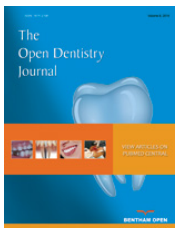

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Metagenomic Assessment of Different Interventions for Treatment of Chronic Periodontitis: A Systematic Review and Meta-Analysis

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Appendix A

Excluded studies by full text

	REFERENCE	REASON OF EXCLUSION
1.	(Badar *et al.*, 2019)	No metagenomics testing (ongoing study)
2.	(Flemmig *et al.*, 2011)	No metagenomics testing
3.	(Grzech-Leśniak, Gaspirc and Sculean, 2019)	No metagenomics testing
4.	(Leonhardt *et al.*, 2007)	No metagenomics testing
5.	(Sajedinejad *et al.*, 2018)	No metagenomics testing
6.	(Sanz-sánchez *et al.*, 2015)	No metagenomics testing
7.	(Ramich *et al.*, 2014)	No metagenomics testing
8.	(RAO, 2008)	No metagenomics testing
9.	(Yilmaz *et al.*, 2012)	No metagenomics testing
10.	(Herrero *et al.*, 2016)	In vitro study
11.	(Kovtun *et al.*, 2012)	In vitro study
12.	(Lee *et al.*, 2015)	In vitro study
13.	(Li *et al.*, 2018)	In vitro and animal study
14.	(Morelli *et al.*, 2017)	In vitro study
15.	(Yamada *et al.*, 2018)	In vitro and animal study
16.	(Zupančič *et al.*, 2018)	In vitro study
17.	('NCT02633345', 2015)	Participants not having periodontitis (Ongoing study)
18.	(Adams *et al.*, 2017)	Participants not having periodontitis
19.	(Rafeek *et al.*, 2019)	Not all participants had periodontitis
20.	(Schwarzberg *et al.*, 2014)	Not all participants had periodontitis
21.	(Teng *et al.*, 2016)	Participants not having periodontitis
22.	(Galimanas, 2014)	Observational study: Case-control study
23.	(Mason, 2016)	Observational study: Cross-sectional study
24.	(Moman, 2017)	Observational study: Case series and in vitro study for probiotics on cell line
25.	(Pozhitkov *et al.*, 2015)	Observational study: Cross sectional study
26.	(Belstrøm *et al.*, 2018)	Included smokers
27.	(Bizzarro *et al.*, 2016)	Included smokers
28.	(Valenza *et al.*, 2009)	Included smokers
29.	(Chen *et al.*, 2018)	Assessed only salivary sample
30.	(Szafrański, Winkel and Stiesch, 2017)	Review article

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Appendix B

1. Characteristics of included studies
(Califf *et al.*, 2017)

Methods	**Study design**: non-randomized clinical trial **Conducted in**: USA **Number of centers:** 1, graduate periodontology clinic at the Ostrow School of Dentistry of the University of Southern California (USC). **Recruitment period**: Not specified **Trial Registry:** Not specified **Funding source**: Not specified
Participants	**Number:** 34 patients; 17 per group **Inclusion criteria:** Patients with at least four separate teeth with a pocket depth of ≥6 mm
Interventions	**Group T:** 15 ml of a fresh solution of 0.25% sodium hypochlorite rinse. Clorox regular bleach (The Clorox Company, Oakland, CA) diluted with tap water served as the source of 0.25% sodium hypochlorite. **Group Ctl:** 15 ml of water rinse Participants were asked to rinse their mouths twice weekly for 30 s. Participants were also instructed in conventional oral hygiene, but they received no subgingival or supragingival scaling prior to the study.
Outcomes	1. 16S rRNA metagenome analysis by NGS 2. Shotgun metagenomics analysis 3. Metabolite extraction and analysis by ultraperformance liquid chromatography (UPLC) system **Sampling technique:** • Microbiological samples from 3- to 12-mm-deep periodontal pockets and from supragingival sites at all three study visits • three teeth were sampled per patient per time point with a sterile Gracey curette per sampled tooth. Samples from individual teeth were analyzed separately. • supragingival sampling, one sample pooled from three teeth with the heaviest plaque accumulation • After sampling, the paper points were stored in 200 µl of 1x phosphate-buffered saline solution and frozen immediately at -80ºC **Microbiome analysis platform:** Assessed by Illumina MiSeq platform + shotgun sequencing Sequence data were analyzed with QIIME version 1.9 **Follow up** at baseline (visit 1), at day 14 (visit 2), and at month 3

2. (Chang, 2012)

Methods	**Study design**: before-and-after study (preliminary of Shi et al 2018) **Conducted in**: Los Angelos, USA **Number of centers:** not specified **Recruitment period**: not specified **Trial Registry:** not specified **Funding source**: NIH/NIDCR PHS Grants RC1DE020298 and 1R01DE021574, and NIH PHS Grant No. U54HG004968.
Participants	**Number:** 4 patients **Inclusion criteria:** • Healthy subjects with generalized moderate to severe chronic periodontitis **Exclusion criteria:** • Subjects with a history of antibiotic therapy in the past 6 months • any history of smoking or diabetes
Interventions	Conventional periodontal therapy including scaling and root planing as well as oral hygiene instructions

(Contd....)

Outcomes	1. Clinical parameters: a. gingival index, b. recession, c. pocket depth d. bleeding on probing 2. Microbiological results: a. subgingival community profile b. abundance **Sampling technique:** • subgingival plaque samples were taken from 4 sites from each patient • The subgingival sample obtained with a sterile curette (Hu-Friedy Mfg. Co., Inc., Chicago, IL). • The sampled plaque was suspended directly in 300 µl of ATL buffer (Qiagen, Inc., Valencia,CA) containing 0.25 ml of 0.1 mm glass beads (BioSpec Products, Inc., Bartlesville, OK) and immediately transported to the laboratory **Microbiological sample analysis:** DNA was extracted from the samples with the QIAamp DNA micro kit (Qiagen, Inc.) 1. Shotgun sequencing Illumina GAIIx sequencing platform (Illumina, Inc., San Diego, CA) 2. 16S rRNA clone library using sanger sequencing (ABI 3730*xl* sequencer) 3. Reference genome alignment sequencing analyses **Follow up** at baseline and 4-6 weeks

3. (Hagenfeld *et al.*, 2018)

