Effect of Periodontal Interventions on Characteristics of the Periodontal Microbiota: A Systematic Review and Meta-analysis

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Abstract

Background: The aim of the systematic review was to evaluate the effect of periodontal interventions on the diversity and composition of periodontal microbiota assessed by high throughput sequencing (HTS) metagenomics analysis.

Methods: An electronic search of all relevant databases was conducted from database inception to May 2021 by two independent reviewers. All clinical trials that evaluated the effect of periodontal interventions on the gingival microbiota through HTS were selected. The measures of alpha diversity was used as primary outcome; richness, Shannon diversity index, and the Chao1 index, whereas relative abundances of bacterial genera was considered as secondary outcome. The Robvis tool was used to assess the risk of bias.

Results: Overall, 19 studies were eligible for the systematic review, of which 10 studies were included in the meta-analysis. Periodontal intervention for test group resulted in decrease in Shannon's diversity, richness and Chao I index as observed from baseline to post-treatment. The most common genera found to increase after periodontal therapy were *Rothia*, *Actinomyces*, *Streptococcus*, *Veillonella* and *Hemophilus* and *Porphyromonas*, *Tannerella*, *Fusobacterium*, and *Treponema* decreased after periodontal therapy.

Conclusion: Periodontal interventions may decrease the bacterial diversity and richness and alter the composition of oral microbiota in the short-term. Periodontal microbiota signatures could potentially be used for the assessment of periodontal intervention in the future. Further studies should implement methodological consistency to provide a more quantitative and mechanistic understanding of these approaches.

Introduction

The human body is a superorganism with trillions of associated microorganisms that are essential for maintaining health, or eliciting disease. These organisms can be either commensal, symbiotic, and pathogenic and are referred to as microbiota [1]. These microbiota easily outnumber the amount of human cells within the body [2]. The total mass of bacteria in an average human is estimated to be about 0.2 kg and the total number of bacteria cells is around 3.8×10^{13} [3]. The activity of the microbiota and expression of its genomic information, known as the microbiome, provides humans with traits that are not usually present within the human genome [4]. In the mouth alone, over 700 bacterial species have been identified in oral samples by DNA based microbiome analysis and form complex mixtures of species in different micro-niches on the teeth, tongue, and soft and hard tissues of the mouth [5].

Periodontal diseases, including gingivitis and periodontitis, results from inflammation caused by ecological disturbances in the periodontal microbiota [6, 7]. In periodontal health, the prevalence of potentially virulent species are present but in lower abundance than species in individuals with disease. Clinical evidence suggests that increased periodontal inflammation may not be associated with a distinct microbiota, but an increase in abundance of potentially virulence abundant species, such as *Porphyromonas gingivalis*, *Treponema denticola*, and *Tannerella forsythia*, as well as others, which increases the total bacterial biomass [8]. As a result, several authors have reported increased bacterial richness, evenness and diversity associated with periodontal disease, although this is unusual, as a disease or microbiota dysbiosis is usually associated with a less diverse microbiota and an increase in certain species in other parts of the body [8, 9].

Periodontal interventions, such as scaling and root planing (SRP) with/out supplementation with antibiotics and chemical disinfectants aims at tampering with the periodontal microflora that would eventually lead to resolution of inflammation. But these procedures can decrease microbial diversity and significantly reduce the relative abundances of both healthy- and gingivitis-associated bacterial species [10, 11]. The development of emerging techniques, such as the potential for oral microbiota transplantation, has also sparked an interest in the response of periodontal microbiota to different therapies to re-establish a healthy microbiota without reducing the bacterial richness and diversity [12]. Previous studies have used low throughput measurement techniques (microbial culture, checkerboard hybridization, PCR analysis) which have resulted in incomplete characterization of the oral microbiome composition [13-15]. Little is known about how the periodontal microbiota composition and diversity changes metagenomically in response to the periodontal treatment [16].

Research can now examine differences in the periodontal microbiota between health and disease through high throughput sequencing (HTS) or next generation sequencing (NGS). The technology is highly scalable and allows the study of the entire genome of the sequence in an automated process. The process involves fragmentation of the genome into smaller pieces, then random sampling for a fragment, which is followed by sequencing [17]. The HTS of 16S rRNA genes allows thousands of sequences per sample and provides the power to comprehensively study bacterial community diversity and composition within a specific niche [9]. Previously, the authors of a systematic review [18], reported a clear separation of microorganisms between dental plaque samples collected before and after periodontal intervention assessed by NGS. But the author's included only 12 studies without any quantitative meta-analysis. To our knowledge there have been no review and meta-analysis conducted on the changes in the periodontal microbiome analysed through HTS exclusively after periodontal intervention. Therefore, the aim of our review is to examine the characteristics of the periodontal microbiota among adults with periodontal disease who received treatments with an active agent, compared to interventions without active agents/placebo/SRP, using HTS methodologies. Thus, we hypothesized that periodontal interventions can influence oral microbiota associated with periodontal disease toward a balanced state consistent with oral health.

