Received: 20 June 2022 | Revised: 10 October 2022 | Accepted: 12 December 2022

DOI: 10.1111/jre.13092

ORIGINAL ARTICLE

Journal of PERIODONTAL RESEARCH WILEY

# Influence of periodontal surgery on the subgingival microbiome—A systematic review and meta-analysis

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## Abstract

**Objective:** The objective of this systematic review and meta-analysis was to evaluate the effect of periodontal surgery on the subgingival microbiome.

**Background:** Periodontitis is a chronic inflammation of the tooth supporting tissues caused by the dysbiosis of the subgingival biofilm. It is managed through different non-surgical and surgical treatment modalities. Recent EFP S3 guidelines recommended performing periodontal surgery as part of Step 3 periodontitis treatment after Step 1 and Step 2 periodontal therapy, with the aim to achieve pocket closure of persisting sites. Changes in the sub-gingival microbiome may explain the treatment outcomes observed at different time points. Various microbiological detection techniques for disease-associated pathogens have been evolved over time and have been described in the literature. However, the impact of different types of periodontal surgery on the subgingival microbiome remains unclear.

**Methods:** A systematic literature search was conducted in Medline, Embase, LILACS and Cochrane Library supplemented by manual search (23DEC2019, updated 21APR2022).

**Results:** From an initial search of 3046 studies, 28 were included according to our specific inclusion criteria. Seven microbiological detection techniques were used to analyse disease-associated species in subgingival plaque samples: optical microscope, culture, polymerase chain reaction (PCR), checkerboard, enzymatic reactions, immunofluorescence and 16S gene sequencing. The included studies exhibited differences in various aspects of their methodologies such as subgingival plaque sample collection or treatment modalities. Clinical data showed a significant decrease in probing pocket depths (PPD) and clinical attachment loss (CAL) after periodontal surgery. Microbiological findings were overall heterogeneous. Meta-analysis was performed on a sub-cohort of studies all using checkerboard as a microbiological detection technique. Random effect models for *Treponema denticola* (*T. denticola*), *Porphyromonas gingivalis* (*P. gingivalis*) and *Tannerella forsythia* (*T. forsythia*) did not show a significant effect on mean counts 3 months after periodontal surgery. Notably, *Aggregatibacter actinomycetemcomitans* (*A. actinomycetemcomitans*) showed a significant increase 3 months after periodontal surgery. 16S gene sequencing was used in one included

study and reported a decrease in disease-associated species with an increase in health-associated species after periodontal surgery at 3 and 6 months.

**Conclusion:** This systematic review has shown that the effect of periodontal surgery on the changes in subgingival microbiome is heterogeneous and may not always be associated with a decrease in disease-associated species. The variability could be attributed to the microbiological techniques employed for the analysis. Therefore, there is a need for well-designed and adequately powered studies to understand how periodontal surgery influences the subgingival microbiome and how the individual's microbiome affects treatment outcomes after periodontal surgery.

**KEYWORDS**
meta-analysis, periodontal surgery, subgingival microbiome, systematic review

## 1 | INTRODUCTION

Periodontal disease is a chronic inflammatory disease of the tooth supporting tissues, which is driven by shifts in the supra- and subgingival microbiome, combined with destructive defence mechanisms of the host.[1] In health, a dynamic balance between health benefiting microbiota and microbial–host interactions (called symbiosis or homeostasis) is present.[2] This host–microbe symbiosis ensures a balance in periodontal tissue and as a result the integrity of the periodontium is maintained. However, changes in the subgingival microbiota towards those associated with disease, known as dysbiosis,[3] may occur. The functional characteristics of microbial communities change whilst they take advantage of the altered nutrition available. In turn, the dysbiotic microbiome can withstand/deregulate the immune and inflammatory response of the host[4] causing chronic inflammation. Thereby, the balance of periodontal tissue turnover changes towards tissue destruction.[5] Whilst dental plaque biofilm is the principal cause for the initial inflammation, it is the individuals' host response that dictates whether the disease progresses.[6] Hence, the scale of tissue destruction varies significantly among individuals and even amongst teeth within the same individual.[7]

Over the years, various studies have tried to establish the association between changes to the subgingival microbiome and the initiation of periodontal inflammation.[5] Earlier microbiological studies focused on either observations under a microscope[8] and/or cultivation of bacteria found in the periodontal pocket.[9] DNA-based techniques followed and led to the development of disease-associated bacterial clusters by Socransky et al.[10] Hereby in particular bacteria from the red complex (*P. gingivalis*, *T. denticola* and *T. forsythia*) and *A. actinomycetemcomitans* have been shown to be associated to periodontal disease[11] and to deeper periodontal pockets of over 6 mm.[12] More recently next generation sequencing techniques have been implemented to analyse subgingival microbial communities. In contrast to polymerase chain reaction (PCR) assays, these techniques can identify the nucleotide sequence of either a target gene or metagenomic sequencing also known as shotgun sequencing. When mapped against a library it is possible to detect, quantify and characterise bacteria, and to develop a detailed picture of composition of subgingival microbiota in health and disease.[13]

The main strategy of periodontitis treatment is to control the dental plaque biofilm and consequently reduce the bacterial load in order to decrease chronic inflammation. The initial phase (Step 1) of periodontal therapy addresses modifiable risk factors, such as supragingival plaque or plaque retention factors, such as suboptimal restorations.[14,15] It is followed by non-surgical periodontitis therapy (Step 2), which aims to remove subgingival biofilm by professional mechanical plaque removal (PMRP). The overall endpoint of periodontitis treatment is to achieve pocket closure defined as PPD ≤4 mm and absence of bleeding on probing (BoP).[16–19] This has shown to provide long-term stability for the periodontal tissues.[20] If after an adequate Step 1 and Step 2 treatment, deep residual pockets (PPD ≥6 mm) are present, periodontal surgery (Step 3) may be suggested.[16,21]

There is concluding evidence that different types of periodontal surgery can lead to periodontal pocket reduction[21,22] together with other indicative parameters for periodontal inflammation, such as bleeding on probing (BoP), defined as clinical endpoints of the treatment. However, the available data on the impact of periodontal surgery on changes in subgingival microbiota are conflicting. It has been shown that increased levels in red complex bacteria at baseline are negatively associated with clinical attachment gain 1 year after surgical periodontitis treatment of intrabony defects[23] and that greater bacterial diversity at baseline is associated to better treatment outcomes after periodontal surgeries.[24] On the other hand, changes of the subgingival microbiome per se before and after periodontal surgery, as reported in different studies, presented with conflicting results. Whilst some studies report a reduction in periodontal pathogens after periodontal surgery[25,26] other studies did not support these findings.[27,28] To date, it is not fully understood how the subgingival microbiome or its changes may influence healing after periodontal surgery or how periodontal surgery affects the subgingival microbiome. Therefore, in this systematic review we aim to appraise the available literature on the effects of periodontal surgery on the subgingival microbiome.

## 2 | MATERIALS AND METHODS

The study protocol has been registered with the International Register of Systematic Reviews, PROSPERO (CRD42020167170; http://www.crd.york.ac.uk/PROSPERO), and it is in line with the Cochrane Handbook.[29] The instructions of the Preferred Reporting Items for Systematic Review and Meta-analysis (PRISMA) were adopted.

### 2.1 | Focused question

The present systematic review addressed the following focus question: *How does periodontal surgery affect the subgingival microbiome (expressed as changes of subgingival bacteria before and after periodontal surgery) in patients with periodontitis*?

#### 2.1.1 | PICO outline

*Participants*

a. *Types of participants*: Adults (≥16 years old), systemically healthy individuals diagnosed with periodontitis.
b. *Types of studies*: Randomised controlled trials (RCTs), controlled trials (CT), longitudinal studies (long.), single arm prospective clinical trials (SCT) and case control studies (CCS).

*Intervention*

Studies evaluating the effect of surgical periodontal therapy, which included treatments such as periodontal regenerative therapies, resective surgical periodontal therapy, periodontal access flap or minimal invasive surgical periodontal flap.