Methods	**Study design**: RCT **Conducted in**: Germany **Number of centers:** multicenter: Medizinische Fakultät der Humboldt Universität Berlin (Charité) [Berlin]; Universitätsklinikum Carl Gustav Carus, Zentrum für Zahn-, Mund- und Kieferheilkunde [Dresden]; Zentrum der Zahn-, Mund- und Kieferheilkunde (Carolinum), Poliklinik für Parodontologie [Frankfurt]; Justus-Liebig-Universität Gießen, Poliklinik für Parodontologie [Giessen]; Universitätsklinikum Greifswald, Poliklinik für Zahnerhaltung, Parodontologie und Kinderzahnheilkunde [Greifswald]; Universitätsklinikum Heidelberg, Poliklinik für Zahnerhaltungskunde, Sektion Parodontologie [Heidelberg]; University Hospital Muenster, Dept. of Periodontology [Muenster]; Universität Würzburg, Poliklinik für Parodontologie [Würzburg] **Recruitment period**: October 2008-December 2011 **Trial Registry:** NCT00707369 **Funding source**: DFG grant: EH 365/1-1
Participants	**Number: 96** patients **Inclusion criteria:** 1. patients with untreated chronic periodontitis (localized: <30% and generalized:≥ 30% of teeth with moderate: ≥ 3mm to <5mm and severe: ≥ 5mm attachment loss. 2. non-smoking patients with localized severe and generalized moderate chronic periodontitis **Exclusion criteria:** 1. if they show confirmed or assumed allergies or hyper-sensitive skin reactions against amoxicillin (or other penicillins or other ingredients of Amoxicillin-ratiopharm® 500mg), metronidazole (or other 5-nitroimidazoles and ingredients of Flagyl® 400mg), systemic diseases or conditions, or show confirmed lactose intolerance; 2. have Down's syndrome; 3. known AIDS/HIV; 4. regularly take systemic medication affecting the periodontal conditions, e.g. phenytoin, nifedipine, and/or steroid drugs; 5. professional periodontal therapy during 6 months prior to baseline; 6. require antibiotic treatment for dental appointments; 7. are undergoing or require extensive dental or orthodontic treatment; 8. are pregnant or breastfeeding; 9. have rampant caries; 10. any oral or extraoral piercing in or around the oral cavity with ornaments or accessory jewelry; 11. are dental students or dental professionals; 12. have participated in a clinical dental trial in the six months preceding the study; 13. cognitive deficits.
Interventions	**Group A:** mechanical debridement + 500 mg amoxicillin and 400 mg metronidazole three times daily for 7 days (antibiotic) **Group P:** mechanical debridement and placebo (placebo) Supportive periodontal therapy was performed in three months intervals over a 24-months period

(Contd....)

Outcomes	1. clinical variables: a. %PPD: percentage of tooth sites with pocket depth ≥5 mm b. %BOP: percentage of tooth sites with bleeding on probing c. %RAL, percentage of tooth sites with further relative attachment loss of ≥1.3mm between baseline and 2 months after therapy; 2. microbiome variables a. Richness b. Evenness c. Diversity d. Dissimilarity **Sampling technique:** • Subgingival specimens from 4 teeth with a probing depth of ≥6 mm, one in each quadrant. • One sterile paper point (ISO45, Roeko Dental, Langenau, Germany) was inserted for 10 seconds in each site and all paper points were removed and pooled in one sterile collection tube • Samples were stored at −20℃ until further use. **Microbiome analysis platform:** DNA Purified by QiaAmp Mini DNA-Isolation Kit (Qiagen, Hilden, Germany) Sequencing by Illumina MiSeq sequencing Sequence processing by R language environment v.3.4.3 and RStudio v.1.0.153, following the DADA2 workflow Follow up 2 months

4. (Hagenfeld *et al.*, 2019) for (Harks *et al.*, 2016)

Methods	**Study design**: RCT **Conducted in**: Germany **Number of centers:** two centers: Dept. of Periodontology and Restorative Dentistry, University Hospital, Muenster, Germany, and Dept. of Periodontology, University Hospital, Wuerzburg, Germany. **Recruitment period**: March 2011- September 2011 **Trial Registry:** NCT02697539 **Funding source**: Kurt Wolff GmbH, Bielefeld, Germany
Participants	**Number:** The main study included **70** patients (sample analysis of a previously published study Harks et al) **Hagefeld et al** analyzed samples from only 41 patients **Inclusion criteria:** • Patients suffering from untreated localized mild-to-moderate chronic periodontitis • pocket probing depths (PPD) of ≥4 mm at a minimum of 4 teeth (except third molars). • Age range: 18-75 years. • Patients must have had at least 10 natural teeth (except third molars) • nonsmokers **Exclusion criteria:** • known systemic diseases that may influence the periodontal conditions • regular consumption of drugs that may interfere with periodontal conditions. • Patients undergoing or requiring extensive dental or • orthodontic treatment, • pregnant or breastfeeding • patients undergoing professional periodontal therapy during the 6 months prior to baseline • patients with periodontal pockets ≥6 mm in more than 2 sextants. Group 1: 35 randomised, 34 analyed (1 loss to follow up) Group 2: 35 randomised, 33 analyzed (2 loss to follow up) For hagenfeld samples from: Group1: 21 patients and Group 2: 21 patients
Interventions	**Group 1:** zinc-substituted carbonated hydroxyapatite dentifrice (mHA group, BioRepair, Wolff, Bielefeld, Germany) **Group 2:** dentifrice containing an amine fluoride/stannous fluoride (AmF/SnF2 group, Meridol, CP GABA, Hamburg, Germany) no further oral hygiene instructions After 4 weeks, mechanical periodontal therapy was performed according to the at baseline recorded clinical measurements. All patients were advised to keep brushing their teeth exclusively with the originally provided toothpaste

(Contd....)

Outcomes	1. clinical variables: (outcomes of the original article Harks et al) a. PFR (plaque formation rate) b. Gingival index (GI) c. Plaque index (PI) d. Bleeding on probing (BOP) e. Pocket probing depth (PPD) f. Recession depth (REC) g. AL (Attachment loss) 2. microbiome variables a. Ribosomal sequence variants (RSV) b. Diversity **Sampling technique:** • supragingival plaque (buccal/lingual and interproximal) from 4 sites per sample tooth and 2 subgingival specimens, one in each quadrant. • One sterile paper point (ISO45 ISO 45, Roeko, Ulm Germany) was inserted for 10 seconds in each site and all paper points were removed and pooled in in transport tubes containing 500 µl Ringer-Glycerin Solution, • Samples were stored in liquid nitrogen until further use. (Harks et al) • Hagenfeld stated samples were stored at −20℃ **Microbiome analysis platform:** • DNA Purified by QiaAmp Mini DNA-Isolation Kit (Qiagen, Hilden, Germany) • Sequencing by Illumina MiSeq sequencing • Sequence processing by R language environment v.3.4.3 and RStudio v.1.0.153, following the DADA2 workflow **Follow up** at Baseline, 4 weeks and 12 weeks

5. (Jünemann *et al.*, 2012)

Methods	**Study design**: RCT ***pilot study of Hagenfeld et al, 2018*** **Conducted in**: Germany **Number of centers:** multi-center **Recruitment period:** **Trial Registry:** ISRCTN64254080 (NCT00707369) **Funding source**: a grant of the German Federal Ministry of Education and Research (BMBF) in the framework of the ParoPhylo project (0313801N).
Participants	**Number: 4** patients (2 per group) **Inclusion criteria:** • non-smokers, • generalized • severe chronic periodontitis, i.e. more than 38% of sites with pocket • probing depths of 6 mm or more
Interventions	**Experimental (Ex):** mechanical debridement + 500 mg amoxicillin and 400 mg metronidazole three times daily for 7 days. **Control (Co):** mechanical debridement + placebo
Outcomes	Same outcomes as **Hagefeld et al, 2018** Same sampling technique as **Hagefeld et al, 2018** **Microbiome analysis platform:** purified with the QiaAmp Mini DNA-Isolation Kit (Qiagen, Hilden, Germany). Sequencing of the amplicon libraries was carried out on the Ion Torrent Personal Genome Machine (PGM) system using the Ion Sequencing 200 kit (all Life Technologies) Sequence processing by UCHIME algorithm and statistical software suite R v 2.9.10 and the vegan R-package **Follow up** at 2 months

6. (Nakano *et al.*, 2017)

Methods	**Study design**: RCT **Conducted in**: Japan **Number of centers:**1: Showa University School of Dentistry, Tokyo, Japan **Recruitment period**: not specified **Trial Registry:** UMIN000015706 **Funding source**: research grants from Morinaga Milk Industry.

(Contd....)