Methods

Study registration

The study review protocol was registered with the PROSPERO database (International Prospective Register of Systematic Reviews) hosted by the Centre for Reviews and Dissemination, University of York, National Institute for Health Research, United Kingdom having the registration number CRD42020188531. This

systematic review and meta-analysis has been prepared according to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines [19].

The research question was based on the PICOS (Population, Intervention, Comparison, Outcome, and Study) format. In clinical trials (S) involving adults for restoration of periodontal health (P), does any treatment with an active agent (I) compared to intervention without active agent/ placebo/ SRP (C) have an effect on the characteristics of the periodontal microbiota (O)?

Research Question:

1. Is there any change in the alpha diversity before and after treatment?
2. Is there any change in the composition of periodontal microbiome before and after periodontal treatment?
3. What are the predominant bacterial species present in the periodontal microbiome identified through HTS?

Search strategy

An electronic search was conducted on the following database to identify eligible studies: MEDLINE, Scopus, and EBSCOHost (Dentistry & Oral Sciences Sources), Cochrane database of systematic review, OpenGrey database and ProQuest dissertation. The articles were searched from database inception to May, 2021. The search strategy was designed to locate both unpublished and unpublished articles.

The search strategy used for the MEDLINE database was ("Metagenomics" [MeSH] OR "Metagenome" [MeSH] OR "High-Throughput Nucleotide Sequencing" [MeSH] OR "microbiota" [MeSH] OR "Genes, Bacterial" [MeSH] OR "metagenomics" [tiab] OR "16s rDNA" [tiab] OR "16s rRNA" [tiab] OR "Pyrosequencing" [tiab] OR "next-generation sequencing" [tiab] OR "Illumina sequencing" [tiab] OR "Functional gene array" [tiab] OR "Oral microbiome" [tiab] OR "Bacteria*" [tiab] OR "Bacterial diversity" [tiab] OR "Bacterial community" [tiab]) AND ("Tooth Diseases" [MeSH] OR "Mouth Diseases" [MeSH] OR "Oral Health" [MeSH] OR "Gingival diseases" [MeSH] OR "Gingivitis" [MeSH] OR "Periodontal diseases" [MeSH] OR "Periodontal debridement" [MeSH] OR "Periodontal index" [MeSH] OR "Periodontal pocket" [MeSH] OR "probing depth" [tiab] OR "periodont*" OR "plaque score").

Our search strategy was not limited only to MeSH term and keywords such as "periodontal microbiome", "oral microbiome", "clinical trial" were also used which resulted in an exhaustive search. A similar search strategies were used for the other databases.

A manual hand searching of reference lists of relevant papers were screened to identify articles that might have been missed on the electronic search. Systematic reviews, narrative reviews, and standard textbooks were additionally searched to identify all the other eligible studies. Whenever possible, citation tracking was performed on search engines.

Study selection criteria

Types of participants

The population selected were adult's ≥ 18 years of age who underwent any procedure or intervention for restoration of periodontal health. The paediatric population was excluded because the oral composition of children is different from that of an adult oral and continues to evolve and mature with age [20].

Type of interventions

Interventions aimed at achieving periodontal health were included. All types of interventions such as standard periodontal therapy (i.e. SRP either as the sole procedure or combined with mouthwash or antibiotics, toothpastes with active agents, and customised diets for achieving periodontal health) were included. Trials that evaluated the effectiveness as a single interventional trial arm by comparing before and after treatment or to any standard treatment, including SRP, antibiotics (Amoxicillin, Metronidazole), toothpaste without active ingredients, placebos, or no treatment, were included as controls.

Types of outcome measures

The primary outcomes were measures of alpha diversity of periodontal microbiota at baseline compared to post treatment values. Alpha diversity summarizes the structure of an ecological community within a particular sample and is commonly measured by the number of different species in a sample (richness), or Shannon's diversity index (combination of richness and diversity), or the Chao1 index (number of species in a community) [21]. The relative abundance level (relative prevalence) for each bacterial genera and species were included as secondary outcomes.