*Comparison*

Subgingival microbiome before and after surgical periodontal therapy; microbiological data at baseline and at a minimum of one follow-up time point. The selection was limited to studies with a minimum follow-up of 6 weeks after periodontal surgery.

*Outcomes*

Primary outcome was the mean value of subgingival bacteria detected with any microbiological detection method and secondary outcomes were clinical parameters such as periodontal probing depth (PPD), clinical attachment loss (CAL), bleeding on probing (BoP) and full mouth plaque scores (FMPS).

### 2.2 | Exclusion criteria

Studies were excluded if patients were affected by systemic disease known to be associated to periodontitis. However, studies were not excluded if pregnant or lactating patients were included or if systemic disease were not specifically mentioned in the methods section. Studies were also excluded if patients had received systemic antibiotics up to 3 months before the onset or during the study. If a control group with healthy patients or a non-antibiotic treatment arm was available, data from these participants were included.

### 2.3 | Search strategy and data management

The literature search was conducted on Medline (via OVID), EMBASE, LILACS and Cochrane databases on the 23rd of December 2019 and updated on the 21st of April 2022. The search strategy included Mesh terms and free text terms related to the Population, the Intervention and the Comparison investigated in this review, connected with the Boolean operator 'AND'. Any study published in English, German, Spanish, Greek or Portuguese was considered. Literature search results were downloaded to Covidence platform (https://www.covidence.org/), which automatically deleted all duplicates from the search.

### 2.4 | Study selection

Two independent reviewers (A.K. and J.P.) carried out a three-stage screening. Prior to the formal screening process, a calibration exercise was undertaken to pilot and refine the screening questions. The first-stage screening of titles and abstracts was carried out to eliminate the irrelevant articles, which did not meet the inclusion criteria. At the second stage screening all studies referring to surgical periodontal procedures were selected for full-text screening. Whenever full text article was not available authors were contacted. Following proof reading of the full text, the study eligibility was verified independently by both reviewers as a third step. Disagreements were resolved by consensus, if necessary, a third reviewer (N.G.) was consulted. The level of agreement between the two reviewers was calculated using Cohen's Kappa statistics.

### 2.5 | Data collection process

Data extraction was also performed in duplicate by two reviewers (A.K. and J.P) and extracted based on the general study characteristics (authors, year of publication, country, setting), population characteristics (number of participants, age, gender, inclusion/exclusion criteria), intervention characteristics, clinical outcomes at different time points and microbiological characteristics (sampling specification, detection technique, pathogens detected and microbiological outcomes at different time points).

Whenever data was not available authors were contacted for clarification. Data which were presented in figures/graphs and without numerical values, was extracted using an online tool WebPlotDigitizer (https://automeris.io/WebPlotDigitizer/) in line with Cochrane handbook.

## 2.6 | Quality assessment and risk of bias

Both examiners (A.K. and J.P.) assessed the quality of the selected studies. For RCTs the Cochrane Risk of Bias Tool 2 was used (RoB 2, updated on the 22 of August 2019, https://sites.google.com/site/riskofbiastool/welcome/rob-2-0-tool/current-version-of-rob-2). The remaining studies were assessed with the 'Risk Of Bias In Non-Randomized Studies - of Interventions tool' (ROBINS-I, https://sites.google.com/site/riskofbiastool/welcome/home/current-version-of-robins-i). Each study was judged as at low, moderate, high or unclear risk of bias. In addition, we extracted data on sample size calculations.

## 2.7 | Data analysis and meta-analysis

For a sub-cohort of our data (checkerboard studies that analysed plaque samples of patients who were submitted to periodontal surgery and prior Step 2) a meta-analysis was performed. A longitudinal random-effects model (DerSimonian-Laird) was implemented for baseline versus 3 months data, and the effect size was measured as Hedges' g. A funnel plot was used to evaluate publication bias within our meta-analysis. For those studies that did not report the standard deviation (SD) values, the authors were contacted and asked to provide the original data necessary for meta-analysis. Statistical analysis was performed with the aid of a software package Stata (version 16.1).

# 3 | RESULTS

## 3.1 | Study selection

The initial search retrieved a total 3050 studies. After removal of duplicates, studies were screened for eligibility. Following first and second stage screening, 46 articles qualified for full-text screening. Twenty-eight (28) articles met the eligibility criteria and were selected for qualitative analysis. Reason for exclusion included: antibiotic use 3 months before onset or during study (10), non-surgical and surgical data combined (4), age (1), no baseline or only baseline data for subgingival bacteria (2) and language (1) (Figure 1).

Kappa scores were calculated for the level of agreement for title/abstract and full-text screening (kappa: 0.68 and 0.82, respectively) showing a good agreement between reviewers.

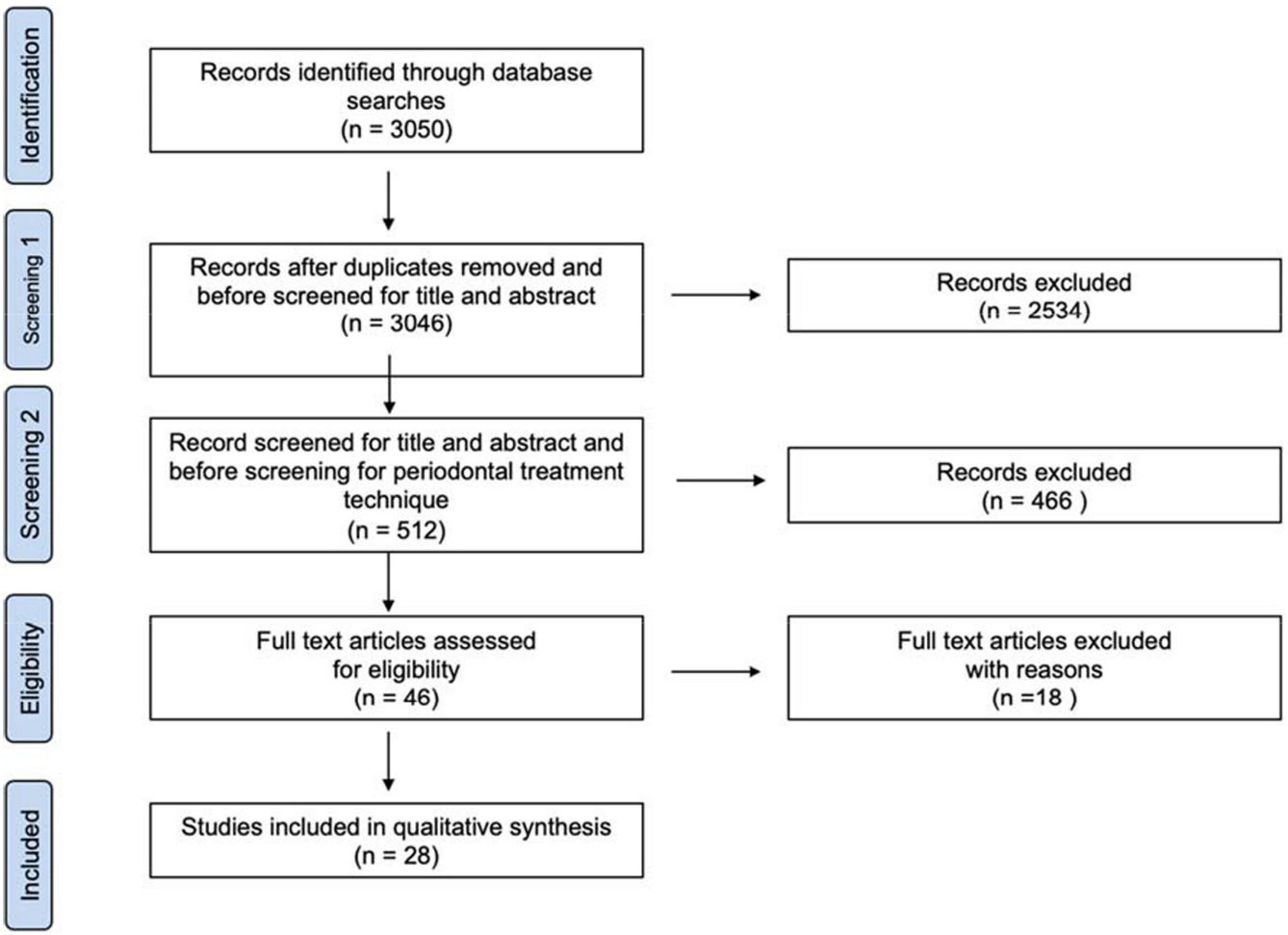


FIGURE 1 PRISMA for screening of studies, three stage screening and selection process.