Participants	**Number:** 46 participants **Inclusion criteria:** Adults aged 65 years and older with tongue coating. **Exclusion criteria:** (i) eating pureed and finely-chopped meals; (ii) receiving parenteral nutrition; (iii) receiving treatment for dental disease (except adjustment of dentures, oral hygiene instructions); (iv) history of allergy to milk; (v) received antibiotic treatment in the past 1 month, or expected to receive it in the near future; (vi) use of oral care products for prevention of oral malodor or improvement of oral hygiene; (vii) regular consumption of LF or LPOcontaining food or oral care products; (viii) Presence of exacerbating diseases of the liver, kidney, heart, lung, gastro-intestine, blood, endocrine system, and metabolic system. **Test:** 24 participants: 1 lost to follow up, 3 violated the eligibility criteria **Placebo:** 22, 5 violated the eligibility criteria.
Interventions	**Test Group:** tablets contain 80 mg of LF+LPO powder (Lactoferrin + lactoperoxidase) (Orabarrier; Morinaga Milk Industry, Tokyo, Japan): 20 mg of LF, 2.6 mg of LPO and 2.6 mg of glucose oxidase. LF and LPO had been purified from bovine milk. Glucose oxidase was obtained from Penicillium chrysogenum. The LF+LPO powder also contained glucose and pH-adjusting agents that support the effects of the active ingredients **Placebo Group:** The placebo tablets contained dextrin and coloring materials instead of LF+LPO powder. Participants were asked to suck a tablet after every meal for 8 weeks. They were instructed not to change their oral hygiene regimens throughout the study period.
Outcomes	1. clinical variables a. O'Leary's plaque control record (O'Leary's PCR) b. probing pocket depth (PPD) c. Bleeding on probing (BOP) d. VSC, volatile sulfur compound in oral air analyzed with a portable gas chromatography device (OralChroma; FIS, Itami, Japan) 2. microbiological variables: a. Diversity indices (Shannon index, Chao1, number of observed species [number of OTUs] and PD whole tree) and distances between samples (UniFrac distance) b. microbiota composition c. abundance **Sampling technique:** • Tongue coating and supragingival plaque were collected using sterile swabs (Puritan Medical Products, Guilford, ME, USA) under constant pressure • The swabs were suspended in 1mL sterile saline stored in vials **Microbiome analysis platform:** • Bacterial DNA was extracted using a commercial kit (QIAamp; Qiagen, Hilden, Germany) • Amplified using PCR with a TaKaRa Ex Taq HS kit (TaKaRa Bio, Shiga, Japan) • QIAquick 96 PCR Purification Kit (Qiagen) • Sequencing by llumina MiSeq: • Analysis processing by QIIME software package version 1.8.0 (25, 26) and BLAST program (ver. 2.2.22) **Follow up** at baseline, 4 and 8 weeks

7. (Queiroz *et al.*, 2017)

Methods	**Study design**: RCT **Conducted in**: Brazil **Number of centers:**1: Graduate Periodontal Clinic of the Piracicaba Dental School, Piracicaba, Brazil **Recruitment period**: March to October 2008 **Trial Registry:** NCT02907528 **Funding source**: grants from Sao Paulo Research Foundation (FAPESP) (process 2013/50389-1), Coordination for the Improvement of Higher Education Personnel (CAPES), and National Institute of Dental and Craniofacial Research (NIDCR) . NIDCR grant T32-DE014320. The sequencing assay was supported through NIDCR grant R01-DE022579

(Contd....)

Participants	**Number: 41** patients **Inclusion criteria:** • systemically healthy • non-smokers • ≥35 years of age • with a buccal Class II furcation defect on mandibular molars due to Chronic Periodontitis, with: 1) a horizontal furcation probing depth (PD) >4 mm; 2) bleeding on probing; 3) <1 mm of gingival recession after non-surgical therapy; 4) >2 mm of keratinized gingiva; and 5) <2 mm of interproximal bone loss were identified **Exclusion criteria:** 1) pregnant or lactating; 2) had received antibiotics in the last 3 months or required antibiotics prior to dental therapy; 3) were taking prescribed anti-inflammatory agents.
Interventions	1**) BONE group:** bone substitute consisting of b-TCP/HA (beta tricalcium phosphate/hydroxyapatite) 2) **BONE+EMD group:** mixture of EMD (Enamel Matrix derivative) and bone substitute consisting of b-TCP/HA; and 3**) EMD group.**
Outcomes	Microbiological analysis of subgingival plaque sample **Sampling technique:** • Sterile paper points were inserted to the base of the furcation defect for 30 seconds, • placed in sterile tubes containing 300 mL of Tris–EDTA 0.1 mM and immediately stored at -20℃. **Microbiome analysis platform:** • DNA purification kiti MiniAmp kit, QIAGEN, Valencia, CA • Multiplexed bacterial tag-encoded FLX amplicon pyrosequencing • Results were visualized with the Python library matplotlib as well as open source programs for statistical analysis and data visualization (R project and Interactive Tree of Life) Samples were collected at baseline and at the 3- and 6-month re-evaluation visits.

8. (Shi *et al.*, 2015)

Methods	**Study design**: Controlled Before-and-after study ***(Full study of Chang, 2012)*** **Conducted in**: USA **Number of centers:** UCLA **Recruitment period**: 2009-2010 **Trial Registry:** not specified **Funding source**: NIH/NIDCR grants RC1 DE020298 and R01 DE021574.
Participants	**Number: 12** patients **Inclusion criteria:** Adult volunteers with chronic periodontitis **Systemically healthy** The age of the patients ranged from 37 to 65 years with an average age of 53 years **Exclusion criteria:** Subjects with a history of antibiotic treatment in the past 6 months, history of smoking, or diabetes were excluded from the study.
Interventions	initial periodontal therapy: scaling and root planing (SRP), and oral hygiene instructions
Outcomes	Same **outcomes** as Chang, 2012 Same **sampling technique** except that sampling was performed for only 2 affected tooth sites rather than 4 in Chang, 2012 Same **metagenomics analysis** using Shotgun Illumina platform. But included seven subgingival samples of healthy individuals from the Human Microbiome Project The study included diseased, resolved and healthy groups. **Follow up:** baseline, 4 and 19 weeks (on average 60 days)

9. (Yamanaka *et al.*, 2012)

Methods	**Study design**: Before and after study **Conducted in**: Japan **Number of centers:**1: YA Dental Clinic in Yonago, Tottori, Japan **Recruitment period**: Not specified **Trial Registry:** IRCT 138904284413 N1 **Funding source**: Grants-in Aid for Young Scientist 23792517 (T.T.) and by Grants-in Aid for Scientific Research 20192403 (Y.Y.) from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

(Contd....)

