as useful adjuncts for the reduction of caries development [19], suppressing oral *Candida* infections [20], and controlling halitosis [21]. Recent publications have also demonstrated the potential benefit of probiotic administration for managing periodontal diseases, especially periodontitis [16,22]. For instance, in one study, probiotic supplements together with conventional mechanical treatment led to clinical improvement of periodontitis [17], similar to

improvements due to antibiotic administration [23]. These and related new findings have led to a totally new paradigm for managing periodontal infections.

Therefore, the aim of this systematic review was to explore the available clinical evidence on the efficacy of probiotic therapy in managing chronic periodontitis. PICOS criteria were adopted in this systematic review (Table 1).

2. MATERIAL AND METHODS

2.1 Protocol and registration

The systematic review was registered in the International Prospective Register of Systematic Reviews 'PROSPERO'; Registration number is: CRD42015020560 [24].

2.2 Eligibility criteria and search strategy

A critical review of the literature was conducted to select pertinent articles published in the literature. An advanced mode electronic search was performed in the MEDLINE (PubMed), SCOPUS and Cochrane Library databases up to March 2016. The search covered all human clinical trials conducted from the year 2000 onwards, wherein the effect of probiotic bacteria on periodontitis was evaluated. The literature search strategy in the PubMed database was performed using the following terms: 'chronic periodontitis OR periodontitis' AND 'probiotic OR probiotics OR prebiotic OR prebiotics OR symbiotic OR symbiotics OR *Bifidobacterium* OR *Lactobacillus*'. The search terms applied to Cochrane Library were 'probiotic' and 'periodontitis'. The search strategy in the Scopus database used the search terms "probiotic" and 'periodontitis', excluding Reviews, Book Chapters and Books. In addition, bibliographies of the selected articles were manually searched.

2.3 Selection criteria

The inclusion criteria were:

- Studies testing probiotic bacteria in the management of periodontitis;
- Humans studies;
- Randomized controlled clinical trials (RCTs).

The exclusion criteria were:

- Absence of information regarding the methodology of the clinical study;
- Reviews and studies on animals;
- Studies devoid of data on clinical parameters of periodontitis and/or associated periodontal pathogens.

2.4 Article review and data extraction

The study selection process was according to PRISMA guidelines [25], as illustrated in Figure 1. Two examiners (V.H.M. and K.H.I) screened the titles and abstracts obtained through the described search strategy. The screening was performed according to the following criteria: randomized clinical trials and clinical and/or microbiological outcome. Full reports were obtained for all the studies that were considered eligible for inclusion in the review. Discrepancies and doubts were resolved in the first instance through data check and discussion. When there was no consensus, a third examiner was consulted. In the absence of pertinent data, the authors of the identified articles were contacted to provide further details. Kappa values for measuring agreement between two authors were equal to 0.89 ($p<0.001$), thus reflecting an almost perfect inter-author agreement.

Data extracted from the randomized clinical trials (RCTs) included the year of publication, the names of authors, the study design, the population demographics, the probiotic bacterial strains used and their mode of administration such as the frequency and duration, and the follow-up procedures, other associated treatment modes, the adverse effects, if any, the key parameters studied, and finally, the outcome measures.

2.5 Risk of bias assessment

A quality assessment of the studies included in the present review was

performed as per the recommendations of the Cochrane Collaboration for systematic reviews of interventions [26]. The quality assessment focused on the following criteria: random sequence generation and allocation concealment (selection bias), blinding of participants and personnel (performance bias), blinding of outcome assessment (detection bias), incomplete outcome data (attrition bias) and selective reporting (reporting bias).

The risk of bias of each study was categorized according to the following criteria: low risk of bias, unclear risk of bias, and high risk of bias.

3. RESULTS

3.1 Study selection

The systematic search yielded 214 studies pertinent to the current review. After excluding duplicate references, a total of 192 articles/studies were screened, and each of the titles and abstracts were scrutinized for selection fitness. A total of 157 studies were excluded during the latter screening phase leading to 35 residual studies that were deemed fit for further analysis.

These 35 studies were further filtered and 23 excluded (Figure 1). A total of 12 studies were included and analyzed in the final review (Table 3).

3.2 Study characteristics

All randomized clinical trials selected were double-blinded studies, except for the study of Shah et al. [23]. The study of Della Ricca et al. [27] was classified as double-blinded by the authors, however its veracity appeared questionable as no placebo group was used. A total of ten studies were placebo-controlled [16-18,22,28-33], whilst the remainder were paired-comparison studies [23,27]. Both parallel [16,17,22,28-30,33] and split-mouth designs [29] were identified within the RCTs.

Four different *Lactobacillus* species, i.e. *Lactobacillus reuteri* in five studies [16,17,22,28,29], *Lactobacillus salivarius* in three studies [18,30,31], *Lactobacillus brevis* in two studies [23,27] and *Lactobacillus rhamnosus* in a single study [33] were evaluated for their probiotic effect against periodontitis. A single study also evaluated the effect of a probiotic product with three different streptococci belonging to *Streptococcus oralis*, *S. uberis*, and *S. rattus* species [32]. The organisms were orally administered either as lozenges, tablets, sachet or as suspensions in soybean oil from four days to 12 weeks. In nine of twelve studies, the probiotic was self-administered

orally and the probiotic concentrations varied between $2.0x10^7$, $2.0x10^8$, $4.0x10^8$ and $2.1x10^9$ colony forming units (CFUs) per day.

In terms of the mode of administration, the patients were asked to keep the probiotic tablets in the mouth and dissolve without chewing [32], whilst in the case of the lozenges the patients were asked to suck the lozenges after they brushed their teeth [22]. When the probiotic-containing sachet was used, the patients were instructed to dissolve the content of one sachet in water and ingest it after brushing their teeth [33]. In one study evaluating the efficacy of probiotic suspended in soybean oil, patients were instructed to place the product in their mouths and spread it over the surface of the oral mucosa and teeth using tongue movements [18].