Selection criteria

Inclusion criteria

Studies with the following criteria were selected: (1) Original studies; (2) evaluation of an intervention compared to a control for restoration of periodontal health; (3) having periodontal disease; (4) analysis of periodontal microbiota through bacterial analysis of 16S rRNA gene sequencing and amplification; (5) randomised clinical trial and/or quasi-experimental.

Exclusion criteria

Studies that analysed saliva instead of supragingival plaque or subgingival plaque for microbial profiling were excluded. Healthy participants without any forms of periodontal disease were excluded. *In vitro* or animal studies, case reports, case series, retrospective studies, literature reviews, opinion papers, letters to conference proceedings and abstracts were also excluded.

Selection of studies

All the articles found through searching of the databases were uploaded in a referencing software, EndNote X9 Version 3.3 (Clarivate Analytics, Philadelphia, PA, USA) and the duplicates were removed. Two independent and calibrated reviewers (SN & SJP) assessed the studies by title and abstract against the eligibility criteria. Full text assessment was done only for selected articles that followed the inclusion and exclusion criteria. In case if there was any disagreement and consensus couldn't be reached among the two reviewers, a third reviewer (LMJ) was referred for the final decision. The reasons for exclusion of an article have been recorded separately.

Data extraction

Data extraction was done independently from the selected studies by the two reviewers (SN & SJP) using a customised data extraction form containing all information necessary to answer the research question. The data extraction form was first pilot tested on random sample (5% of included studies) and modified until all the reviewers agreed upon the key variables and outcomes. When the included studies had any missing information, the authors were contacted via email correspondence for further details and information. The data extraction form included details on: (1) Study Characteristics: surname of the first author, year and country of study, study design; (2) Patient Characteristics: disease type and definition, age, number of participants, treatment description, and duration; (3) Outcome measures: Alpha diversity (richness, Shannon diversity, and Chao1 index) and relative abundance levels for genera identified in the study. Any one of the following recorded in a study was measured as outcome.

For the purpose of pooled data and meta-analysis, data extraction from graphs or charts was done using WebPlotDigitizer tool (Version 4.2 GNU Affero General Public License). In studies that reported only the median, the estimates were converted to mean and standard deviation [22].

Assessment of risk of bias

For quality assessment of clinical trials, the "Risk of Bias Assessment 2" (RoB 2.0) guidelines formed by the Cochrane Collaboration was used [23]. For each study, bias was assessed on five domains: (1) bias due to randomisation; (2) bias due to deviations from intended intervention; (3) bias due to missing data; (4) bias due to outcome measurement and; (5) bias due to selection of reported results. Each clinical trial was scored as 'high risk', 'some concerns' or 'low risk' and given an overall score. The robvis visualization tool was used for visualizing the risk of bias assessment.

The risk of bias and quality of evidence assessment was performed independently and in duplicates by two reviewers (SN & SJP). Any disagreements was resolved through discussion between the two reviewers or a third reviewer was consulted (LMJ).

Statistical Analysis

All data were extracted and collated in spreadsheet. All the findings are represented in narrative table form and where possible the data was meta-analysed. Meta-analysis using a random effects model was implemented. The pooled standardised mean difference (SMD) and 95% confidence intervals were estimated for measures of alpha diversity before and after treatment in both test and control group. Random effects model was used and forest plots were constructed for visualization of difference between the two groups. Heterogeneity was measured using I^2 statistics, which represents the percentage of variation attributable to statistical heterogeneity and was categorized as low (25%–50%), moderate (51%–75%), or high (>75%) [24]. Sub-group analysis and sensitivity analysis could not be performed due to limited data. Publication bias could not be assessed due to a limited number of eligible studies [25]. The meta-analysis was performed using STATA 15 software (StataCorp. 2017, Stata Statistical Software: Release 15. StataCorp LLC, College Station, TX, USA).

Results

Search strategy and screening process

The search details are provided in the PRISMA flow chart (Fig. 1). The search strategy resulted in 2077 potential articles: 725 articles from PubMed (MEDLINE), 872 articles from Scopus, 480 articles from the Dentistry and Oral Sciences database, and one article from reference searching [26]. After removal of duplicates, 1627 articles were screened. All articles were assessed according to the inclusion and exclusion criteria and this led to full text analysis of 22 articles [10, 11, 26–45]. One study did not report the complete sequencing of the subgingival microbiome [36] and one study did not report baseline data [45]

and while one study assessed on healthy participants [30] and were all 3 thus excluded. Overall, 19 studies were included for descriptive analysis and 10 studies [10, 11, 27–29, 32, 34, 35, 37, 44] were meta-analysed.

Characteristics of included studies

Characteristics of the included studies were examined in the context of study design, periodontal disease definition, age, description of test and control group, plaque collection method, hypervariable region of 16S rRNA gene and follow up period (Table 1).