Participants	**Number: 19** patients **Inclusion criteria:** • All subjects had at least 19 teeth. • generally healthy adults, • with no use of antibiotics or periodontal surgery during the preceding 6 months or during the periodontal therapy.
Interventions	periodontal therapy including scaling, curettage, tooth brushing instruction, and professional mechanical tooth cleaning, but not surgical intervention or antibiotics.
Outcomes	Bacterial composition of saliva and supragingival plaque samples **Sampling technique:** 1. Stimulated saliva samples 2. Sterile curettes were used to collect supragingival plaque from all tooth surfaces 3. Samples were stored at -30℃ until further **Metagenomic analysis:** Pyrosequencing (FLX instrument of Roche) **Follow up** at baseline and approximately 2 years after the first sample collection

1. ChiCTR-IOR-16008194

Characteristics of ongoing studies

Study name	Probiotic modulation of oral ecology.
Methods	**Study design**: case-control, for cases, cross-over study **Conducted in**: Hong Kong **Number of centers:** Faculty of Dentistry, University of Hong Kong **Funding source**: Faculty of Dentistry, University of Hong Kong
Participants	**Number:** 14 **Inclusion criteria:** 1. Systemically healthy, 2. non-smoker, 3. Adult subjects (30-65 years) **Exclusion criteria:** 1. A recent history of trauma or tooth extractions; 2. Pregnant or lactating females; 3. Diagnosis of periimplantitis (PD >=5mm with signs of supporting bone loss); 4. Implant sites treated with augmentation procedures; 5. Subjects who underwent antibiotic therapy within past 3 months; 6. Radiation therapy in the head and neck area, HIV, Tuberculosis, hepatitis or other infectious diseases or uncontrolled diabetes, ischaemic heart disease, thyroid disorders or psychological problems.
Interventions	**Group1:** Diagnosed as having generalized chronic periodontitis (CP); **Group2:** Diagnosed as periodontally healthy controls (H); **Group3:** Partially edentulous and have received implant therapy, with periimplant health and ready to receive a definitive prosthesis. **Intervention:** Probiotic supplement containing Lactobacillus strains; DSM 17938 and ATCC PTA 5289 (ProdentisTM, BioGaia, Lund Sweden) is regulated as food supplement in Hong Kong **Control:** placebo
Outcomes	Microbiome Analysis from saliva and plaque samples around teeth and implants
Starting date	1/5/2016
Contact information	Principal investigator: Aneesha Acharya. Oral Rehabilitation Department (Implant Dentistry), The Prince Philip Dental Hospital, 34- Hospital Road, Hong Kong. **Telephone:** +85251307181. **Email:** aneesha.a2@gmail.com

Appendix C

Risk of bias assessment

NON- RCTs:

The Risk Of Bias In Non-randomized Studies – of Interventions (ROBINS-I) assessment tool

(Califf et al., 2017)

Risk of bias assessment (cohort-type studies)

Risk of bias assessment (cohort-type studies)

Responses underlined in green are potential markers for low risk of bias, and responses in red are potential markers for a risk of bias. Where questions relate only to sign posts to other questions, no formatting is used.

Bias domain	Signalling questions	Elaboration	Response options
Bias due to confounding	1.1 Is there potential for confounding of the effect of intervention in this study? **If N/PN to 1.1:** the study can be considered to be at low risk of bias due to confounding and no further signalling questions need be considered	In rare situations, such as when studying harms that are very unlikely to be related to factors that influence treatment decisions, no confounding is expected and the study can be considered to be at low risk of bias due to confounding, equivalent to a fully randomized trial. There is no NI (No information) option for this signalling question.	Y/PY/PN/N
	If Y/PY to 1.1: determine whether there is a need to assess time-varying confounding:		
	1.2. Was the analysis based on splitting participants' follow up time according to intervention received? **If N/PN**, answer questions relating to baseline confounding (1.4 to 1.6) **If Y/PY**, proceed to question 1.3.	If participants could switch between intervention groups then associations between intervention and outcome may be biased by time-varying confounding. This occurs when prognostic factors influence switches between intended interventions.	NA/Y/PY/PN/N/ NI
	1.3. Were intervention discontinuations or switches likely to be related to factors that are prognostic for the outcome? **If N/PN**, answer questions relating to baseline confounding (1.4 to 1.6) **If Y/PY**, answer questions relating to both baseline and time-varying confounding (1.7 and 1.8)	If intervention switches are unrelated to the outcome, for example when the outcome is an unexpected harm, then time-varying confounding will not be present and only control for baseline confounding is required.	NA/Y/PY/PN/N/ NI
	Questions relating to baseline confounding only		
	1.4. Did the authors use an appropriate analysis method that controlled for all the important confounding domains?	Appropriate methods to control for measured confounders include stratification, regression, matching, standardization, and inverse probability weighting. They may control for individual variables or for the estimated propensity score. Inverse probability weighting is based on a function of the propensity score. Each method depends on the assumption that there is no unmeasured or residual confounding.	NA/Y/PY/PN/N/ NI
	1.5. **If Y/PY to 1.4**: Were confounding domains that were controlled for measured validly and reliably by the variables available in this study?	Appropriate control of confounding requires that the variables adjusted for are valid and reliable measures of the confounding domains. For some topics, a list of valid and reliable measures of confounding domains will be specified in the review protocol but for others such a list may not be available. Study authors may cite references to support the use of a particular measure. If authors control for confounding variables with no indication of their validity or reliability pay attention to the subjectivity of the measure. Subjective measures (e.g. based on self-report) may have lower validity and reliability than objective measures such as lab findings.	NA/Y/PY/PN/N/ NI
	1.6. Did the authors control for any post-intervention variables that could have been affected by the intervention?	Controlling for post-intervention variables that are affected by intervention is not appropriate. Controlling for mediating variables estimates the direct effect of intervention and may introduce bias. Controlling for common effects of intervention and outcome introduces bias.	NA/Y/PY/PN/N/ NI

(Contd....)

Bias domain	Signalling questions	Elaboration	Response options
	Questions relating to baseline and time-varying confounding		
	1.7. Did the authors use an appropriate analysis method that adjusted for all the important confounding domains and for time- varying confounding?	Adjustment for time-varying confounding is necessary to estimate the effect of starting and adhering to intervention, in both randomized trials and NRSI. Appropriate methods include those based on inverse probability weighting. Standard regression models that include time-updated confounders may be problematic if time-varying confounding is present.	NA/Y/PY/PN/N/ NI
	1.8. **If Y/PY to 1.7**: Were confounding domains that were adjusted for measured validly and reliably by the variables available in this study?	See 1.5 above.	NA/Y/PY/PN/N/ NI
	Risk of bias judgement	See Table 1.	**Low** / Moderate / Serious / Critical / NI
	Optional: What is the predicted direction of bias due to confounding?	Can the true effect estimate be predicted to be greater or less than the estimated effect in the study because one or more of the important confounding domains was not controlled for? Answering this question will be based on expert knowledge and results in other studies and therefore can only be completed after all of the studies in the body of evidence have been reviewed. Consider the potential effect of each of the unmeasured domains and whether all important confounding domains not controlled for in the analysis would be likely to change the estimate in the same direction, or if one important confounding domain that was not controlled for in the analysis is likely to have a dominant impact.	Favours experimental / Favours comparator / Unpredictable

Bias in selection of participants into the study	2.1. Was selection of participants into the study (or into the analysis) based on participant characteristics observed after the start of intervention? **If N/PN to 2.1:** go to 2.4 2.2. **If Y/PY to 2.1**: Were the post- intervention variables that influenced selection likely to be associated with intervention? 2.3 **If Y/PY to 2.2**: Were the post-intervention variables that influenced selection likely to be influenced by the outcome or a cause of the outcome?	This domain is concerned only with selection into the study based on participant characteristics observed *after* the start of intervention. Selection based on characteristics observed *before* the start of intervention can be addressed by controlling for imbalances between experimental intervention and comparator groups in baseline characteristics that are prognostic for the outcome (baseline confounding). Selection bias occurs when selection is related to an effect of either intervention or a cause of intervention **and** an effect of either the outcome or a cause of the outcome. Therefore, the result is at risk of selection bias if selection into the study is related to both the intervention and the outcome	Y/PY/PN/N/NI NA/Y/PY/PN/N/ NI NA/Y/PY/PN/N/ NI
	2.4. Do start of follow-up and start of intervention coincide for most participants?	If participants are not followed from the start of the intervention then a period of follow up has been excluded, and individuals who experienced the outcome soon after intervention will be missing from analyses. This problem may occur when prevalent, rather than new (incident), users of the intervention are included in analyses.	Y/PY/PN/N/NI

(Contd....)