3.3 Risk of bias within studies

The Cochrane Collaboration's tool for assessing the risk of bias in randomized trials was used to identify papers with intrinsic flaws in methodology and design [34], thus ascertaining the quality of each study. As shown in Table 2, all studies had a random sequence generation, using computer-based randomization programs, block approach or tables. However, the allocation concealment was not totally clear in four studies [18,23,27,30]. The studies of Della Riccia et al. [27] and Shah et al. [23] also had an unclear risk of bias in blinding of patients, as well as the researchers, and outcome assessment. One study was classified as having an unclear risk of bias for the "incomplete outcome data" parameter [28], as the authors did not report the reason/s for excluding patients data from the analysis.

3.4 The effect of probiotics in periodontitis

The clinical outcome measures that were used to evaluate the effect of probiotic bacteria in treating periodontal diseases, included probing pocket depth (PPD),

gingival recession (REC), gingival index (GI), gingival bleeding index (GBI), plaque index (PI), bleeding on probing (BOP) and clinical attachment (CAL), as well as quantitative and qualitative analysis of the burden of intraoral periodontal pathogens.

Of the five studies that compared the mechanical periodontal treatment alone or associated with probiotic administration in managing chronic periodontitis [16,17,22,32,33], two studies [16,22] showed higher reduction of PPD, BOP, GI and PI in the probiotics groups ($p<0.05$) during a long-term follow up period of up to 360 days. In the study of Laleman et al. [32], only PI was significant lower ($p<0.05$) after mechanical procedures and probiotic administration compared with the control group, at 24-weeks. In turn, Teughels et al. [17] found a significant reduction in full-mouth PPD after administration of two strains of *L. reuteri* (DSM17938 and ATCC PTA5289), as well as an associated significant gain in CAL after 12 weeks of follow up, in lesions with moderate to deep pockets ($p<0.05$). The PI and BOP were both consistently lower in the probiotic group, although these differences were statistically significant only in a few occasions over the time course of the clinical trial. The single trial testing *L. rhamnosus* (SP-1) found no difference in the clinical improvement between the probiotic and the control groups [33]. However, in the same study, at initial visits and after 1-year follow up, the probiotics induced a statistically significant ($p<0.05$) reduction in number of patients with PPD $\geq$ 6mm, reducing the need for surgery, compared with the placebo group. [33] .

Only one of 12 selected studies evaluated the effect of probiotics in aggressive periodontitis [23]. Here, they also performed scaling and root planning prior to *L. brevis* and/or antibiotic administration. Their results indicated that either probiotic alone, probiotic plus antibiotic, and antibiotic alone were able to reduce significantly ($p<0.05$) the PPD, GI, PI and CAL after 2 months of follow up.

A total of five studies [18,27,28,30,31] tested the effect of probiotics without prior mechanical periodontal treatment, in patients with periodontitis. Della Riccia et al. [27] detected a highly significant reduction in gingival inflammation, calculus levels and temperature sensitivity after treatment with *L. brevis* containing lozenges ($p<0.0001$). Additionally, all patients with gingival inflammation rated as moderate/diffuse before treatment, showed no signs of gingivitis after probiotic administration. For instance, bleeding on probing had an impressive reduction from 1.38 ± 0.19 to 0.05 ± 0.05 ($p<0.0001$) after probiotic therapy. Similarly, Vicario et al. [28] also showed a significant ($p<0.05$) improvement of periodontal parameters (PPD, PI, BOP) after 30 days of treatment with two *L. reuteri* strains (ATCC 55730 and ATCC PTA5289).

Suzuki et al. [18] found a significant reduction of BOP, but not in PPD, after the application of *L. salivarius* WB21 containing soybean oil in comparison to the placebo ($p=0.010$). Incidentally, they noted, a significant increase in the volume of stimulated salivary flow after two weeks of probiotic consumption ($p<0.05$). On the contrary, Shimauchi et al. [31] were unable to show a significant improvement in periodontal health in terms of clinical parameters between the test group (*L. salivarius* WB21/xylitol) and the placebo group (xylitol alone). However, they described a significant improvement in PI and PPD within the cohort of current smokers in the probiotic group in comparison to those in the placebo group [31].

As opposed to the aforementioned studies, Vivekananda et al. [29] performed a split mouth study, in which the effects of *L. reuteri* strains (DSM17938 and ATCC PTA5289) were tested in quadrants with or without prior scaling and root planning (SRP), within the same subject. They noted a maximum reduction of PPD, CAL indices in the group that had a combination therapy with SRP and the probiotic

($p<0.001$). The probiotic alone also showed better results than SRP alone for three parameters, PI, GI and GBI.

A total of seven studies assessed microbiological parameters to evaluate the effect of probiotics on the oral burden of periodontal pathogens [16-18,23,29,30,32]. In one study, the proportion of obligate anaerobes was reduced after the probiotic treatment ($p<0.005$), although this difference could not be maintained after a 12-months follow up period [16]. In another study by Teughels et al. [17], *L. reuteri* administration was shown to promote a large and significant reduction in *Porphyromonas gingivalis* numbers in subgingival and supragingival environment, as well as in saliva over a 12-week period [17]. In one more study using *L. salivarius*, the burden of *Tannerella forsythia* tended to decrease ($p=0.073$) after probiotic consumption [18]. The same group found that the number of *Prevotella intermedia* increased in the placebo group ($p=0.045$), but did not change in the group receiving *L. salivarius.* Vivekananda et al. [29] also noted a beneficial effect of probiotics, either alone or in combination with the mechanical treatment, in reducing *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, and *Prevotella intermedia* in relatively deep pockets of 5-7 mm ($p<0.01$). On the other hand, Shah et al. [23] found no significant reduction of *A. actinomycetemcomitans* counts in saliva after treatment with *L. brevis*.