Table 1
Characteristic of Included Studies

First author and Year	Study design	Disease	Disease definition	Age	Case (n)	Control (n)	Test description	Control description	
Kruse et al. 2020	Single-blinded, randomised controlled split mouth study	Chronic periodontitis patient undergoing maintenance therapy	2 single-rooted with PPD = 5 mm and positive BOP or > 5 mm with or without positive BOP	61.4 ±10.6	10		Air polishing using trehalose powder	Ultrasonic scaling	
Al-Kamel et al. 2019 (Prevention study Yemen)	Triple blinded, placebo-controlled, parallel-arm RCT	Experimental gingivitis	21 days gingivitis model	22.22 ± 1.73	10	10	T1:1.25% NAC mouthwash T2: 0.2% CHX mouthwash	Placebo mouthwash	
Al-Kamel et al. 2019 (Treatment study) Yemen					10	10	1.25% N-acetyl cysteine mouthwash	0.2% CHX mouthwash	
Hagenfeld et al. 2019 Germany	Double-blind, two centre RCT	Mild to moderate periodontitis	PPD of ≥ 4 mm in at least four teeth except third molars	54.22	20	21	Toothpaste containing zinc substituted carbonated hydroxyapatite	Toothpaste with amine fluoride/stannous fluoride	

RCT: Randomized clinical trial; SRP: Scaling and root planing; PPD: periodontal probing depth; CAL: clinical attachment level; BOP: Bleeding on probing; NAC: Mazza Gingival index; AAP: American Association of Periodontology; CPC: Cetylpyridinium Chloride; EMD: emodogain

First author and Year	Study design	Disease	Disease definition	Age	Case (n)	Control (n)	Test description	Control description	
Lu et al. 2019 China	Clinical trial	Periodontitis patients on maintenance therapy	Maintained subjects that had stable condition with more than 20 teeth, bop (%) $\leq 25\%$, PPD ≤ 5 mm	50.18 $\pm$ 12.08	17	17	Ultrasonic scaling	Air polishing	
Woelber et al. 2019 Germany	Single blinded RCT	Gingivitis	Mean GI index ≥ 0.5	27.2 (4.7)	15	15	Anti-inflammatory diet low in processed carbohydrates and animal proteins and rich in omega-3 fatty acids, vitamin c, vitamin d, antioxidants, plant nitrates and fibre	Western diet	
Hagenfeld et al. 2018 Germany	Double-blind, parallel group RCT	AAP 1999 definition for moderate/severe chronic periodontitis	3mm to < 5mm/_5mm attachment loss	NA	42	47	SRP + 50 mg amoxicillin and 400 mg metronidazole	SRP + placebo	
Chen et al. 2018 USA	Pre/post intervention	Chronic periodontitis	NA	NA	19		SRP	Before SRP	

RCT: Randomized clinical trial; SRP: Scaling and root planing; PPD: periodontal probing depth; CAL: clinical attachment level; BOP: Bleeding on probing; NAC: Mazza Gingival index; AAP: American Association of Periodontology; CPC: Cetylpyridinium Chloride; EMD: emodogain

First author and Year	Study design	Disease	Disease definition	Age	Case (n)	Control (n)	Test description	Control description	
Belstrom et al. 2018 Denmark	Pre/post intervention	AAP 2015 definition for moderate/severe chronic periodontitis	PD $\geq$ 5 & <7 mm and CAL 3–5 mm/ PD $\geq$ 7 mm and CAL $\geq$ 5 mm	47–75	25		SRP	Before SRP	
Queiroz et al. 2017 Brazil	Single blinded RCT	Chronic periodontitis with Class II furcation on molars	Horizontal furcation PD $\geq$ 4 mm	53.14	13	13	Beta-tricalciumphosphate/hydroxyapatite graft/ EMD + BONE	EMD only	
Han et al. 2017 China	Pre/post intervention	AAP 1999 definition for generalized Aggressive Periodontitis	Rapid attachment loss and bone destruction, and possible familial aggregation of disease.	28 $\pm$ 1.41	2		SRP	No treatment	
Liu et al. 2017 China	Pre/post intervention	AAP 1999 definition for generalized Aggressive Periodontitis	Rapid attachment loss and bone destruction, and possible familial aggregation of disease.	30.75 $\pm$ 3.17	12		SRP	No treatment	

RCT: Randomized clinical trial; SRP: Scaling and root planing; PPD: periodontal probing depth; CAL: clinical attachment level; BOP: Bleeding on probing; NAC: Mazza Gingival index; AAP: American Association of Periodontology; CPC: Cetylpyridinium Chloride; EMD: emodogain