### 3.2 | Study characteristics

Out of 28 included studies, all were reported in English. They were conducted in United States ($n = 10$), Brazil ($n = 4$), India ($n = 4$) and Norway, Switzerland, Greece, Sweden, Denmark, Germany, Italy, UK, Netherlands and Iceland (each $n = 1$). The majority of studies were randomised clinical trials (RCTs = 19), the remaining were either Controlled Trials (CTs = 5) or longitudinal studies (long. = 4). The sample size of the included studies ranged from 7[30,31] to 41 patients.[32] About half of the studies[25,32–40] had a split-mouth design. Follow-up time points varied and ranged from 6 weeks to 12 months after surgical procedure. Microbiological follow-up time points were reported as follows: 3 months in 75% (21 out of 28),[25,28,30,32,34,36–51] 6 months in 57% (16 out of 28),[8,25,27,30,32–34,36,38,39,42,44–46,50,51] 12 months in 29% (8 out of 28),[8,25,27,31,33,34,42,50] 9 months in 11% (3 out of 28),[25,30,34] 1.5 months[26] and 2 months[52] in 7% (2 out of 28) and 4 months[52] and 10 months[35] in 4% (1 out of 28)[35] of the studies.

The studies were published in a timespan of over 30 years, from 1985[8] to 2019[28,45] which is reflected in both selection of treatment modalities provided and microbiological analysis of the subgingival plaque samples. In 21 out of 28 studies, periodontal surgery was performed following completion of non-surgical therapy (Step 2). Seven out of 28 studies performed periodontal surgery (Step 3) after the initial periodontal therapy (Step 1).[8,35,41,42,49,50,52] There were various definitions for periodontitis, also owing to the different years of publication, but patients were generally suffering from stage III to IV periodontitis[53] and selected surgical sites were sites with PPD >5 mm.

In the studies included in the present systematic review, different surgical interventions were described such as Modified Widman Flap[8,30,36,40,46,49,50] (MWF, $n = 8$),[8,30,35,36,40,46,49,50] access flap surgeries (AFS, $n = 4$),[27,34,38,42] resective surgeries ($n = 3$),[26,51,52] laser as an adjunct to periodontal surgery ($n = 2$),[43,44] Kirkland flap[8,45] ($n = 2$), apically positioned flap surgeries[25,37] (APF, $n = 6$),[25,37,39,41,49,50] regenerative procedures (GTR, $n = 4$)[31–33,48] and AFS in combination with photodynamic therapy (PDT, $n = 2$).[28,47] Primary outcomes of clinical data were PPD (89%), CAL 79%, PI (75%) and BoP (60%).

Seven different microbiological analysis techniques were used in the included studies: dark field microscopy[8,30,46] ($n = 3$), culture[26,27,31,41,43,48,51,52] ($n = 8$), PCR/qPCR[33,42,45] ($n = 3$), checkerboard[25,28,34–37,44,47,49,50] ($n = 10$), enzymes[38,39] ($n = 2$), immunofluorescence[40] ($n = 1$) and 16S gene sequencing[32] ($n = 1$). Microbiological outcomes were reported as either positive (bacterium detected) or negative (bacterium not detected) per case (or site) or as mean values and changes over time. Table 1 presents the characteristics and treatment modalities for all included studies.

### 3.3 | Sampling method for subgingival plaque samples

Studies provided detailed description of the sampling method including removal of supragingival plaque prior subgingival plaque sample collection, type of curette used and sampling site. In the majority of the studies, subgingival plaque samples were collected with a sterile periodontal curette (57%).[8,25,26,28,30,33,34,36,37,44–48,52,54] The second most common sampling method used was sterile paper points (35%).[27,31,32,40–43,49–51] One study collected subgingival plaque samples with a toothpick[39] and one study did not provide any information about the sampling methodology.[38] Variation was observed within this sampling method in regard to the number of paper points per site, size of paper points and length of time the paper points were kept in the periodontal pocket. The length of time varied from 10 s[27,49,50] to 30 s.[32,42] Whilst some studies collected samples only from one site of the mouth, for example the deepest site or the surgical site,[28,30,45,46] other studies collected samples on various or even all teeth present.[25,35,37] Table 2 summarises the methods used for collection of subgingival plaque samples.

### 3.4 | Clinical findings

Most studies reported clinical outcomes before and after periodontal surgery. Two studies did not report any clinical outcome[49,50] and one study reported only baseline data.[33] Table 3 presents clinical outcomes at baseline and at 3 months after surgical periodontal therapy. The mean values for all clinical parameters given in the original studies were averaged to the nearest 1/100 mm. The different periodontal surgeries reduced full mouth PPD (FMPPD) and CAL (FMCAL) by a mean of 1.95 and 0.74 mm, respectively, after 3 months (PPD baseline: 5.28 ± 1.21 mm; 3 months: 3.33 ± 0.93 mm and CAL baseline: 6.10 ± 2.26 mm; 3 months: 5.35 ± 1.74 mm).

Reporting on plaque index (PI) was heterogeneous. Four studies (4 out of 28, 14%)[34,38,39,46] did not describe data collection in regard to plaque levels in the methodology section. From the remaining 24 studies, three studies (3 out of 24, 13%) did not present the data in the article[30,49,50] and one study (1 out of 24, 4%) only reported baseline data.[36] Among the 24 studies which collected data on plaque levels, two different plaque indices were used. 67% of the studies (16 out of 24)[25,27,28,32,33,35–37,39,41,42,44,47,48,51,52] used a dichotomous index system and 33% of the studies (8 out of 24)[8,30,31,40,43,45,49,50] used the Silness and Löe Index.[55] At baseline, the dichotomous index reporting had a range from 11%[51] to 100%[41] with an average of 40.9 ± 32.65% and 3 months after periodontal surgeries the range was from 15.63%[28] to 79%[37] with a mean of 43.83 ± 15.63%.

Full mouth BoP was reported in 15 studies with an average of 55.7 ± 28.65% at baseline that reduced to 37.18 ± 17.57% at 3 months. BoP ranged from 8.69%[32] to 99.70%[27] at baseline and 9.37%[28] to 62.73%[34,39] at 3 months follow-up.

### 3.5 | Microbiological findings

The reporting of the microbiological outcomes was heterogeneous amongst studies and the presence of periodontal disease-associated species was dependant on the microbiological detection technique