The study evaluating *L. salivarius* in combination with xylitol in tablet formulations [30] assessed the effect of the probiotic at 4- and 8-weeks after two-months intervention period. The authors reported a significant numerical decrease in five selected periodontal pathogens (*A. actinomycetemcomitans*, *P. intermedia*, *P. gingivalis*, *T. forsythia*, and *Treponema denticola*) in the subgingival plaque after probiotic administration at the four-weeks time point ($p=0.012$). Individually, a

significant reduction in *T. forsythia* at both time points (four- and eight-weeks) was found in the subgingival plaque of the probiotic group (four-weeks $p<0.001$, and eight-weeks $p=0.006$).

Only one of the 12 selected studies, used streptococci as probiotics for managing periodontal disease [32]. In that study, Laleman et al [32] using three different strains belonging to *Streptococcus oralis*, *S. uberis*, and *S. rattus* species found no significant difference in the PPD, REC, CAL, BOP and GI after probiotic therapy. However, the percentage of sites with dental plaque was significantly lower ($p<0.05$) in the probiotic group than in the control group 12 weeks after the end of the treatment, and also the level of *P. intermedia* levels in the periodontal pockets was significantly reduced after therapy.

3.5 Compliance and adverse effects of probiotics

No adverse effects of probiotic administration were reported in the RCTs included in this review. Positive patient compliance with probiotics administration was noted in seven studies [16,17,28-30,32,33].

4. DISCUSSION

The mainstay of the management of periodontal disease is the reduction and elimination of specific periodontal pathogens (periodontopathogens/pathobionts) by debridement which includes scaling and root planning [27]. Antimicrobial agents can also be used in conjunction with mechanical procedures to reduce the pathogenic microbial burden and provide a satisfactory clinical outcome particularly in chronic situations [35,36]. However, the frequent recolonization of treated sites by periodontal pathogens and recurrence of disease, and the emergence of antibiotic resistance in these pathobionts have led to calls for new approaches for the management of periodontitis, especially chronic periodontitis that affects millions of people worldwide.

One rather radical or innovative approach that has been attempted during the last few decades is to manage a number of infectious diseases through administration of probiotics, so that the disease causing pathogens are eliminated, promoting the development of a healthy flora, thus leading to restoration of health [20,37,38]. Consequently, there has been an increasing interest in the use of probiotics for oral infections [39], including periodontal diseases. In this systematic review, we attempted to collate the available clinical and microbiological evidence on the utility of probiotics in the management of chronic periodontitis.

As this is a relatively new area of study, only a handful of randomized clinical trials that fitted the well-defined exclusion and inclusion criteria were found. On analysis of the data from the twelve selected studies it is safe to conclude that three *Lactobacillus* species, i.e. *L. reuteri*, *L. salivarius*, and *L. brevis* that have been selected as probiotics improved the clinical parameters of periodontal disease in the

targeted patient cohorts. However, the same beneficial results could not be noticed with *L. rhamnosus* SP-1 and the *Streptococcus* species, demonstrating the species specificity of probiotics in managing periodontitis. The reduction in probing pocket depth and clinical attachment loss, the two most common clinical parameters of periodontitis [40], were significantly reduced after administration of all three *Lactobacillus* probiotics. Gingival and plaque index [41], and bleeding on probing [42] also improved due to the probiotic therapy in most of the studies.

Clearly, due to the lack of consensus on probiotic administration procedures, a number of protocols varying in dosage, frequency and duration have been used in the different studies. These include frequency of tablet, lozenge or suspension administration that varied from one [28] to four times [27] per day, and the intervention period ranging from four days [27] to 12 weeks [16,22,32]. Although not comparable, these variables did not seem to significantly influence the outcome, as the PI, BOP and gingival inflammation improved in all patient cohorts reported. Nevertheless, it is important in future studies to agree on a standardized protocol, such as the period, the dosage and the frequency of probiotic administration so as to yield broadly comparable data.

The range of *Lactobacillus* and *Streptococcus* concentrations used in the 12 studies evaluated is noteworthy. Depending on the frequency and concentration of bacteria in the probiotic product, patients received suspensions ranging from 2-4 x 10^8 CFU/day of *L. reuteri* and *L. brevis* to 1.2-2.0 x 10^9 CFU/day of *L. salivarius*, 2 x 10^7 CFU/day of *L. rhamnosus* and 6.0 x 10^8 CFU/day of *Streptococcus* spp. Concentration of the probiotic in the products was not specified in three studies [16,22,27]. The PPD of patients receiving probiotic therapy was significantly reduced ($p<0.05$) in two different studies that used *L. reuteri* at a concentration of 2 x 10^8 [28]

and 4 x 10^8 CFU/day [17]. On the other hand, in the most recent study where *L. rhamnosus* was tested at a low concentration (2 x 10^7 CFU/day), no significant differences were noted between the test and control groups for PPD, CAL, PI and BOP [33]. Thus, it is difficult to surmise the optimal concentration of the probiotic dose and further research is warranted to ascertain the ideal dose profile for the improvement of clinical parameters of periodontitis.

Mechanical periodontal treatment prior to probiotic intervention was another variation of the protocols used in five studies [16,17,22,29,33]. The initial periodontal therapy included scaling and root planning with an ultrasonic scaler and hand instruments. The adjunct probiotic therapy after debridement led to improvement in clinical parameters including PPD and CAL compared with the control population, which did not receive probiotics, but only SRP [22,29]. Such attachment gain in moderate and deep pockets, and the consequent reduction of pocket depth obviates the necessity for surgical intervention and it is tempting to suggest that this may be a further benefit of the probiotic therapy [17,33].