First author and Year	Study design	Disease	Disease definition	Age	Case (n)	Control (n)	Test description	Control description	
Califf et al. 2017 USA	Clinical trial	Periodontitis	At least four separate teeth with a pocket depth of 6 mm	41	17	17	0.25% sodium hypochlorite	Water rinse	
Bizzaro et al. 2016 Netherland	Single blinded RCT	Chronic periodontitis	Proximal attachment loss of ≥ 3 mm at ≥ 2 non-adjacent teeth + below median responders	NA	9	10	SRP + 0.12% CHX rinse (2 × day × 28 days) and antibiotics (amoxicillin 375 mg + metronidazole 250 mg, 3 times a day × 7 days)	SRP + CHX 0.12% only	
Huang et al. 2016 China	Double-blind randomised controlled clinical trial	Moderate gingivitis	At least 10 bleeding sites; mean MGI is from 1.0–2.5	33.80 ± 7.86	47	44	The brush-plus-rinse group: manual toothbrush and a toothpaste containing 0.321% sodium fluoride and 1.16% stannous chloride and 20 ml rinse with 0.0747% CPC for 30 seconds after brushing.	Brush twice-daily with a manual brush and a 0.243% sodium fluoride toothpaste	
Teng et al. 216	Double blind randomised controlled trial	Experimental gingivitis	21 days gingivitis model	18–53	41	50	CPC mouthrinse, two times daily	Water rinse	

First author and Year	Study design	Disease	Disease definition	Age	Case (n)	Control (n)	Test description	Control description	
Shi et al. 2015	Pre/post intervention	Chronic periodontitis	N/A	53	17		SRP	Before SRP	
Laksmmana et al. 2012 USA	Pre/post intervention	AAP definition of Aggressive Periodontitis	Rapid attachment loss and bone destruction, and possible familial aggregation of disease.	N/A	2		SRP + 500mg amoxicillin + 500mg metronidazole, three times daily x 8 days	N/A	
Junemann et al. 2012 Germany	Double blind, parallel group, placebo controlled RCT	Generalized severe chronic periodontitis	More than 38% of sites with pocket probing depths of 6 mm or more	N/A	2	2	SRP + 500mg amoxicillin + 400mg metronidazole, three times daily x 7 days	SRP	
Yamanaka et al. 2012 Japan	Pre/post intervention	Periodontitis	N/A	35–73	19		SRP	Before SRP	
RCT: Randomized clinical trial; SRP: Scaling and root planing; PPD: periodontal probing depth; CAL: clinical attachment level; BOP: Bleeding on probing; NAC: Mazza Gingival index; AAP: American Association of Periodontology; CPC: Cetylpyridinium Chloride; EMD: emodogain									

Study type and intervention:

Of the 19 studies, only half were RCT [10, 27–29, 31, 32, 34, 37, 40, 44], whereas seven studies [11, 26, 33, 38, 39, 42, 43] were pre and post intervention studies (before and after SRP) and two studies [35, 41] were clinical trials without clearly defining randomisation. For the purposes of this analysis, one RCT [10] was split into three sub-studies; two prevention and one treatment sub-study, while another clinical trial [27] described their results as above or below median responses; thus we analysed this data separately. In total, six types of interventions were observed: (a) six studies [11, 26, 33, 38, 42, 43] were single arm studies comparing pre and post SRP; (b) four studies [27, 29, 39, 40] supplemented SRP with antibiotics; (c) four studies [10, 28, 37, 41] used mouthwash rinses only; (d) two studies [35, 44] compared ultrasonic scaling to air polishing; (e) one study [32] used toothpastes with active ingredients; (f) one study used a regenerative material (Beta-tricalcium phosphate/EMD/Hydroxyapatite) for furcation therapy; and (g) one study used an anti-inflammatory diet. The

duration of the interventions ranged from immediately after treatment [43] to three weeks, [10, 28], 4 weeks [26, 37, 38], 6 weeks [11, 34], 8 weeks [29, 39, 40], 12 weeks [32, 33, 35, 41, 44], 6 months [31] to 12 months [27].

Study participants:

All the study subjects were generally systematically healthy patients and the age ranged from 18–73 years. All the studies included both the male and female gender except for one study [38] that only included females. For this review, we included studies on all forms of periodontal diseases, including generalised gingivitis [34, 37], chronic periodontitis [26, 27, 29, 31–33, 40, 42, 44], aggressive periodontitis [11, 38, 39], periodontitis (unclassified) [35, 41, 43] and experimental gingivitis [10, 28].