**TABLE 1** Characteristics of the included studies according to microbiological detection technique

| Authors (year), Country | Study design | Sample size | Disease classification | Surgical sites | NSPT | Treatment | Follow-up (months) |
|---|---|---|---|---|---|---|---|
| Dark-field microscope | | | | | | | |
| Lindhe (1985), Sweden | RCT | 15 | Advanced periodontitis | >8 sites with PPD 6 mm | No | PMPR vs. MWF vs. Kirkland flap surgery | 6 & 12 |
| Mahmood (1987), UK | RCT | 15 | Mod. to sev. periodontitis | PPD >4 mm after PMPR | Yes | MWF with metronidazole[a] or placebo | 3 & 6 |
| Paul (2010), India | RCT | 7 | Mod. to sev. periodontitis | >2 sites with PPD >5 mm | No | MWF vs. PMPR | 3, 6 & 9 |
| Culture | | | | | | | |
| Ali (1992), Norway | CT | 10 | Mod. to sev. periodontitis | >7 sites with PPD >6 mm | No | PMPR vs. ST | 3 |
| Gokhale (2012), India | RCT | 10 | Gen. periodontitis | PPD >5 mm after PMPR | Yes | ST with or without diode laser application | 3 |
| Nagarjuna (2016), India | RCT | 15 | Gen. chronic periodontitis | Intrabony defects | Yes | Hydroxyapatite composite graft with moxifloxacin[a] or placebo gel | 3 |
| Newman (1989), USA | RCT | 40 | Mod. chronic periodontitis | *Not recorded* | Yes | Osseous periodontal surgery with or without wound dressing | 1 to 1.5 |
| Pedrazzoli (1992), Denmark | CT | 11 | Mod. to sev. periodontitis | >2 sites with PPD >5 mm single rooted | No | Reversed bevel flap surgery vs. PMPR | 2 & 4 |
| Sbordone (1999), Italy | RCT | 15 | Adult periodontitis | Interproximal defects | Yes | GTR (membrane) with or without tetracycline fibres | 12 |
| Sigurdsson (1994), Iceland | CT | 11 | Sev. periodontitis | PPD >6 mm<br>50% bone loss | Yes | ST vs. PMPR | 1.5, 6 & 12 |
| Tuan (2000), USA | RCT | 14 | Sev. periodontitis | Interproximal defects | Yes | ST with or without osseous recontouring | 3 & 6 |
| PCR/qPCR | | | | | | | |
| Cirino (2019), Brazil | RCT/ | 16 | Gen. aggr. periodontitis | >2 sites with PPD >7 mm | No | PMPR vs. ST | 3, 6 & 12 |
| Karthikeyan (2019), India | RCT | 20 | Gen. chronic periodontitis | PPD >5 mm after PMPR | Yes | Kirkland flap surgery with or without diode laser | 3 & 6 |
| Rudiger (2003), Germany | Long. | 29 | *Not reported* | Intrabony defects | Yes | GTR in intra-osseous defects | 6 & 12 |
| DNA–DNA checkerboard | | | | | | | |
| Gapski (2004), USA | RCT | 24 | Sev. periodontitis | PPD of 5–12 mm | Yes | AFS low dose doxycycline vs. placebo | 1, 2, 3, 6, 9 & 12 |
| Cadore (2019), Brazil | RCT | 16 | Sev. periodontitis | PPD >5 mm after PMPR | Yes | AFS with or without photodynamic therapy | 3 |
| Haffajee (1995), USA | RCT | 40 | Chronic periodontitis | >4 sites with PPD >4 mm | No | ST with Augmentin[a], tetracycline[a], ibuprofen or placebo | 10 |

(Continues)

TABLE 1 (Continued)

| Authors (year), Country | Study design | Sample size | Disease classification | Surgical sites | NSPT | Treatment | Follow-up (months) |
|---|---|---|---|---|---|---|---|
| Jensen (2010), Switzerland | RCT | 8 | Chronic periodontitis | >2 sites with PPD >5 mm | Yes | Gingivectomy with or without Nd:YAG laser | 3 & 6 |
| Kyriazis (2013), Greece | RCT | 30 | Gen. sev. periodontitis | >3 sites with PPD >6 mm | Yes | MWF vs. APF | 3 & 6 |
| Levy (1999), USA | Long. | 11 | *Not reported* | >8 sites with PPD 4 mm | Yes | APF | 3 |
| Levy (2002), USA | Long | 18 | *Not reported* | >8 sites with PPD 4 mm | Yes | APF | 3, 6, 9 & 12 |
| Martins (2017), Brazil | RCT | 20 | Sev. chronic periodontitis | Sites with PPD >5 mm | Yes | AFS vs. AFS with PDT | 3 |
| Shiloah (1997), USA | CT | 10 | Mod. periodontitis | 1 site per quadrant with PPD >5 mm | No | MWF and ST with or without citric acid | 3 |
| Shiloah (1998), USA | CT | 10 | Moderate periodontitis | 1 site per quadrant with PPD >5 mm | No | MWF and ST with or without citric acid | 3, 6 & 12 |
| Enzymes | | | | | | | |
| Neiva (2005), USA | RCT | 30 | Mod. to sev. periodontitis | 2 sites with PPD >5 mm | Yes | AFS with Vitamin B or placebo | 3 & 6 |
| Dastoor (2007), USA | RCT | 30 | Mod. to sev. periodontitis | 2 molars with >PPD 5 mm | Yes | ST with Azithromycin or placebo | 3 & 6 |
| Gene sequencing | | | | | | | |
| Queiroz (2017), Brazil | RCT | 41 | Chronic periodontitis | Lower jaw class II furcation defect | Yes | ST with Emdogain, bone graft or both | 3 & 6 |
| Immunofluorescence | | | | | | | |
| Danser (1996), Netherlands | Long. | 15 | Mod. to sev. periodontitis | 1 site per quadrant with PPD >5 mm | Yes | MWF | 3 |

Abbreviations: AFS, access flap surgery; APF, apical positioned flap; CT, controlled trial; GTR, guided tissue regeneration; Long., longitudinal study; MDW, Modified Widman Flap; mod, moderate; NSPT, non-surgical periodontal therapy; PCR, polymerase chain reaction; PDT, photodynamic therapy; qPCR, quantitative polymerase chain reaction; RCT, randomized controlled trial; sev., severe; ST, surgical therapy.

[a]Antibiotic treatment arm which was not included in the data analysis.

**TABLE 2** Summary of sampling methods for subgingival plaque samples, according to microbiological detection technique

| Author/year | Sampling sites | Method |
|---|---|---|
| Dark-field microscopy | | |
| Lindhe (1985) | Each quadrant three approximal sites with BoP. 1st site: PPD <4 mm, 2nd site: PPD = 4–6 mm, 3rd site: PPD >6 mm | Curette |
| Mahmood (1987) | Deepest site in treatment quadrant | Curette |
| Paul (2010) | Sampling of test and control site in seven selected patients | Curette |
| Culture | | |
| Ali (1992) | Seven to ten sites per participant | Paper point |
| Gokhale (2012) | Ten randomly selected patients, deepest pocket from test and control side | Paper point |
| Nagarjuna (2016) | Test and control site | Curette |
| Newman (1989) | Two interproximal surgical sites | Curette |
| Pedrazzoli (1992) | Two approximal sites with PPD ≥5 mm on single rooted teeth in each quadrant (split-mouths study design) | Curette |
| Sbordone (1999) | Surgical sites | Paper point |
| Sigurdsson (1994) | Selected sites with Bop and PPD ≥6 mm | Paper point |
| Tuan (2000) | Three deepest pockets in the mouth | Paper point |
| PCR | | |
| Cirino (2019) | Randomly selected, two moderate (PPD = 5–6 mm) and two deep (PPD ≥7 mm) sites | Paper point |
| Karthikeyan (2019) | Deepest periodontal site | Curette |
| Rudiger (2003) | Test sites | Curette |
| Checkerboard | | |
| Cadore (2019) | Deepest site | Curette |
| Gapski (2004) | Mesiobuccal site of each surgery tooth | Curette |
| Haffajee (1995) | Mesial aspect of all teeth | Curette |
| Jensen (2010) | Two deepest pockets in each experimental quadrant | Curette |
| Kyriazis (2013) | Mesial and distal site of selected teeth | Curette |
| Levy (1999) | Mesiobuccal aspect of all teeth | Curette |
| Levy (2002) | Mesiobuccal aspect of all teeth | Curette |
| Martins (2017) | Mesial and distal of selected teeth | Curette |
| Shiloah (1998) | One site in each quadrant of the patients | Paper point |
| Shiloah (1998) | One site in each quadrant of the patients | Paper point |
| BANA test | | |
| Dastoor (2007) | Two posterior teeth, mesiobuccal aspect, in surgical quadrant | Toothpick |
| Neiva (2005) | Three sites with PPD ≥5 mm | NI |
| Immunofluorescence | | |
| Danser (1996) | Four deepest sites | Paper point |
| Gene sequencing | | |
| Queiroz (2017) | Furcation site | Paper points |

used. The three studies[8,30,46] using dark field microscopy described the bacteria based on their morphological category. The eight studies[26,27,31,41,43,48,51,52] reporting microbiological data from culture techniques used morphological categories but also cultivated specific pathogens to assess the effect of periodontal surgery on the subgingival bacteria. The two enzyme-based studies[38,39] estimated levels of red complex bacteria based on an enzymatic reaction. In addition, DNA-based techniques[25,28,33–37,42,44,45,47,49,50] (PCR, checkerboard and immunofluorescence) detected specific disease-associated species based on known DNA sequences. More recently, a 16S gene sequencing study[32] reported microbiological data on bacterial species levels by comparing detected DNA sequences with microbiome libraries. Standard deviation (SD) or other measures of variation in the microbiological data was reported in 68% of the included studies. Many of the findings did not achieve statistical significance.