Microbiological parameters were used as outcome measures in seven of the twelve studies. A total of six studies showed the capacity of probiotics to reduce the aggregate number of periodontal pathogens when probiotic therapy and mechanical procedures were either combined or not [16-18,29,30,32]. These observations are supported in *in vitro* investigations that demonstrated the antimicrobial activity of lactobacilli against *A. actinomycetemcomitans*, *P. gingivalis*, *P. intermedia* and *Prevotella nigrescens* [43,44].

The effect of probiotic therapy in combination with xylitol was investigated by two groups [30,31]. Xylitol, a naturally occurring a low-calorie pentose sugar

substitute, is known for its ability to suppress growth and metabolism of cariogenic mutans streptococci in particular [45]. However, a little known effect of xylitol is its ability to launch an anti-inflammatory response in macrophages infected with the periodontopathogen *P gingivalis* [46,47]. Shimauchi et al. [31] evaluated this phenomenon, and they did not find any significant improvement in clinical parameters of periodontitis between the control group (placebo + xylitol) and the test group (probiotic + xylitol). On the other hand, Mayanagi et al. [30] noted a greater reduction in *T. forsythia* in the subgingival plaque after treatment with probiotic and xylitol in comparison with xylitol alone, despite the claims of its antimicrobial effect.

The mechanisms underlying the observed salutary effect of probiotics in reducing the burden of periodontopathic organisms as reported by several investigators is unclear, as yet. As per the ecological plaque hypothesis [48], the ability of probiotics to tip the overall composition of the periodontal biofilm in favor of commensals and eliminate the dysbiosis caused by periodontopathogens (the so called `red complex bacteria`) could be one reason for such observations. Other possible mechanisms of probiotic action may include the competitive inhibition of adhesion sites, and nutrient depletion [49,50]; modulation of the immune system [22,51]; modulation of cell proliferation and apoptosis [52]; production of antimicrobial substances, such as lactic acid, hydrogen peroxide and reuterin [49,53]; and modulation of the pH and/or the oxidation–reduction potential of the plaque biofilm [9] (Figure 2). Some examples of the above could be gleaned from the evaluated studies. For instance, Ince et al. [22] reported a reduction of matrix metalloproteinase-8 (MMP-8) levels in the gingival crevicular fluid in chronic periodontitis after probiotic treatment. Active MMP-8 is the main host cell-derived collagenase that leads to periodontal tissue destruction and it is mostly found in sites

with progressive periodontitis [54]. An increased level of tissue inhibitor of metalloproteinases-1 (TIMP-1), which has inhibitory effect against all MMPs, was also noted in patients treated with probiotic [22].

L. brevis possesses arginine-deaminase that metabolizes arginine to citrulline and ammonia [27]. The competition inhibition of nitric oxide synthase (NOS) by arginine-deaminase may cause the probiotic to suppress nitric oxide generation, an important inflammatory mediator in periodontitis [55]. Indeed, Della Riccia et al. [27] in their studies noted that the probiotic treatment was associated with a significant decrease in the NOS activity. The variation of all these inflammation-associated markers strengthens the evidence of the anti-inflammatory capacity of probiotics. Also noteworthy is the study of Shimauchi et al. [31], which showed that probiotics significantly decreased the salivary lactoferrin levels in smokers with chronic periodontitis. Lactoferrin is a member of the transferrin family of iron-binding proteins and its high salivary concentration is associated with periodontal inflammation [56].

As regards safety, probiotics are traditionally considered to be safe for human consumption with negligible adverse side effects [57]. None of the RCTs reported adverse effects, such as stomach upset, gastrointestinal disorder, headache and nausea/vomiting [28]. This is a critical requirement if probiotics are proposed as an adjunct in the management of periodontal disease, particularly, because they need to be continuously administered for prolonged periods for the maintenance of a healthy periodontal flora.

To conclude, our systematic review indicates that the oral administration of probiotics is an effective adjunct in the management of periodontitis, reducing

periodontopathogenic bacteria and improving the clinical signs of the disease, although strain specificity of the probiotic appears to be important for the observed beneficial effects. Clinical improvement was achieved with three *Lactobacillus* species, (*L. reuteri, L. salivarius*, and *L.* brevis), but not by a *Streptococcus* strains and *L. rhamnosus*. The mechanisms underpinning the effect of probiotics in alleviating periodontal infection is unclear as yet, although it is highly likely that probiotics play a critical role in nurturing a healthy gingival flora and deterring the colonization of the periodontal niche by the periodontopathogens. Further studies are warranted in order to confirm the findings of the relatively few, appropriately controlled studies available in the literature, as probiotic therapy could be an attractive supplementary adjunct for traditional therapies in managing periodontal disease.

5. EXPERT COMMENTARY

Periodontal diseases are arguably the commonest human affliction. Of the periodontal diseases, periodontitis, a chronic inflammatory disease that compromises supporting structures of the tooth (gingivae, periodontal ligament and alveolar bone), is particularly insidious due to its slow relentless, silent and chronic progression leading to eventual tooth and bone loss and deteriorating the overall quality of life of the affected patient.

The aetiology of periodontitis is complex and involves polymicrobial interrelationships that disrupt the fine balance of the oral microbiome associated with periodontal tissue homeostasis. Periodontal disease development is intimately connected to a shift from a symbiotic dental biofilm, composed mostly of facultative anaerobic bacteria (e.g. *Actinomyces* and *Streptococci*), to a dysbiotic microbial

community of anaerobic microbes from the phyla essentially belonging to Spirochaetes, Bacteroidetes, Firmicutes, Proteobacteria, and Synergistetes.