	2.5. **If Y/PY to 2.2 and 2.3, or N/PN to 2.4**: Wer djustment techniques used that are likely to correct for the presence of selection biases?	It is in principle possible to correct for selection biases, for example by using inverse probability weights to create a pseudo-population in which the selection bias has been removed, or by modelling the distributions of the missing participants or follow up times and outcome events and including them using missing data methodology. However such methods are rarely used and the answer to this question will usually be "No".	NA/Y/PY/PN/N/ NI
	Risk of bias judgement	See Table 1.	**Low**/ Moderate / Serious / Critical / NI
	Optional: What is the predicted direction of bias due to selection of participants into the study?	If the likely direction of bias can be predicted, it is helpful to state this. The direction might be characterized either as being towards (or away from) the null, or as being in favour of one of the interventions.	Favours experimental / Favours comparator/ C) Towards null /Away from null /// Unpredictable

Bias in classification of interventions	3.1 Were intervention groups clearly defined?	A pre-requisite for an appropriate comparison of interventions is that the interventions are well defined. Ambiguity in the definition may lead to bias in the classification of participants. For individual-level interventions, criteria for considering individuals to have received each intervention should be clear and explicit, covering issues such as type, setting, dose, frequency, intensity and/or timing of intervention. For population-level interventions (e.g. measures to control air pollution), the question relates to whether the population is clearly defined, and the answer is likely to be 'Yes'.	Y/PY/PN/N/NI
	3.2 Was the information used to define intervention groups recorded at the start of the intervention?	In general, if information about interventions received is available from sources that could not have been affected by subsequent outcomes, then differential misclassification of intervention status is unlikely. Collection of the information at the time of the intervention makes it easier to avoid such misclassification. For population-level interventions (e.g. measures to control air pollution), the answer to this question is likely to be 'Yes'.	Y/PY/PN/N/NI
	3.3 Could classification of intervention status have been affected by knowledge of the outcome or risk of the outcome?	Collection of the information at the time of the intervention may not be sufficient to avoid bias. The way in which the data are collected for the purposes of the NRSI should also avoid misclassification.	Y/PY/PN/N/NI
	Risk of bias judgement	See Table 1.	Low / Moderate / **Serious** / Critical / NI
	Optional: What is the predicted direction of bias due to measurement of outcomes or interventions?	If the likely direction of bias can be predicted, it is helpful to state this. The direction might be characterized either as being towards (or away from) the null, or as being in favour of one of the interventions.	Favours experimental / Favours comparator Towards null /Away from null / Unpredictable

Bias due to deviations from intended interventions	**If your aim for this study is to assess the effect of assignment to intervention, answer questions 4.1 and 4.2**		
	4.1. Were there deviations from the intended intervention beyond what would be expected in usual practice?	Deviations that happen in usual practice following the intervention (for example, cessation of a drug intervention because of acute toxicity) are part of the intended intervention and therefore do not lead to bias in the effect of assignment to intervention. Deviations may arise due to expectations of a difference between intervention and comparator (for example because participants feel unlucky to have been assigned to the comparator group and therefore seek the active intervention, or components of it, or other interventions). Such deviations are not part of usual practice, so may lead to biased effect estimates. However these are not expected in observational studies of individuals in routine care.	Y/PY/PN/N/NI

(Contd....)

	4.2. **If Y/PY to 4.1**: Were these deviations from intended intervention unbalanced between groups *and* likely to have affected the outcome?	Deviations from intended interventions that do not reflect usual practice will be important if they affect the outcome, but not otherwise. Furthermore, bias will arise only if there is imbalance in the deviations across the two groups.	NA/Y/PY/PN/N/
	If your aim for this study is to assess the effect of starting and adhering to intervention, answer questions 4.3 to 4.6		
	4.3. Were important co-interventions balanced across intervention groups?	Risk of bias will be higher if unplanned co-interventions were implemented in a way that would bias the estimated effect of intervention. Co-interventions will be important if they affect the outcome, but not otherwise. Bias will arise only if there is imbalance in such co-interventions between the intervention groups. Consider the co-interventions, including any pre-specified co-interventions, that are likely to affect the outcome and to have been administered in this study. Consider whether these co-interventions are balanced between intervention groups.	Y/PY/PN/N/NI
	4.4. Was the intervention implemented successfully for most participants?	Risk of bias will be higher if the intervention was not implemented as intended by, for example, the health care professionals delivering care during the trial. Consider whether implementation of the intervention was successful for most participants.	Y/PY/PN/N/NI
	4.5. Did study participants adhere to the assigned intervention regimen?	Risk of bias will be higher if participants did not adhere to the intervention as intended. Lack of adherence includes imperfect compliance, cessation of intervention, crossovers to the comparator intervention and switches to another active intervention. Consider available information on the proportion of study participants who continued with their assigned intervention throughout follow up, and answer 'No' or 'Probably No' if this proportion is high enough to raise concerns. Answer 'Yes' for studies of interventions that are administered once, so that imperfect adherence is not possible. We distinguish between analyses where follow-up time after interventions switches (including cessation of intervention) is assigned to (1) the new intervention or (2) the original intervention. (1) is addressed under time-varying confounding, and should not be considered further here	Y/PY/PN/N/NI
	4.6. **If N/PN to 4.3, 4.4 or 4.5**: Was an appropriate analysis used to estimate the effect of starting and adhering to the intervention?	It is possible to conduct an analysis that corrects for some types of deviation from the intended intervention. Examples of appropriate analysis strategies include inverse probability weighting or instrumental variable estimation. It is possible that a paper reports such an analysis without reporting information on the deviations from intended intervention, but it would be hard to judge such an analysis to be appropriate in the absence of such information. Specialist advice may be needed to assess studies that used these approaches. If everyone in one group received a co-intervention, adjustments cannot be made to overcome this	NA/Y/PY/PN/N/ NI
	Risk of bias judgment	See Table 2	**LOW**
	Optional: What is the predicted direction of bias due to deviations from the intended interventions?	If the likely direction of bias can be predicted, it is helpful to state this. The direction might be characterized either as being towards (or away from) the null, or as being in favour of one of the interventions.	

Bias due to missing data	5.1 Were outcome data available for all, or nearly all, participants?	"Nearly all" should be interpreted as "enough to be confident of the findings", and a suitable proportion depends on the context. In some situations, availability of data from 95% (or possibly 90%) of the participants may be sufficient, providing that events of interest are reasonably common in both intervention groups. One aspect of this is that review authors would ideally try and locate an analysis plan for the study.	Y/PY/PN/N/NI
	5.2 Were participants excluded due to missing data on intervention status?	Missing intervention status may be a problem. This requires that the *intended* study sample is clear, which it may not be in practice.	Y/PY/PN/N/NI

(Contd....)