Figure 2 summarises the effect of periodontal surgery on disease-associated species. Commonly reported disease-associated species were *A. actinomycetemcomitans* ($n = 15$), *P. gingivalis* ($n = 17$), *T. denticola* ($n = 7$), *T. forsythia* ($n = 11$), *Prevotella*

**TABLE 3** Clinical outcomes including full mouth BoP, PPD and CAL at baseline and 3 months after periodontal surgery, PI of the studies that reported dichotomous FMPS, Mean values, SD, Min and Max were calculated by averaging the mean values reported in the original studies

| Clinical outcome | Baseline | | | | 3 months | | | |
|---|---|---|---|---|---|---|---|---|
| | Mean | SD | Min | Max | Mean | SD | Min | Max |
| PI (%) | 42.29 | 31.02 | 11.30 | 100 | 41.78 | 19.73 | 15.63 | 79 |
| BoP (%) | 55.70 | 28.65 | 8.69 | 99.70 | 37.18 | 17.57 | 9.37 | 62.73 |
| PPD (mm) | 5.28 | 1.21 | 3.21 | 7.53 | 3.33 | 0.93 | 2.00 | 5.60 |
| CAL (mm) | 6.10 | 2.26 | 3.35 | 11.50 | 5.35 | 1.74 | 3.29 | 9.80 |

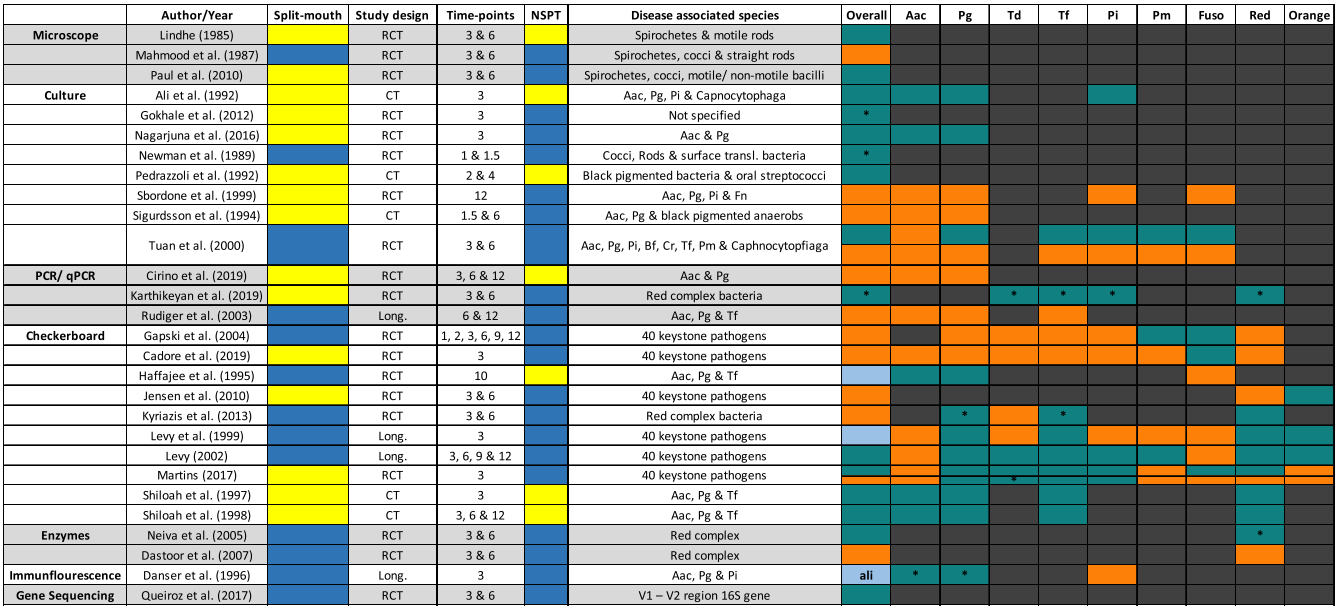


**FIGURE 2** Overview of microbiological findings before and after periodontal surgery according to the microbiological detection technique; blue fields: yes; yellow fields: no; turquoise fields: decrease in disease-associated species following periodontal surgery; orange fields: same or increase of disease-associated species; light blue fields: inconclusive findings; Aac, *A. actinomycetemcomitans*; Fuso, Fusobacteria; Pg, *P. gingivalis*; Pi, *P. intermedia*; Pm, *P. micros*; Td, *T. denticola*; Tf, *T. forsythia*.

*intermedia* (*P. intermedia*, *n* = 10), *Peptostreptococcus micros* (*P. micros*, *n* = 6) and *Fusobacteria* (*n* = 8). The results were heterogeneous with some studies reporting a decrease of mean counts of the selected pathogens whilst other did not. *P. gingivalis* was commonly associated with a decrease of mean counts after periodontal surgery as it decreased in 59% (10 out of 17)[25,35,37,40,41,47–51] studies after periodontal surgery. *A. actinomycetemcomitans* decreased in 40% (6 out of 15)[35,40,41,48–50] studies.

Changes in the subgingival microbiota after periodontal surgery and the influence of different clinical approaches on the microbiological outcomes were also investigated. In seven studies periodontal surgery (Step 3) was performed directly after the initial periodontal therapy (Step 1).[8,35,41,42,49,50,52] Three months after periodontal surgery, five of these seven studies (5 out of 7, 72%)[8,41,49,50,52] reported a reduction in mean counts of periodontal pathogens. Meanwhile, one study (1 out of 7, 14%)[35] reported in-conclusive results and one study (1 out of 7, 14%)[42] showed an increase or similar levels in mean counts of periodontal pathogens after periodontal surgical procedure. In contrast, among the 21 studies performing non-surgical periodontal therapy (Step 2) before periodontal surgery,[25–28,30–34,36–40,43–48,51] only eight (8 out of 21, 38%)[25,26,30,32,38,43,45,48] reported a reduction in mean counts of periodontal pathogens 3 months after periodontal surgeries, five studies (5 out of 21–23%)[36,37,40,47,51] reported inconclusive results and eight studies (8 out of 21–38%)[27,28,31,33,34,39,44,46] reported an increase or similar levels in mean counts of periodontal pathogens (Figure 3).

Another clinical aspect of study methodology is whether studies applied a split-mouth design. Thirteen studies had a split-mouth design,[25,26,32–40,46,51] and 15 studies presented a whole-mouth design with different treatment arms.[8,27,28,30,31,41–45,47–50,52] Five of the split-mouths design studies (5 out of 13, 38%)[25,26,32,38,40] reported a decrease in subgingival periodontal pathogens after periodontal surgery, four studies (4 out of 13, 31%)[33,34,39,46] reported same levels or increase in periodontal pathogens, and four studies (4 out of 13, 31%)[35–37,51] had inconclusive results. In comparison, out of the 15 whole-mouth design studies, nine studies (9 out of 15, 60%)[8,30,41,43,45,48–50,52] reported a decrease of periodontal