Periodontal pathogens, besides causing tooth loss, may adversely affect the systemic health of patients. Bacteremias, atherosclerotic vascular disease, adverse pregnancy outcomes, diabetes and nosocomial pneumonias have all been linked to periodontal pathogens and their bioproducts. Hence, maintaining the homeostasis of the oral microbiome, as well as the conversion of a diseased, dysbiotic microbiome to a symbiotic state is critically important to maintain oral and systemic health.

The conventional treatment modalities of periodontitis include non-surgical management, which entails debridement of the pathogenic, dysbiotic biofilm from the tooth surfaces by mechanical procedures, such as scaling, together with improved oral hygiene regimens, and in some cases accompanied by antibiotic therapy. However, the high incidence of recurrence of periodontitis caused by frequent recolonization of treated sites by periodontopathogens, and the adverse side effects of antibiotics, including the potential emergence of resistant organisms, have led to a call for new therapeutic approaches for managing periodontal diseases.

Consequently, the use of probiotic bacteria has emerged as an alternative therapeutic technique for infectious diseases in the oral cavity. Probiotics are live microorganism able to combat pathogens without being harmful to the host. To be considered a probiotic, the microorganism needs to be of human origin, preferably isolated from the human gut, and devoid of intrinsic and transmissible antibiotic resistance genes. The safety of probiotic bacteria has been clearly demonstrated in all the clinical trials included in the present review, as no adverse effects were reported by the patients during probiotic therapy.

Bacteria belonging to the genera *Lactobacillus* have been traditionally used as probiotics for many decades. Now, they are deemed worthy as an alternative biological approach to combat periodontopathogens, as shown by the randomized controlled trials included in this systematic review.

Probiotic bacteria, especially lactobacilli, were effective adjunct for treating periodontal disease, particularly when combined with mechanical removal of pathogenic biofilms. Emerging data reviewed here indicate that adjunctive use of specific probiotic supplements leads to significant amelioration of disease indices (probing pocket depth, gingival index, plaque index, bleeding on probing, and clinical attachment level), and reduces the need for antibiotics and surgery procedures. These clinical improvements were closely associated with significant alterations in the microbial profile of the periodontal pocket, reducing the burden of traditional periodontopathogens, such as *Porphyromonas gingivalis*, *Tannerella forsythia*, *Prevotella intermedia*, and *Aggregatibacter actinomycetemcomitans*. However, it was disappointing to note that only a few attempted to investigate the mechanisms underlying the action of probiotics against oral pathogens. This area is worthy of further exploration and may yield clues to microbial behavior in the oral ecosystem that could be exploited for further therapeutic purposes in future.

To conclude, the data presented in this systematic review conclusively imply that probiotic bacteria can be used as an adjuvant to conventional treatment of periodontitis with no side effects on the host. They may augment the antimicrobial effect of mechanical plaque removal, reducing the necessity for surgical intervention, and replace antibiotics in the management of human periodontal infections in future.

6. FIVE-YEAR VIEW

The clinical efficacy of probiotic administration in the war against periodontal disease and its perpetrating bacterial flora will gain popularity based on underlying scientific evidence. The mechanisms of action of probiotics against oral pathogens will be better understood, nurturing further the development of commercial probiotic products and their use to combat oral diseases such as periodontitis.

7. KEY ISSUES

- Periodontitis is one of the commonest human afflictions leading to tooth loss, but may also affect the systemic health by increasing the patients' risk for atherosclerotic vascular disease, adverse pregnancy outcomes, diabetes and nosocomial pneumonias.
- Adverse side effects of antibiotics, including the potential emergence of resistant organisms, limit their routine and long term use in managing the chronic periodontal diseases. The frequent recolonization of treated sites by periodontopathogens is also a clinical dilemma.
- Of the 12 clinical trials selected (from 214) in this systematic review, through a rigorous filter procedure, all trials that used *Lactobacillus* species as probiotics yielded favorable clinical outcomes in terms of a reduction of the conventional disease indices such as probing pocket deep (PPD), bleeding on probing (BOP), gingival index (GI) and plaque index (PI), with probiotic administration and concomitant scaling and root planning (conventional mechanical treatment).
- One randomized controlled trial testing *Steptococcus* spp. as a probiotic showed no significant difference between the probiotic and the placebo groups regarding PPD, BOP, GI and clinical attachment level (CAL), although *P.*

intermedia counts were reduced in the probiotic group after 12 weeks of treatment.

- In some studies, significant reduction in gingival inflammation (gingivitis) was observed after treatment with *Lactobacillus* alone, without prior mechanical treatment.
- Oral administration of lactobacilli significantly reduced ($p<0.05$) the oral burden of periodontal pathogens, such as *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, and *Prevotella intermedia*. The maintenance of this beneficial effect was dependent on continuous probiotic consumption.
- The mechanisms of action of probiotics are unclear, as yet, although it is likely that they play a critical role in nurturing a healthy gingival flora and deterring the recolonization of the periodontal niche by periodontopathogens.
- Probiotics may serve as adjunct or replacement therapy to substitute antibiotics in managing human periodontal infections in future.

Declaration of interest

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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Figure 1 : Flow diagram of the screening and selection process.