Study methodology and metagenomics analysis:

The plaque collection methods varied across studies, the most popular methods for collection of plaque was sterile paper points [10, 26, 27, 29, 31, 32, 39, 40, 44] and sterile gracey curettes [28, 33–35, 37, 38, 41–43]. Only one author used filter paper for plaque collection [11]. All the included studies used amplicon sequencing of 16S rRNA except for one study that used shotgun approach for sequencing. The most commonly used hypervariable region observed was the V3-V4/ V4 [11, 26, 29, 33–35, 38, 39] region of 16S rRNA, while some authors used V1-V3 [10, 28, 37, 43], V4-V6 [32], V4-V5 [41] V5-V7 [27] and V6 [40]. All the studies used operational taxonomic units as the basis of metagenomics analysis, except for two studies [29, 32] that used ribosomal sequencing variants (RSVs).

Measures of Alpha diversity:

Alpha diversity is a measure of within sample diversity of community, described in terms of the number (richness) or distribution (evenness) [46]. The bacterial richness (number of observed species) was assessed by three authors [10, 11, 28, 29, 32, 40, 43, 44]. The commonly used indices to measure alpha diversity was Shannon Diversity Index [10, 11, 26–29, 37, 38, 40, 42, 43]. Some studies used Chao1 index [10, 11, 35, 38, 41, 43], Simpson index [11, 26, 40], Pious evenness [29, 40] and Faith's phylogenetic diversity [41].

Measure of Beta diversity:

Beta diversity is a measure of the between sample differences of pairs of communities. The beta diversity was observed by all studies except for four studies [31, 33, 39, 40]. It can be measured either using principal coordinates analysis (PCoA) or principal component analysis (PCA) [46]. To assess the beta-diversity, PCoA was used by 11 studies [10, 11, 29, 32, 35, 37, 38, 41–43, 47] and only three studies used the PCA [27, 28, 34]. Meta-analysis could not be conducted for beta diversity and was not considered as an outcome as the findings were in an accessible format. We tried contacting authors of the papers for raw primary data for beta-diversity but failed to receive any additional information.

Relative abundance of bacterial genera:

One of the main findings of the review was consistency of the identified bacteria among the studies. We tried to identify the most abundant species before and after periodontal intervention and tried to find the common species that decreased and increased after periodontal intervention (Additional file S1). There was more prevalence of bacteria of red complex in pre-intervention stage and more health associated bacteria after intervention. Post intervention *Porphyromonas*, *Fusobacterium*, *Tannerella*, *Treponema*, *Selenomonas*, *Parvimonas*, *TM7*, *Fillibactor*, *Fretibacterium*, and *Campylobacter* decreased in number. While *Escherichia*, *Neisseria*, *Prevotella*, *Capnocytophaga*, *Lautropia*, *Haemophilus*, *Veillonella*, *Actinomyces*, *Streptococcus* and *Rothia* increased in number post intervention (Fig. 2).

Synthesis of results:

Alpha diversity compared within test and control groups from baseline to post treatment:

Meta-analysis could not be conducted for studies that did not include mean and SD. The difference in alpha diversity from baseline to post intervention was compared independently for both pooled test and pooled control groups. Post intervention only the pooled test group showed significant changes in richness, Shannon's diversity and Chao 1 index. Seven trials [10, 11, 28, 29, 32, 43, 44] revealed a decrease in richness in the treatment group (SMD = 0.24; 95% CI= -0.03, 0.46) (Fig. 3). In contrast, the richness resembled baseline in the control group (SMD=-0.35, 95% CI= -0.59, 0.12). Eight studies were meta-analysed for Shannon's diversity [7, 10, 11, 27–29, 35, 43] (Fig. 4). The test group showed decrease in Shannon diversity in the post treatment samples (SMD: -0.39, 95% CI: 0.20, 0.58), whereas the control group (SMD: -0.24, CI: -0.45, -0.04) resembled baseline. While four trials examined the Chao1 of observed species [10, 11, 35, 43] (Additional File S2), and there was a decrease in the Chao 1 Index between baseline and post treatment (T = SMD: 0.42, 95% CI: 0.09, 0.75) in the test group than the control group (C = SMD: -0.28, 95% CI: -0.14, 0.70).

Risk of Bias Assessment:

The robvis tool was used for visualizing the risk of bias assessment (Additional File S3). The risk of bias for each study was assessed for all domains, as described in the Cochrane Handbook [23]. From the 19 studies, the overall risk of bias was high for all but three trials [10, 32, 44]. The main reason for high risk bias was unclear reporting about random sequence generation and allocation concealment.