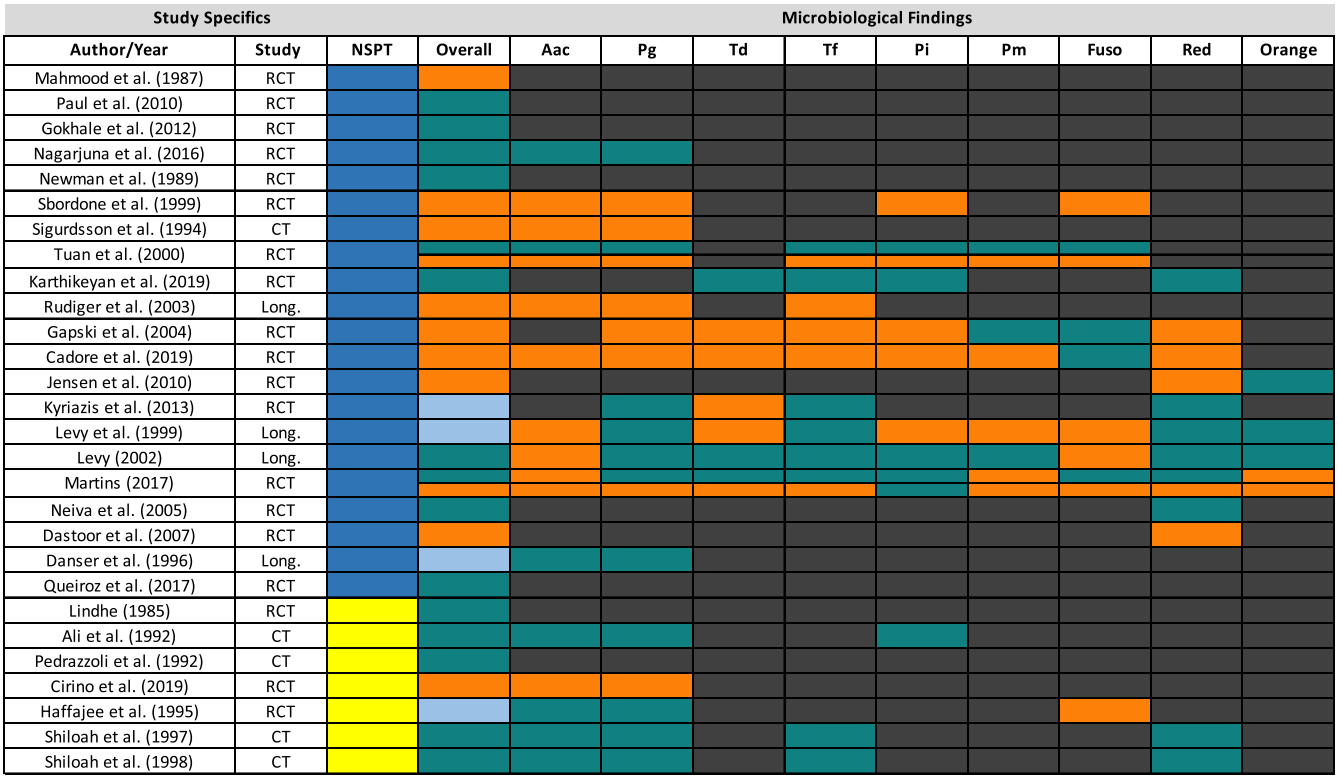


**FIGURE 3** Overview of microbiological findings before and after periodontal surgery organised to NSPT before surgery or no NSPT before surgery; blue fields: yes; yellow fields: no; turquoise fields: decrease of disease-associated species following periodontal surgery; orange fields: same or increase of disease-associated species; light blue fields: inconclusive findings; Aac, *A. actinomycetemcomitans*; Fuso, *Fusobacteria*; Pg, *P. gingivalis*; Pi, *P. intermedia*; Pm, *P. micros*; Td, *T. denticola*; Tf, *T. forsythia*.

pathogens after periodontal surgery, five studies (5 out of 15, 33%)[27,28,31,42,44] showed same levels or increase in periodontal pathogens after surgical intervention and one study (1 out of 15, 6%)[47] had inconclusive results.

## 3.6 | Meta-analysis

Owing to the heterogeneity and complexity of the data reported and methodologies applied, an overall quantitative data synthesis of the changes in subgingival microbiota after periodontal surgery was not feasible. Checkerboard was the microbiological technique used by a considerable number of studies, making it sufficient for a meta-analysis. Studies were included in the meta-analysis, if patients received Step 2 periodontal treatment prior to periodontal surgery (Step 3) and if there were quantitative data available at baseline and at 3 months after periodontal surgery for any of the following periodontal pathogens: *A. actinomycetemcomitans*, *P. gingivalis*, *T. denticola* or *T. forsythia*.[25,28,34,36,47] When all studies reporting on *A. actinomycetemcomitans* (Figure 4) were included in a random-effect model for small sample sizes (DerSimonian-Laird), the overall effect was significant towards an increase of mean counts 3 months after periodontal surgery ($p = .03$) with a Hedges's $g$ −0.39 (95% Confidence interval −0.74 to −0.03). None of the red complex bacteria showed a significant effect towards decrease or increase of mean counts 3 months after periodontal surgery. *P. gingivalis* (Figure 5) had a Hedges's $g$ overall effect of 0.49 (95% Confidence interval −0.03 to 0.96), *T. denticola* (Figure 6) had a Hedges's g overall effect of −0.10 (95% Confidence interval −0.47 to 0.27) and *T. forsythia* (Figure 7) had a Hedges's g overall effect was −0.12 (95% Confidence interval −0.38 to 0.15).

## 3.7 | Risk of bias assessment

Figures 8 and 9 present the results of the risk of bias assessment. Some concerns with the randomisation process,[8,27,30,43,48,51] because of deviations from the intended intervention,[30,43,48] bias in the measurement of the outcome[8,30,43,51] and some concerns about bias in the reporting[30,48,51] were identified. Furthermore, only eight of the included studies reported on sample size calculation.[28,32,34,36,42,47,48] None of those samples size calculations were based on microbiological outcomes.

# 4 | DISCUSSION

To the best of the authors' knowledge, this is the first systematic review summarising the effect of periodontal surgery on the changes

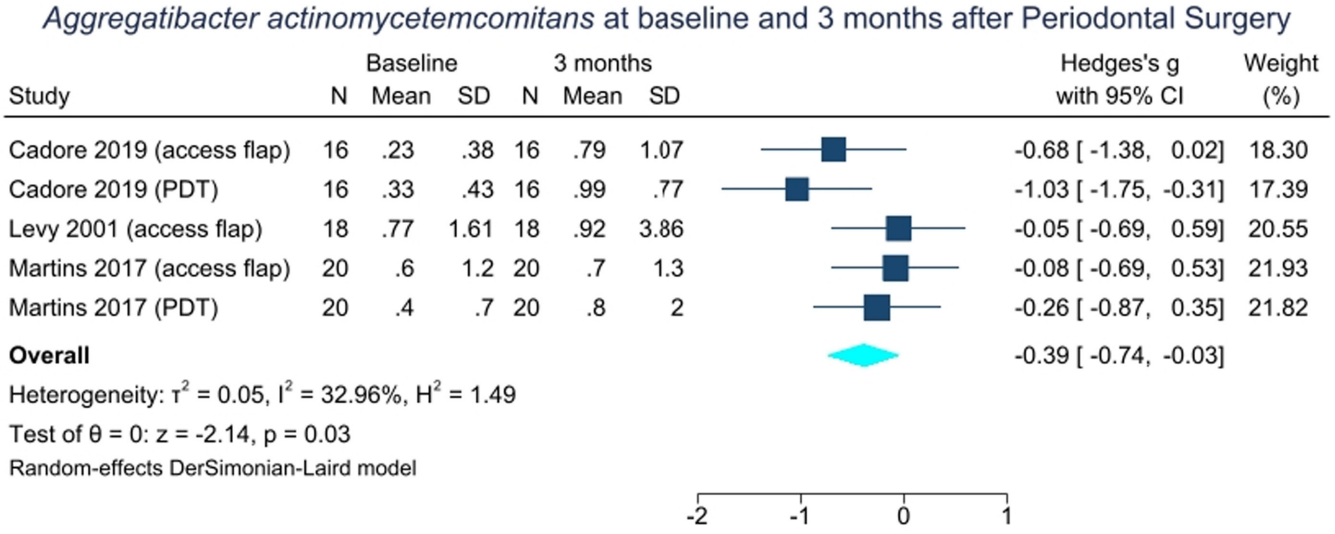


FIGURE 4 Forest plot representing the effect size of periodontal surgery on *A. actinomycetemcomitans* levels detected in subgingival plaque at baseline and 3 months after periodontal surgery.