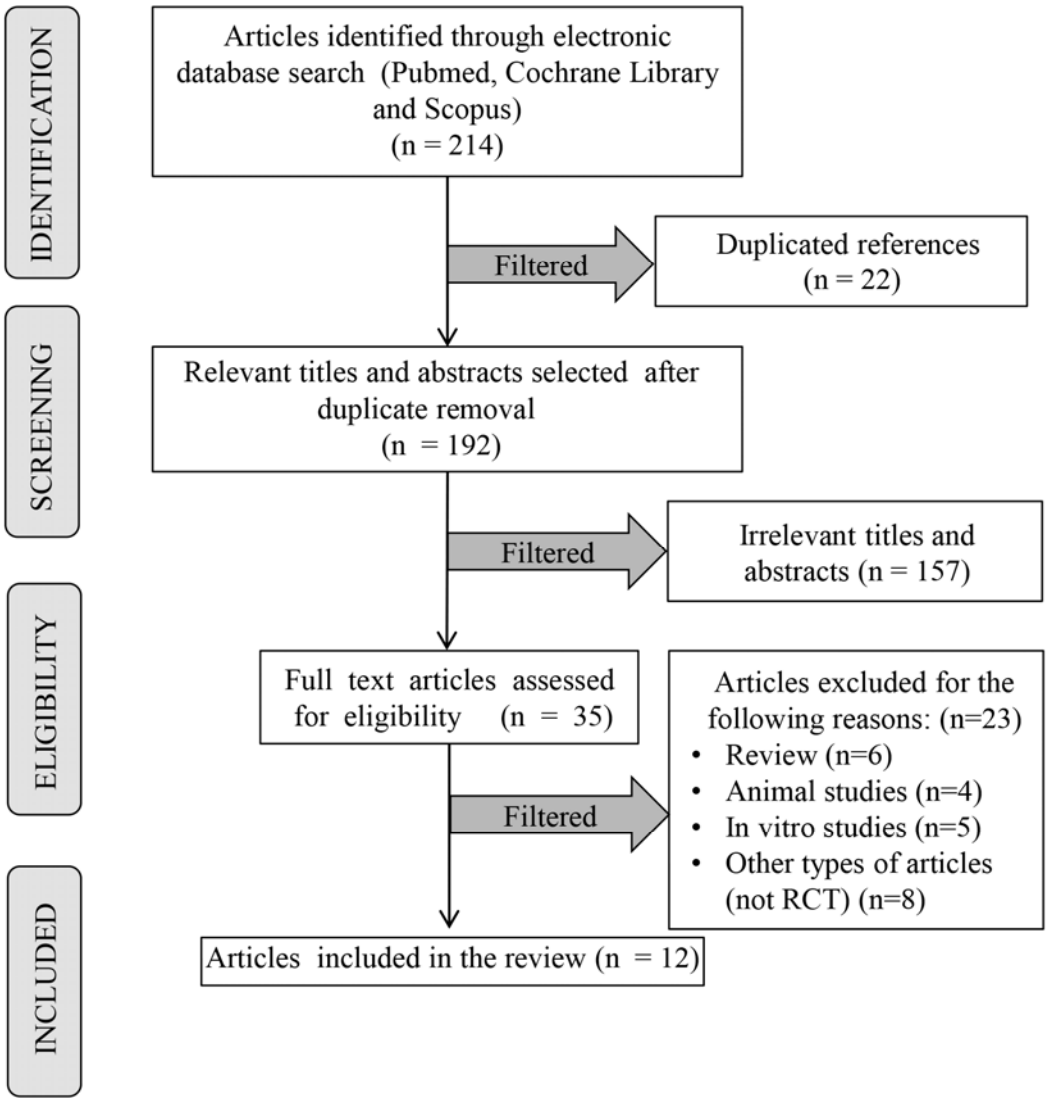

Figure 2 : Proposed mechanisms of the suppression of periodontopathogens by probiotics; Dysbiosis of the pathogenic periodontal microbiome is likely to be shifted in favour of development of a more host-freindly symbiotic biofilm due to probiotic therapy. A number of mechanisms, as illustrated here, have been proposed for probiotic activity and includes competitive inhibition of pathogen attachment, direct and indirect immune response, secretion of antimicrobial substances, modulation of pathogen proliferation and apoptosis.