	5.3 Were participants excluded due to missing data on other variables needed for the analysis?	This question relates particularly to participants excluded from the analysis because of missing information on confounders that were controlled for in the analysis.	Y/PY/PN/N/NI
	5.4 **If PN/N to 5.1, or Y/PY to 5.2 or 5.3**: Are the proportion of participants and reasons for missing data similar across interventions?	This aims to elicit whether either (i) differential proportion of missing observations or (ii) differences in reasons for missing observations could substantially impact on our ability to answer the question being addressed. "Similar" includes some minor degree of discrepancy across intervention groups as expected by chance.	NA/Y/PY/PN/N/
	5.5 **If PN/N to 5.1, or Y/PY to 5.2 or 5.3**: Is there evidence that results were robust to the presence of missing data?	Evidence for robustness may come from how missing data were handled in the analysis and whether sensitivity analyses were performed by the investigators, or occasionally from additional analyses performed by the systematic reviewers. It is important to assess whether assumptions employed in analyses are clear and plausible. Both content knowledge and statistical expertise will often be required for this. For instance, use of a statistical method such as multiple imputation does not guarantee an appropriate answer. Review authors should seek naïve (complete-case) analyses for comparison, and clear differences between complete-case and multiple imputation-based findings should lead to careful assessment of the validity of the methods used.	NA/Y/PY/PN/N/
	Risk of bias judgement	See Table 2	**Low** / Moderate / Serious / Critical / NI
	Optional: What is the predicted direction of bias due to missing data?	If the likely direction of bias can be predicted, it is helpful to state this. The direction might be characterized either as being towards (or away from) the null, or as being in favour of one of the interventions.	Favours experimental / Favours comparator/ Towards null /Away from null / Unpredictable

Bias in measurement of outcomes	6.1 Could the outcome measure have been influenced by knowledge of the intervention received?	Some outcome measures involve negligible assessor judgment, e.g. all-cause mortality or non-repeatable automated laboratory assessments. Risk of bias due to measurement of these outcomes would be expected to be low.	Y/PY/PN/N/NI
	6.2 Were outcome assessors aware of the intervention received by study participants?	If outcome assessors were blinded to intervention status, the answer to this question would be 'No'. In other situations, outcome assessors may be unaware of the interventions being received by participants despite there being no active blinding by the study investigators; the answer this question would then also be 'No'. In studies where participants report their outcomes themselves, for example in a questionnaire, the outcome assessor is the study participant. In an observational study, the answer to this question will usually be 'Yes' when the participants report their outcomes themselves.	Y/PY/PN/N/NI
	6.3 Were the methods of outcome assessment comparable across intervention groups?	Comparable assessment methods (i.e. data collection) would involve the same outcome detection methods and thresholds, same time point, same definition, and same measurements.	Y/PY/PN/N/NI
	6.4 Were any systematic errors in measurement of the outcome related to intervention received?	This question refers to differential misclassification of outcomes. Systematic errors in measuring the outcome, if present, could cause bias if they are related to intervention or to a confounder of the intervention-outcome relationship. This will usually be due either to outcome assessors being aware of the intervention received or to non-comparability of outcome assessment methods, but there are examples of differential misclassification arising despite these controls being in place.	Y/PY/PN/N/NI
	Risk of bias judgement	See Table 2	**Low** / Moderate / Serious / Critical / NI
	Optional: What is the predicted direction of bias due to measurement of outcomes?	If the likely direction of bias can be predicted, it is helpful to state this. The direction might be characterized either as being towards (or away from) the null, or as being in favour of one of the interventions.	Favours experimental / Favours comparator / Towards null /Away from null / Unpredictable

(Contd....)

Bias in selection of the reported result	Is the reported effect estimate likely to be selected, on the basis of the results, from... 7.1. ... multiple outcome *measurements* within the outcome domain?	For a specified outcome domain, it is possible to generate multiple effect estimates for different measurements. If multiple measurements were made, but only one or a subset is reported, there is a risk of selective reporting on the basis of results.	Y/PY/PN/N/NI
	7.2 ... multiple *analyses* of the intervention-outcome relationship?	Because of the limitations of using data from non-randomized studies for analyses of effectiveness (need to control confounding, substantial missing data, etc), analysts may implement different analytic methods to address these limitations. Examples include unadjusted and adjusted models; use of final value vs change from baseline vs analysis of covariance; different transformations of variables; a continuously scaled outcome converted to categorical data with different cut-points; different sets of covariates used for adjustment; and different analytic strategies for dealing with missing data. Application of such methods generates multiple estimates of the effect of the intervention versus the comparator on the outcome. If the analyst does not pre-specify the methods to be applied, and multiple estimates are generated but only one or a subset is reported, there is a risk of selective reporting on the basis of results.	Y/PY/PN/N/NI
	7.3 ... different *subgroups*?	Particularly with large cohorts often available from routine data sources, it is possible to generate multiple effect estimates for different subgroups or simply to omit varying proportions of the original cohort. If multiple estimates are generated but only one or a subset is reported, there is a risk of selective reporting on the basis of results.	Y/PY/PN/N/NI
	Risk of bias judgement	See Table 2	**Low** / Moderate / Serious / Critical / NI
	Optional: What is the predicted direction of bias due to selection of the reported result?	If the likely direction of bias can be predicted, it is helpful to state this. The direction might be characterized either as being towards (or away from) the null, or as being in favour of one of the interventions.	Favours experimental / Favours comparator 1 Towards null /Away from null / Unpredictable

Overall bias	**Risk of bias judgement**	See Table 3.	Low / Moderate / **Serious**/ Critical / NI
	Optional: What is the overall predicted direction of bias for this outcome?		Favours experimental / Favours comparator / Towards null /Away from null / Unpredictable

FOR BEFORE AND AFTER STUDIES

QUALITY ASSESSMENT TOOL OR QUANTITATIVE STUDIES

1. (Chang, 2012)

COMPONENT RATINGS

A) SELECTION BIAS

(Q1) Are the individuals selected to participate in the study likely to be representative of the target population?

1 Very likely

2 Somewhat likely

3 Not likely

4 Can't tell

(Q2) What percentage of selected individuals agreed to participate?

1 80 - 100% agreement

2 60 – 79% agreement

3 less than 60% agreement

4 Not applicable

5 Can't tell

RATE THIS SECTION	STRONG	MODERATE	WEAK
See dictionary	1	2	3

B) STUDY DESIGN

Indicate the study design

1 Randomized controlled trial

2 Controlled clinical trial

3 Cohort analytic (two group pre + post)

4 Case-control

5 Cohort (one group pre + post (before and after))

6 Interrupted time series

7 Other specify _____

8 Can't tell

Was the study described as randomized? If NO, go to Component C.

No Yes

If Yes, was the method of randomization described? (See dictionary)

No Yes

If Yes, was the method appropriate? (See dictionary)

No Yes

RATE THIS SECTION	STRONG	MODERATE	WEAK
See dictionary	1	2	3

C) CONFOUNDERS

(Q1) Were there important differences between groups prior to the intervention?

1 Yes

2 No

3 Can't tell

The following are examples of confounders:

1 Race

2 Sex

3 Marital status/family

4 Age

5 SES (income or class)

6 Education

7 Health status

8 Pre-intervention score on outcome measure

(Q2) If yes, indicate the percentage of relevant confounders that were controlled (either in the design (e.g. stratification, matching) or analysis)?

1 80 – 100% (most)

2 60 – 79% (some)

3 Less than 60% (few or none)

4 Can't Tell

RATE THIS SECTION	STRONG	MODERATE	WEAK
See dictionary	1	2	3

D) BLINDING

(Q1) Was (were) the outcome assessor(s) aware of the intervention or exposure status of participants?

1 Yes

2 No

3 Can't tell

(Q2) Were the study participants aware of the research question?

1 Yes

2 No

3 Can't tell

RATE THIS SECTION	STRONG	MODERATE	WEAK
See dictionary	1	2	3

E) DATA COLLECTION METHODS

(Q1) Were data collection tools shown to be valid?