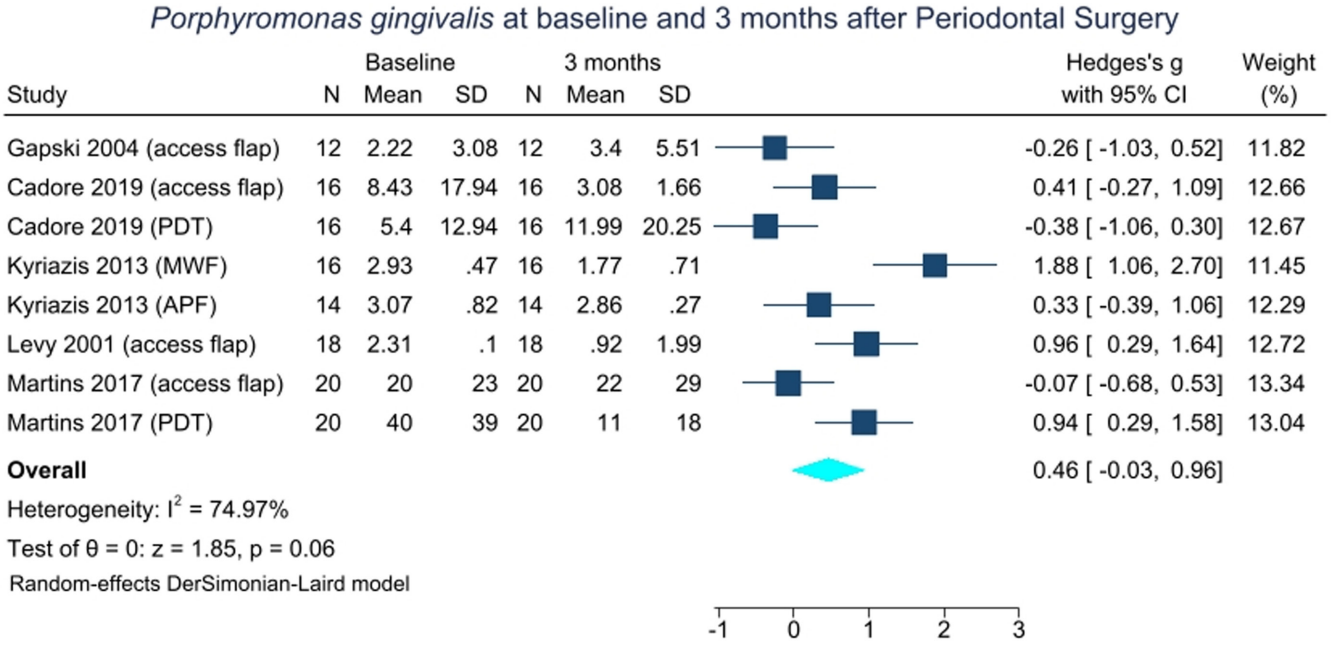


FIGURE 5 Forest plot representing the effect size of periodontal surgery on *P. gingivalis* levels detected in subgingival plaque at baseline and 3 months after periodontal surgery.

in the subgingival microbiome following the use of the different techniques for microbiological analysis.

The findings of the present systematic review have shown that surgical treatment of periodontitis leads to a reduction in BoP, PPD and CAL. PPD and CAL decreased by an average of 1.95 and 0.74 mm 3 months after periodontal surgery, respectively. This is in agreement with previous publications.[16,17,21,56] Despite the clinical improvement, the microbiological changes following periodontal surgery were heterogeneous. Some studies reported a decrease in mean counts of disease-associated pathogens,[8,25,26,30,32,38,41,43,48–50,52] whilst other studies did not find changes in the bacterial load.[27,28,31,33–35,37,39,40,42,44,46,47,51] Seven different microbiological analysis techniques have been used and whilst earlier studies predominately used dark-field microscopy[8,30,46] and culture techniques[27,31,41,52] to describe microbiological shifts, recent publications were more likely to use DNA based detection methods such as PCR,[33,42,45] checkerboard[28,47] and 16S gene sequencing.[32]

There seemed to be an association between microbiological detection techniques and findings to the overall effect of periodontal surgery on disease-associated species. For example, 75% (6 out of 8) of culture-based studies[26,41,43,48,51,52] reported a decrease of disease-associated pathogens versus only 33% (1 out of 3) of the

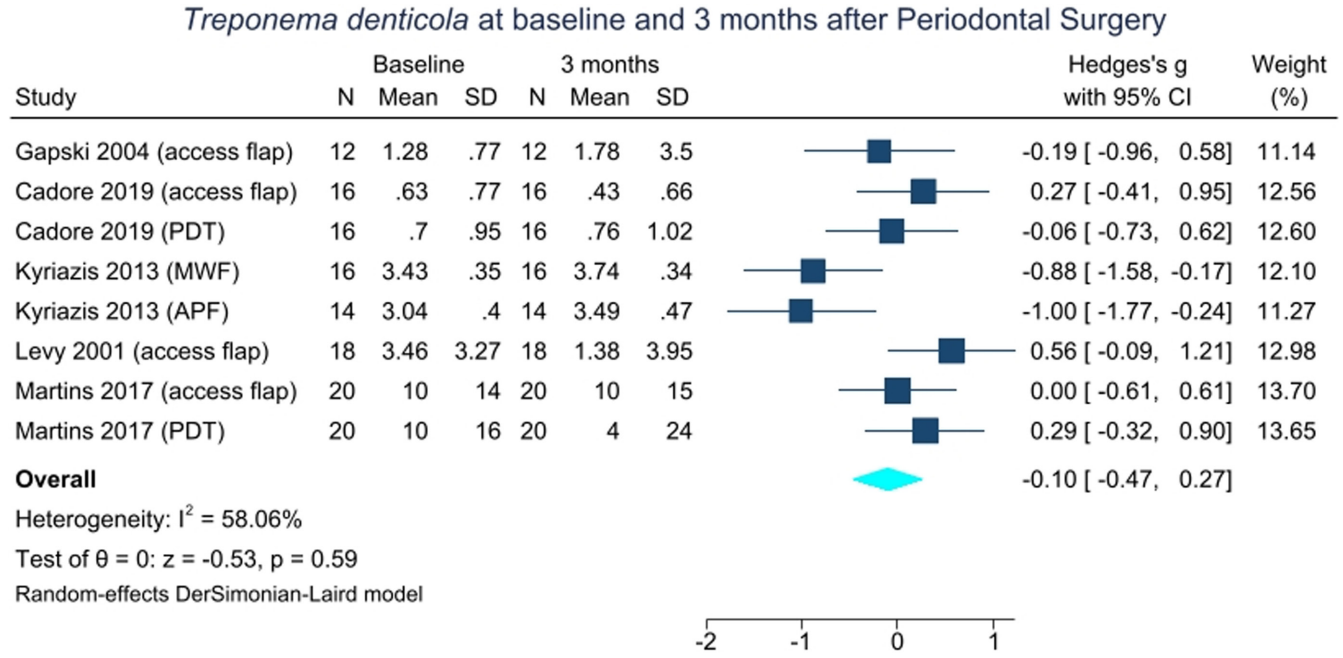


FIGURE 6 Forest plot representing the effect size of periodontal surgery on *T. denticola* levels detected in subgingival plaque at baseline and 3 months after periodontal surgery.