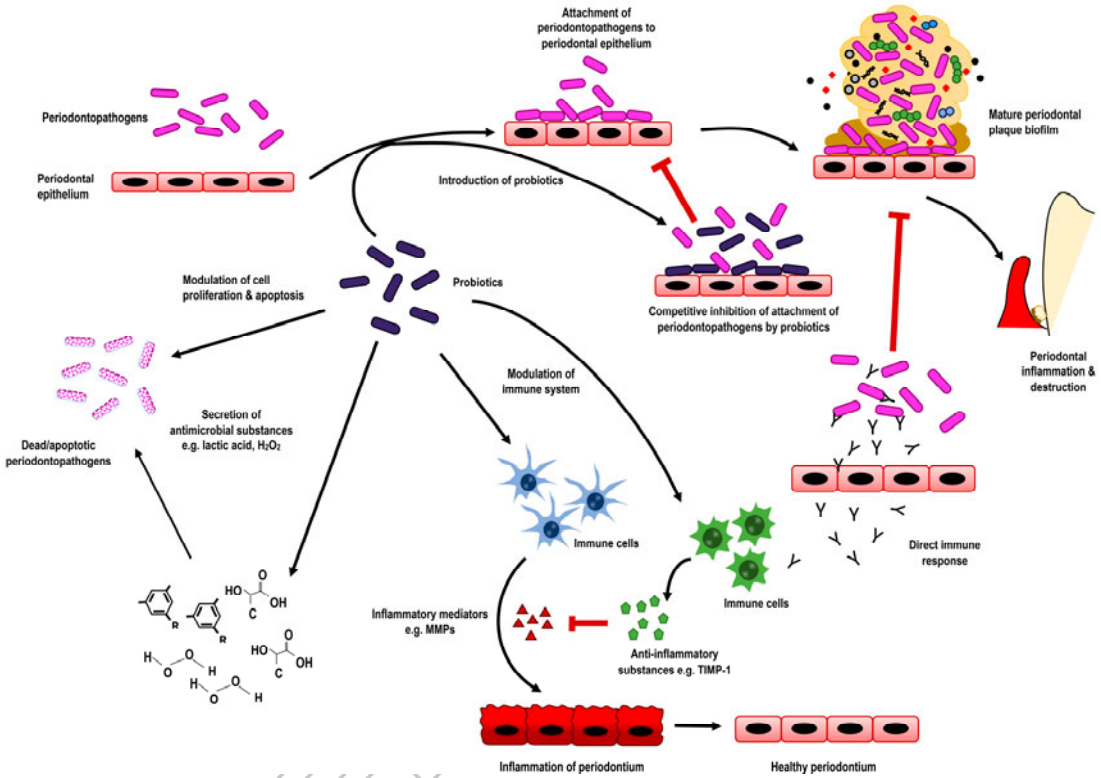

Table 1. PICOS criteria for the systematic review.

Population (P)	Patients affected by periodontitis
Intervention (I)	Oral administration of probiotic bacteria
Comparison (C)	Placebo treatment or absence of probiotic treatment
Outcome (O)	Clinical parameters of periodontitis evaluation and/or incidence of periodontal pathogenic bacteria
Study design (S)	Randomized controlled trials
Focused question	Is the oral administration of probiotic bacteria helpful in the management of periodontitis?

Table 2. Assessment of the risk of bias of studies included. Low risk of bias (+), unclear risk of bias (?) and high risk of bias (-).

Study	Random sequence generation (selection bias)	Allocation concealment (selection bias)	Blinding of participants and personnel (performance bias)	Blinding of outcome assessment (detection bias)	Incomplete outcome data (attrition bias)	Selective reporting (reporting bias)
Morales et al. 2016	+	+	+	+	+	+
Laleman al. 2015	+	+	+	+	+	+
Ince et al. 2015	+	+	+	+	+	+
Tekce et al. 2015	+	+	+	+	+	+
Shah et al. 2013	+	?	?	?	+	+
Vicario et al. 2013	+	+	+	+	?	+
Teughels et al. 2013	+	+	+	+	+	+
Suzuki et al. 2012	+	?	+	+	+	+
Vivekananda et al. 2010	+	+	+	+	+	+
Mayanagi et al. 2009	+	?	+	+	+	+
Shimauchi et al. 2008	+	+	+	+	+	+
Della Riccia et al. 2007	+	?	?	?	+	+

Table 3. Treatment of patients affected by periodontitis with probiotic bacteria. Resume of data collected from the randomized clinical trials included in the systematic review.

Study	Study design *	Population	Probiotic	Probiotic therapy	Follow-up	Concomitant treatment	Adverse effects **	Periodontal assessment †	Outcomes
Morales et al. 2016 [33]	RD, PL, DB, PC	N = 28 Chronic periodontitis F/M gender ratio: 14/14 Age range: 36-60 years	*Lactobacillus rhamnosus* SP1 Sachet 2 x 10^7 UFC/sachet	3 months 1x per day	0, 3, 6, 9, 12 months	Mechanical periodontal treatment	None	PPD, CAL, PI, BOP	Control and test groups presented similar clinical improvements. The probiotics induced a significant reduction ($p<0.05$) in subjects with PPD ≥ 6mm, indicating a reduced need for surgery.
Laleman et al. 2015 [32]	RD, PL, DB, PC	N = 48 Advanced periodontitis F/M gender ratio: 22/26 Age range: ≥36 years	*Streptococcus oralis* KJ3, *S. uberis* KJ2, *S. rattus* JH145 Tablet 10^8 CFU each strain/tablet	3 months 2x per day	0, 4, 8, 12 and 24 weeks	Mechanical periodontal treatment	None	PPD, REC, CAL, BOP, PI, GI Microbiological parameters	PI was significant lower ($p<0.05$) in the probiotic group than the control at 24-week. *P. intermedia* counts were reduced in the probiotic group at 12-week. No significant difference was observed between the two groups (probiotic x placebo) regarding PPD, REC, CAL, BOP, GI.
Ince et al. 2015 [22]	RD, PL, DB, PC	N = 30 Chronic periodontitis F/M gender ratio: 13/17 Age range: 35-50 years	*L. reuteri* Lozenges	3 weeks 2x per day	21, 90, 180 and 360 days	Mechanical periodontal treatment	None	PPD, GI, PI, BOP, CAL; Biochemical parameters	PI, GI, BOP, and PPD significant improved in the probiotic group at all time points ($p<0.05$). Mean values of attachment gain significantly higher in probiotic group on days 90, 180 and 360. Decreased GCF MMP-8 levels and increased TIMP-1 levels significant up to day 180 ($p<0.05$).
Tekce et al. 2015 [16]	RD, PL, DB, PC	N = 40 Chronic periodontitis F/M gender ratio: 22/18 Age range: 35-50 years	*L. reuteri* Lozenges	3 weeks 2x per day	21, 90, 180 and 360 days	Mechanical periodontal treatment	None	PPD, GI, PI, BOP, CAL; Microbiological parameters	PI, GI, BOP and PPD significantly lower in the probiotic group at all time points ($p<0.05$). The proportions of obligate anaerobes were reduced with the exception of day 360 after probiotic consumption.
Shah et al. 2013 [23]	RD	N = 30 Aggressive periodontitis F/M gender ratio: 16/14 Age range: 14-35 years	*L. brevis* Lozenges 10^8 CFU/gram	14 days 2x per day	0, 2 weeks and 2 months	Mechanical periodontal treatment Antibiotic: doxycycline (100 mg), 1x per day	NR	PPD, GI, PI, CAL Microbiological parameters	All groups (probiotic alone, probiotic + antibiotic, antibiotic alone) reduced significantly ($p<0.05$) PPD, GI, PI and CAL at 2 months. Lactobacilli counts in saliva increased after probiotic consumption. Reduction of *A. actinomycetemcomitans* counts in saliva was not significant in all groups ($p > 0.05$).
Vicario et al. 2013 [28]	RD, PL, DB, PC	N = 19 - Chronic periodontitis F/M gender ratio: 7/12 Age range: 44-64 years	*L. reuteri* ATCC 55730 + *L. reuteri* ATCC PTA5289 Tablets 2.0 x 10^8 CFU/tablet	30 days 1x per day	30 days	None	None	PPD, PI, BOP	PPD, PI, BOP significantly reduced in the probiotic group ($p<0.05$).
Teughels et al. 2013 [17]	RD, PL, DB, PC	N = 30 Chronic periodontitis F/M gender ratio: 15/15 Age range:	*L. reuteri* DSM17938 + *L. reuteri* ATCC PTA5289 Lozenges 1.0 x 10^8 CFU	12 weeks 2x per day	3, 6 9 and 12 weeks	Mechanical periodontal treatment	None	PPD, GI, PI, BOP, CAL; Microbiological parameters	Reduction of full-mouth PPD in moderate and deep pockets after probiotic administration ($p<0.05$). Significantly greater gain of CAL in probiotic group ($p<0.05$). Reductions in *P.*