Discussion

To the best of our knowledge this is the first systematic review that assessed the effects of periodontal interventions on the diversity of periodontal microbiota meta-analytically. The effects of periodontal interventions on diversity and abundance of certain genera were assessed to test if periodontal interventions could alter the microbial composition of the periodontal plaque to a resolved or healthier state. The findings from our review suggests that periodontal intervention could lead to low diversity, richness and community evenness as we observed a decrease in the alpha diversity.

When interpreting the results of this systematic review the following limitations should be considered. The heterogeneity could be primarily due to methodological variation (study design, disease levels, interventions and study population) and sequencing techniques. There were limited number of clinical trials reporting on the periodontal microbiome. For this reason, as long as the sample collection was done from the gingival area, we aggregated all studies on patients with gingivitis and periodontitis. Periodontal pathogens found in saliva may not be reflective of periodontal microbiota [33] and were excluded from this review. As observed from our review, the method of plaque collection varied from use of paper point and sterile Gracey curette, but both the methods are equally effective for collection of samples [48]. The included studies also used a wide-range of different interventions to restore periodontal health from common procedures like SRP to diet. The patient population also differed in geographic and ethnic variability.

The composition and diversity of the microbiome could be dependent on factors such as the hypervariable region selected, DNA extraction methods, sampling, the sequencing platform used, and the database for taxonomy [25, 49]. The OTUs, or RAVs, the number of reads per sample, the quality of filtering and the normalization of data could also be the reason for heterogeneity. The authors of this review found methodological inconsistencies for HTS in all the included studies. For example, the inconsistencies in DNA extraction and library preparation method and variability in marker selection (e.g. V_1 - V_3 vs V_3 - V_4). The laboratory environment should be standardized and applied across studies. Our systematic review and meta-analysis, included studies with similar methods and outcome measures for microbial profiling. We excluded one study which did not report the results of the complete genome sequencing [36]. Systematic and comparable methodologies and rigorous statistical analysis are required for a more accurate and precise estimation of these effects. The latest use of HTS of 16S rRNA allows a deeper analysis of the subgingival microbiota, but also has limited our ability to interpret data sets with wide-ranges in methodologies, techniques, and analysis methods [50]. Further, studies should also examine more than one niche in the oral microbiota and allow for technical comparisons between different types of oral microbiota, including saliva, periodontal pockets, the tongue, and throat in relationship to periodontal disease [33, 51].

Periodontal disease leads to an oral microbiota with higher richness, evenness, and diversity. There is a shift the microbial communities at the species and even at strain/clone level. A diverse combination of species have been reported, but oral health-associated species are usually lower in frequency [8, 9]. Whereas, periodontal health is related to lowered diversity and evenness [42]. Our findings suggest that the richness and diversity were significantly lower in the pooled (all studies) treatment group compared to baseline. Periodontal interventions resulted in a less rich and diverse microbiota, which is a normal characteristic seen in a periodontally healthy individual. Therefore, periodontal interventions might have resulted in a shift in oral microbiota with less variations in the microbial community. Our findings are in contrast to those of Galimanas *et al.* [52] and Kirst *et al.* [53], who failed to capture any differences in microbial diversity and overall composition between healthy (no periodontitis) and periodontitis patients. While an overriding host defence could limit the community composition to non-pathogenic commensals, technical and methodological implications also need to be further investigated when comparing studies [9]. Overall the diversity depends on mean number of reads. A lot of information might be lost if the number of reads are too low due to extraction methods and low abundance OTU might be discarded or the reads might be too short and might not provide a good identification of the microbial community [54, 55]. The low abundance organisms may make all the difference among the studies. NGS is moving to other platform that may produce larger reads and therefore better identification. Studies analysing larger reads (> 500–700 bp) for one single 16s RNA could make a huge difference. Microbiome are resilient due to richness, kind of interactions and functional redundancy.