1 Yes

2 No

3 Can't tell

(Q2) Were data collection tools shown to be reliable?

1 Yes

2 No

3 Can't tell

RATE THIS SECTION	STRONG	MODERATE	WEAK
See dictionary	1	2	3

F) WITHDRAWALS AND DROP-OUTS

(Q1) Were withdrawals and drop-outs reported in terms of numbers and/or reasons per group?

1 Yes

2 No

3 Can't tell

4 Not Applicable (*i.e.* one time surveys or interviews)

(Q2) Indicate the percentage of participants completing the study. (If the percentage differs by groups, record the lowest).

1 80-100%

2 60-79%

3 Less than 60%

4 Can't tell

5 Not Applicable (i.e. Retrospective case-control)

RATE THIS SECTION	STRONG	MODERATE	WEAK	
See dictionary	1	2	3	Not Applicable

G) **INTERVENTION INTEGRITY**

(Q1) What percentage of participants received the allocated intervention or exposure of interest?

1 80-100%

2 60-79%

3 Less than 60%

4 Can't tell

(Q2) Was the consistency of the intervention measured?

1 Yes

2 No

3 Can't tell

(Q3) Is it likely that subjects received an unintended intervention (contamination or co-intervention) that may influence the results?

4 Yes

5 No

6 Can't tell

H) ANALYSES

(Q1) Indicate the unit of allocation (circle one)

community organization/institution practice/office individual

(Q2) Indicate the unit of analysis (circle one)

community organization/institution practice/office individual

(Q3) Are the statistical methods appropriate for the study design?

1 Yes

2 No

3 Can't tell

(Q4) Is the analysis performed by intervention allocation status (i.e. intention to treat) rather than the actual intervention received?

1 Yes

2 No

3 Can't tell

GLOBAL RATING

COMPONENT RATINGS

Please transcribe the information from the gray boxes on pages 1-4 onto this page. See dictionary on how to rate this section.

A	**SELECTION BIAS**	**STRONG**	**MODERATE**	**WEAK**	-
-	-	1	2	3	-
B	STUDY DESIGN	**STRONG**	**MODERATE**	**WEAK**	-
-	-	1	2	3	-
C	CONFOUNDERS	**STRONG**	**MODERATE**	**WEAK**	-
-	-	1	2	3	-
D	BLINDING	**STRONG**	**MODERATE**	**WEAK**	-
-	-	1	2	3	-
E	DATA COLLECTION METHOD	**STRONG**	**MODERATE**	**WEAK**	-
-	-	1	2	3	-
F	WITHDRAWALS AND DROPOUTS	**STRONG**	**MODERATE**	**WEAK**	-
-	-	1	2	3	Not Applicable

GLOBAL RATING FOR THIS PAPER (circle one):

1 STRONG (no WEAK ratings)

2 MODERATE (one WEAK rating)

3 WEAK (two or more WEAK ratings)

With both reviewers discussing the ratings:

Is there a discrepancy between the two reviewers with respect to the component (A-F) ratings?

No Yes

If yes, indicate the reason for the discrepancy

1 Oversight

2 Differences in interpretation of criteria

3 Differences in interpretation of study

Final decision of both reviewers (circle one):

1 STRONG

2 MODERATE

3 WEAK

1. **(Shi *et al.*, 2015)**

COMPONENT RATINGS

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(Q4) Is the analysis performed by intervention allocation status (i.e. intention to treat) rather than the actual intervention received?

1 Yes

2 No

4 Can't tell

GLOBAL RATING

COMPONENT RATINGS

Please transcribe the information from the gray boxes on pages 1-4 onto this page. See dictionary on how to rate this section.

A	**SELECTION BIAS**	**STRONG**	**MODERATE**	**WEAK**	
		1	2	3	
B	**STUDY DESIGN**	**STRONG**	**MODERATE**	**WEAK**	
		1	2	3	
C	**CONFOUNDERS**	**STRONG**	**MODERATE**	**WEAK**	
		1	2	3	
D	**BLINDING**	**STRONG**	**MODERATE**	**WEAK**	
		1	2	3	
E	**DATA COLLECTION METHOD**	**STRONG**	**MODERATE**	**WEAK**	
		1	2	3	
F	**WITHDRAWALS AND DROPOUTS**	**STRONG**	**MODERATE**	**WEAK**	
		1	2	3	Not Applicable

GLOBAL RATING FOR THIS PAPER (circle one):

1 STRONG (no WEAK ratings)

2 MODERATE (one WEAK rating)

3 WEAK (two or more WEAK ratings)

With both reviewers discussing the ratings:

Is there a discrepancy between the two reviewers with respect to the component (A-F) ratings?

No Yes

If yes, indicate the reason for the discrepancy

1 Oversight

2 Differences in interpretation of criteria

3 Differences in interpretation of study

Other biases:

The reviewers agreed upon lowering the risk of bias in this study by 1 grade as the authors did not provide any information about one of the outcomes (the clinical condition) and did not provide any numbers indicating the microbiological outcomes. They also only included in the analysis the samples of periodontal pockets that showed clinical improvement.

Final decision of both reviewers (circle one):

1 STRONG

1. MODERATE

2. WEAK

2.(Yamanaka *et al.* 2012)

COMPONENT RATINGS

A) SELECTION BIAS

(Q1) Are the individuals selected to participate in the study likely to be representative of the target population?

1 Very likely

2 Somewhat likely

3 Not likely

4 Can't tell

(Q2) What percentage of selected individuals agreed to participate?

1 80 - 100% agreement

2 60 – 79% agreement

3 less than 60% agreement

4 Not applicable

5 Can't tell

RATE THIS SECTION	STRONG	MODERATE	WEAK
See dictionary	**1**	**2**	**3**

B) STUDY DESIGN

Indicate the study design

1 Randomized controlled trial

2 Controlled clinical trial

3 Cohort analytic (two group pre + post)

4 Case-control

5 Cohort (one group pre + post (before and after))

6 Interrupted time series

7 Other specify _____

8 Can't tell

Was the study described as randomized? If NO, go to Component C.

No Yes

If Yes, was the method of randomization described? (See dictionary)

No Yes

If Yes, was the method appropriate? (See dictionary)

No Yes

RATE THIS SECTION	STRONG	MODERATE	WEAK
See dictionary	1	2	3

C) CONFOUNDERS

(Q1) Were there important differences between groups prior to the intervention?

1 Yes

2 No

3 Can't tell

The following are examples of confounders

1 Race

2 Sex

3 Marital status/family

4 Age

5 SES (income or class)

6 Education

7 Health status

8 Pre-intervention score on outcome measure

(Q2) If yes, indicate the percentage of relevant confounders that were controlled (either in the design (e.g. stratification, matching) or analysis)?

1 80 – 100% (most)

2 60 – 79% (some)

3 Less than 60% (few or none)

4 Can't Tell

RATE THIS SECTION	STRONG	MODERATE	WEAK
See dictionary	1	2	3

D) BLINDING

(Q1) Was (were) the outcome assessor(s) aware of the intervention or exposure status of participants?

1 Yes

1 No

2 Can't tell

Q2) Were the study participants aware of the research question?

1 Yes

2 No

3 Can't tell

RATE THIS SECTION	STRONG	MODERATE	WEAK
See dictionary	1	2	3

E) DATA COLLECTION METHODS

(Q1) Were data collection tools shown to be valid?

1 Yes

2 No

3 Can't tell

(Q2) Were data collection tools shown to be reliable?