		≥35 years	each strain/lozenges						*gingivalis* numbers in the subgingival, supragingival and saliva samples in probiotic group ($p < 0.05$).
Suzuki et al. 2012 [18]	RD, DB, PC	N = 42 Periodontitis F/M gender ratio: 32/10 Age range: 21-76 years	*L. salivarius* WB21 Soybean oil 4.0×10^8 CFU per day	15 days 3x per day	15 days	None	NR	PPD, BOP; Volume of stimulated salivary flow; Saliva pH; Microbiological parameters	Reduced BOP in the probiotic group compared with the control group ($P = 0.010$). Total bacterial numbers decreased in the probiotic group ($p = 0.033$). Number of *P. intermedia* increased in the placebo group, but not in the probiotic group ($p = 0.045$).
Vivekanand a et al. 2010 [29]	RD, PL, SMD, DB, PC	N = 30 Chronic periodontitis F/M gender ratio: 11/19 Age range: 34-50 years	*L. reuteri* DSM17938 + *L. reuteri* ATCC PTA5289 Lozenges 1.0×10^8 CFU each strain/lozenges	3 weeks 2x per day	0, 21 and 42 days	With or without mechanical periodontal treatment	None	PPD, GI, PI, GBI, CAL; Microbiological parameters	Higher reduction of PI, GI and GBI in the probiotic group. PPD reduced from 5.08 ± 0.75 to 3.78 ± 0.61 mm ($p<0.001$) and CAL from 3.93 ± 0.93 to 2.85 ± 0.74 mm ($p<0.001$) in the group treated with probiotic and mechanical procedure. Probiotic, either alone or following mechanical treatment, significantly reduced *A. actinomycetemcomitans*, *P. gingivalis*, and *P. intermedia* ($p<0.01$).
Mayanagi et al. 2009 [30]	RD, PL, DB, PC	N = 67 Chronic periodontitis F/M gender ratio: 9/58 Age range: 32-61 years	*L. salivarius* WB21 + xylitol Tablet Bacteria - 6.7×10^8 CFU/tablet Xylitol - 280 mg/tablet	8 weeks 3x per day	0, 4 and 8 weeks	None	None	Microbiological parameters	Reduction in numbers of five periodontal pathogens (*A. actinomycetemcomitans*, *P. intermedia*, *P. gingivalis*, *T. denticola*, and *T. forsythia*) in the subgingival plaque at 4W ($p<0.05$). Significant reduction of *T. forsythia* in subgingival plaque of the probiotic group at both 4W ($p<0.001$) and 8W ($p = 0.006$).
Shimauchi et al. 2008 [31]	RD, DB, PC	N = 67 Chronic periodontitis F/M gender ratio: 9/58 males Age range: 32-61 years	*L. salivarius* WB21 + xylitol Tablet Bacteria - 6.7×10^8 CFU/tablet Xylitol - 280 mg/tablet	8 weeks 3x per day	0, 4 and 8 weeks	None	None	PPD, GI, PI, BOP; Lactoferrin levels in saliva	Periodontal clinical parameters improved in both groups after intervention. Significant improvement of PI, GI and PPD in current smokers in the probiotic group ($p<0.05$). Significant decreased of salivary Lf level in the test group smokers ($p<0.01$).
Della Riccia et al. 2007 [27]	RD, DB, PCS	N = 21 Chronic periodontitis F/M gender ratio: 5/16 Age range: 30-51 years N = 8 Health volunteers Age range: 24-47 years	*L. brevis* Lozenges	4 days 4x per day	0 and 4 days	None	NR	PI, BOP; Gingival inflammation; Calculus; Temperature sensitivity; Molecular analysis	All clinical parameters reached statistically significant differences after treatment with probiotic ($p<0.0001$). *L. brevis*-containing lozenges was associated with a strong decrease in the levels of inflammation- associated molecules ($P<0.01$)

* RD = Randomized, PL = Parallel, DB = Double-blinded, PC = Placebo-controlled, SMD = Split-mouth design, PCS = Paired-comparison study

** NR = Not reported

† PPD = Probing pocket depth, REC = Gingival recession, GI = Gingival index, PI = Plaque index, BOP = Bleeding on probing, CAL = Clinical attachment level, Gingival bleeding index = GBI