While there is considerable debate about the presence of a core human microbiota, many microbes are generally common among most individuals and comprise dominant species that exist under healthy conditions [1]. The variable sets of species are exclusive to an individual are often linked to lifestyle and environmental changes. It additionally depends on phenotypic and genotypic determinants, as well as one's evolutionary history [56]. This signal is as unique across individuals as a human fingerprint [4]. In our review, we found few species increased and decreased post intervention and these species could be used as periodontal based signatures for assessment of efficacy of periodontal intervention. The species *Porphyromonas*, *Tannerella*, *Fusobacterium*, *Treponema* and *Selenomonas* was found consistently to decrease post intervention among all the pooled studies. Collectively these species are associated with gingivitis and chronic periodontitis [1, 7, 10, 53]. Decrease in abundance of these species has been associated with recovery from periodontal disease. While *Streptococcus*, *Actinomyces*, *Rothia*, *Veillonella*, *Prevotella* and *Veillonella* increased post intervention. Among these, the class *Firmicutes* (e.g. *Streptococcus*) and *Actinobacteria* (e.g. *Actinomyces*) were most commonly found (Fig. 2). Previous studies have shown that *Firmicutes* and *Actinobacteria* are routinely found in dental plaque from healthy individuals [57] and *Streptococcus* have also been associated with healthy oral conditions and is an indicator of periodontal stability [50]. Similarly *Actinomyces* is generally associated with health [1, 7, 10]. Due to a limited number of eligible studies, the abundance could not be calculated at species level, which is a more robust technique to discriminate the role of specific community members in driving the ecological shift [30]. A combination of both amplicon and shotgun sequencing could identify bacteria more in depth to species level. The main advantage of HTS is the ability to discriminate species at the clonal level.

The current interventions for periodontal treatment aims at reducing the bacterial biomass but do not necessarily return it to a state of periodontal health [8]. Our review highlights the fact there is not a single composition of bacteria that represents either a healthy state or diseased state. For e.g. the genus *Prevotella*

and *Streptococcus* are detected in health and some species are detected in disease. There has been increase in interest towards therapeutic interventions that can restore periodontal health by modulating the microbial ecology [58]. Oral microbiome transplantation (OMTs) could be potentially beneficial in replacing diseased microbiota with healthy microbes [12]. This could ensure that the diseased site is repopulated with microbiota associated with health.

Conclusion

There is a need of more standerized clinical trial and NGS methods and validated reference databases for better taxonomy. Here, we conduct a preliminary, qualitative comparison to begin to understand the overarching trends of how periodontal microbiota respond to treatment.

Decreased alpha diversity indicates a shift of the periodontal microbiota towards periodontal health following periodontal intervention. Few genera were consistently present in many of the included studies and could be part of the core microbiota. There was consistency in the microbiota species that increased and decreased post intervention and could serve as microbiota based signatures for understanding and comparing different periodontal interventions in the future.

FUTURE RESEARCH

Future research in this area should be oriented towards examine different treatments while adopting the same methodologies for oral microbial profiling. Our findings highlight the importance of complete characterization of the periodontal microbiota to accurately evaluate the effectiveness of new therapeutic and diagnostic methods for periodontal disease treatment. The periodontal microbiota could be an alternative target for new therapies and should be monitored to better understand the efficacy of periodontal treatment outcomes.

Abbreviations

HTS: High throughput Sequencing

SRP: Scaling and root planing

PCR: Polymerase chain reaction

NGS: Next generation sequencing

PRISMA: Preferred Reporting Items for Systematic Review and Meta-Analysis

ROB: Risk of Bias

RCT: Randomised controlled clinical trial

RSV: ribosomal sequencing variant

PCoA: Principal coordinate analysis

PCA: Principal component analysis

SD: Standard deviation

SMD: Standardized mean difference

OMT: Oral microbiome transplant

OTU: Operation taxonomic unit

Declarations

• Ethics approval and consent to participate:

This was systematic review and was approved by the institution.

• Consent for publication:

Not applicable.

• Availability of data and materials:

All data generated or analysed during this study will be included in a published article [and its supplementary information files].

• Competing interests:

The authors declare that they have no competing interests.

• Funding:

Not applicable.

• Authors' contributions:

SN, study conception and design, search and selection, statistical evaluation and data interpretation, manuscript writing; SJP, Study conception, search and selection, analysis, data interpretation, manuscript preparation and writing; LW, study concept and design, critical reading the manuscript; PZ, data interpretation, critical reading the manuscript; KK, statistical analyses, data interpretation, critical reading the manuscript; LJ, statistical analyses, data interpretation, critical reading the manuscript. All authors have read and approved the manuscript.

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Figures

Figure 1

PRISMA flow chart of the included trials.

Figure 2

The most abundant species present across all the studies post intervention. The size of the bubble is related to the abundance. A) Decrease in species post intervention B) Increase in species post intervention

Figure 3

Meta-analysis of alpha diversity richness of pooled test (pre vs. post treatment) and pooled control groups (pre vs. post treatment) independently from baseline to post treatment

Figure 4

Meta-analysis of alpha diversity Shannon diversity index of pooled test (pre vs. post treatment) and pooled control groups (pre vs. post treatment) independently from baseline to post treatment.

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