1 Yes

2 No

3 Can't tell

RATE THIS SECTION	STRONG	MODERATE	WEAK
See dictionary	1	2	3

F) WITHDRAWALS AND DROP-OUTS

(Q1) Were withdrawals and drop-outs reported in terms of numbers and/or reasons per group?

1 Yes

2 No

3 Can't tell

4 Not Applicable (i.e. one time surveys or interviews)

(Q2) Indicate the percentage of participants completing the study. (If the percentage differs by groups, record the lowest).

1 80 - 100% agreement

2 60 – 79% agreement

3 less than 60% agreement

4 Can't tell

5 Not Applicable (i.e. Retrospective case-control)

RATE THIS SECTION	STRONG	MODERATE	WEAK	
See dictionary	1	2	3	Not Applicable

G) INTERVENTION INTEGRITY

(Q1) What percentage of participants received the allocated intervention or exposure of interest?

1 80 - 100% agreement

1 60 – 79% agreement

2 less than 60% agreement

4 Can't tell

(Q2) Was the consistency of the intervention measured?

1 Yes

2 No

3 Can't tell

(Q3) Is it likely that subjects received an unintended intervention (contamination or co-intervention) that may influence the results?

4 Yes

5 No

6 Can't tell

J) ANALYSES

(Q1) Indicate the unit of allocation (circle one)

Community organization/institution practice/office individual

(Q2) Indicate the unit of analysis (circle one)

Community organization/institution practice/office individual

(Q3) Are the statistical methods appropriate for the study design?

1 Yes

2 No

3 Can't tell

(Q4) Is the analysis performed by intervention allocation status (i.e. intention to treat) rather than the actual intervention received?

1 Yes

2 No

3 Can't tell

GLOBAL RATING

COMPONENT RATINGS

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3 Differences in interpretation of study

Final decision of both reviewers (circle one):

1STRONG

2 MODERATE

3 WEAK

For RCTs:

1. (Hagenfeld *et al.*, 2018)

Bias	Authors' Judgment	Support for Judgment
Random sequence generation (selection bias)	Unclear risk	**Quote:** "multicenter randomized, double-blinded, parallel group, and placebo-controlled study " **Comment:** no further details
Allocation concealment (selection bias)	Unclear risk	**Comment:** not mentioned
Blinding of participants and personnel (performance bias)	Unclear risk	**Quote:** "multicenter randomized, double-blinded, parallel group, and placebo-controlled study." **Comment:** no further details
Blinding of outcome assessment (detection bias)	Unclear risk	**Comment:** not mentioned
Incomplete outcome data (attrition bias)	low risk	**Quote:** "After repetition, still 6 post-treatment samples and 1 pre-treatment sample showed an insufficient quality and were thus removed from further analysis. To maintain pairwise comparisons, the related 7 paired samples were also excluded from further analysis. **Comment:** The recruited patients were 96 and the authors analyzed the results of 89 only. The unreported data of the 7 patients were not due to attrition, but rather due to technical problems with analysis.
Selective reporting (reporting bias)	Low risk	**Comment:** all outcomes were reported
Other bias	Low risk	**Comment:** No other sources of bias identified

2. (Hagenfeld *et al.*, 2019) and (Harks *et al.*, 2016)

Bias	Authors' Judgment	Support for Judgment
Random sequence generation (selection bias)	Low risk	**Quote:** "Participants were randomly assigned to the test or the control group by the use of computerized center-specific randomization lists. Quad-block randomization lists were generated for each center by a statistician who was not involved in any other aspect of the trial."
Allocation concealment (selection bias)	Low risk	**Quote:** "Randomization lists were stored exclusively at the study centers. Randomization was performed by 2 study nurses who were not involved in measurements or treatment of the participating patients."
Blinding of participants and personnel (performance bias)	Unclear risk	**Quote:** " the blinded dentifrices" " randomized, double-blind" **Comment:** no further details at either articles
Blinding of outcome assessment (detection bias)	Unclear risk	**Comment:** not mentioned
Incomplete outcome data (attrition bias)	High risk	**Comment:** The main study of (Harks *et al*., 2016) randomized 35 patients for each group, from which a balanced loss to follow up at the end of the study occurred (1 patient *vs* 2 patients). In the study of (Hagenfeld *et al.*, 2019), only samples from 41 patients (20 patients in Group 1 vs 21 patients in Group 2) were included with no justification of the difference in number of participants
Selective reporting (reporting bias)	Low risk	**Comment:** all outcomes were reported
Other bias	Low risk	**Comment:** No other sources of bias identified

3.(Jünemann *et al.*, 2012)

Bias	Authors' Judgment	Support for Judgment
Random sequence generation (selection bias)	Unclear risk	**Quote:** "Patients were part of a double-blind, parallel group, randomized, placebo-controlled multi-center efficacy study." **Comment:** no further details at either articles
Allocation concealment (selection bias)	Unclear risk	**Comment:** not mentioned

Blinding of participants and personnel (performance bias)	Unclear risk	**Quote:** " Patients were part of a double-blind, parallel group, randomized, placebo-controlled multi-center efficacy study." **Comment:** no further details at either articles
Blinding of outcome assessment (detection bias)	Unclear risk	**Comment:** not mentioned
Incomplete outcome data (attrition bias)	Low risk	**Comment:** No losses to follow up
Selective reporting (reporting bias)	Low risk	**Comment:** all outcomes were reported
Other bias	Low risk	**Comment:** No other sources of bias identified

4. (Nakano *et al.*, 2017)

Bias	Authors' Judgment	Support for Judgment
Random sequence generation (selection bias)	Unclear risk	**Quote:** " Participants were randomly assigned to receive either placebo or test tablets" **Comment:** no further details.
Allocation concealment (selection bias)	Unclear risk	**Comment:** not mentioned
Blinding of participants and personnel (performance bias)	Low risk	**Quote:** "These placebo and test tablets were identical in weight, texture and appearance and were of readily soluble perforated type to prevent asphyxia and aspiration."
Blinding of outcome assessment (detection bias)	Unclear risk	**Comment:** not mentioned
Incomplete outcome data (attrition bias)	High risk	**Quote:** "Five participants in the placebo group and three in the test group failed to comply with the suggested intake rate (less than 75%). Therefore, 37 subjects with full analysis sets were included in the efficacy analysis." **Comment:** patients who violated the eligibility criteria were excluded from analysis rather than performing an intention to treat analysis at least for the clinical variables.
Selective reporting (reporting bias)	Low risk	**Comment:** all outcomes were reported
Other bias	Low risk	**Comment:** No other sources of bias identified

5.(Queiroz *et al.*, 2017)

Bias	Authors' Judgment	Support for Judgment
Random sequence generation (selection bias)	Unclear risk	**Quote:** "Patients were randomized to the three groups described below" **Comment:** No further details.
Allocation concealment (selection bias)	Low risk	**Quote:**"Patients were randomized to the three groups described below and treatment for each buccal furcation defect was revealed after flap elevation and root/ defect debridement"
Blinding of participants and personnel (performance bias)	Low risk	**Comment:** blinding of the personnel is not possible due to difference in surgical procedure. No information about the participants' blinding
Blinding of outcome assessment (detection bias)	Unclear risk	**Comment:** Not mentioned
Incomplete outcome data (attrition bias)	Low risk	**Comment:** No losses to follow up
Selective reporting (reporting bias)	Low risk	**Comment:** all outcomes were reported
Other bias	Low risk	**Comment:** No other sources of bias identified