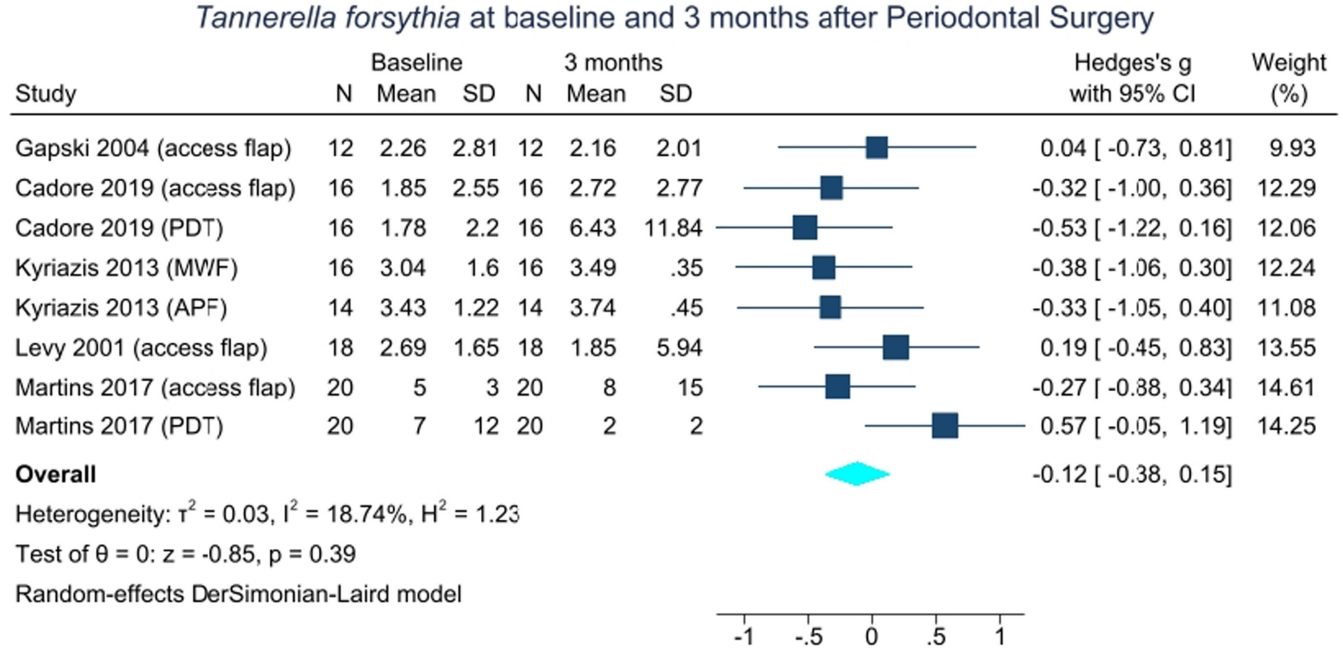


FIGURE 7 Forest plot representing the effect size of periodontal surgery on *T. forthysia* levels detected in subgingival plaque at baseline and 3 months after periodontal surgery.

PCR/qPCR studies.[45] The only gene sequencing study[32] that was included in this systematic review reported an increase in health-associated bacteria 3 and 6 months after periodontal surgeries. On a species level, *P. gingivalis* was the bacterium which was most often associated with a decrease in mean counts after periodontal surgery.[25,35,37,40,41,47–51] The meta-analysis on the checkerboard studies did not find a significant effect of periodontal surgery on *P. gingivalis*, *T. denticola* and *T. forsythia* mean counts 3 months after periodontal surgery. The only significant effect shown, was an increase in *A. actinomycetemcomitans* 3 months after periodontal surgery.

In this systematic review, differences in the included studies were noted between sampling methods for subgingival plaque samples, clinical approach, time points for subgingival plaque sample

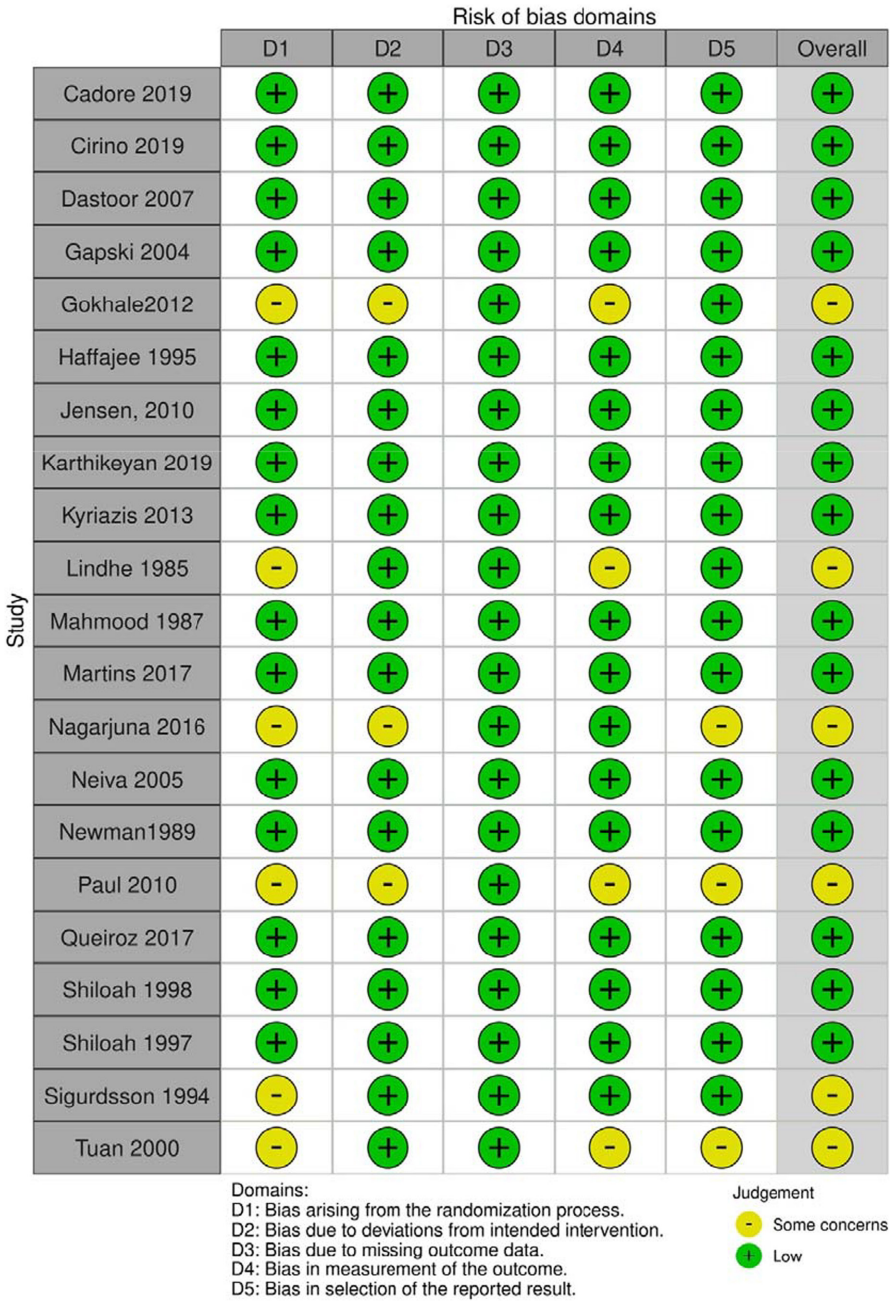


**FIGURE 8** Risk of bias assessment of RCTs.

collection and microbiological detection techniques. Nine different surgical techniques were applied in the 28 studies included in this systematic review. These were combined with seven different microbiological detection techniques used to analyse subgingival microbiota before and after periodontal surgery. For the analysis, data from different surgical techniques were pooled and therefore the results should be interpreted with caution in terms of the impact of specific surgical techniques on the subgingival microbiota.

In regard to subgingival plaque sample collection methodology, the most common method used were curettes (57%)[8,25,26,28,30,33,34,36,37,44–48,52,54] followed by sterile paper points (36%).[27,31,32,40–43,49–51] One study collected the subgingival plaque samples with a toothpick.[39] Jervoe-Storm et al.[57] investigated how differences in subgingival plaque-sampling techniques (curette vs. paper point) influence microbiological results. They reported that sampling with curettes leads to more bacteria detected in each sample. However, when assessing the composition of the collected plaque samples, both methods present similar results. Hence, both techniques can be recommended for clinical research. All studies included in the present meta-analysis collected subgingival plaque samples with a curette. Therefore, the sampling technique would unlikely be a contributing factor to the results of our meta-analysis.

In addition to the sampling technique, there are also variations regarding the sites selected for sampling. Some studies used the deepest site per quadrant,[28,40,44–46,51] a defined test site[30,31,33,36,48] or collected samples from the whole mouth and analysed them as one pooled sample.[25,35,37] This factor could have affected the amount

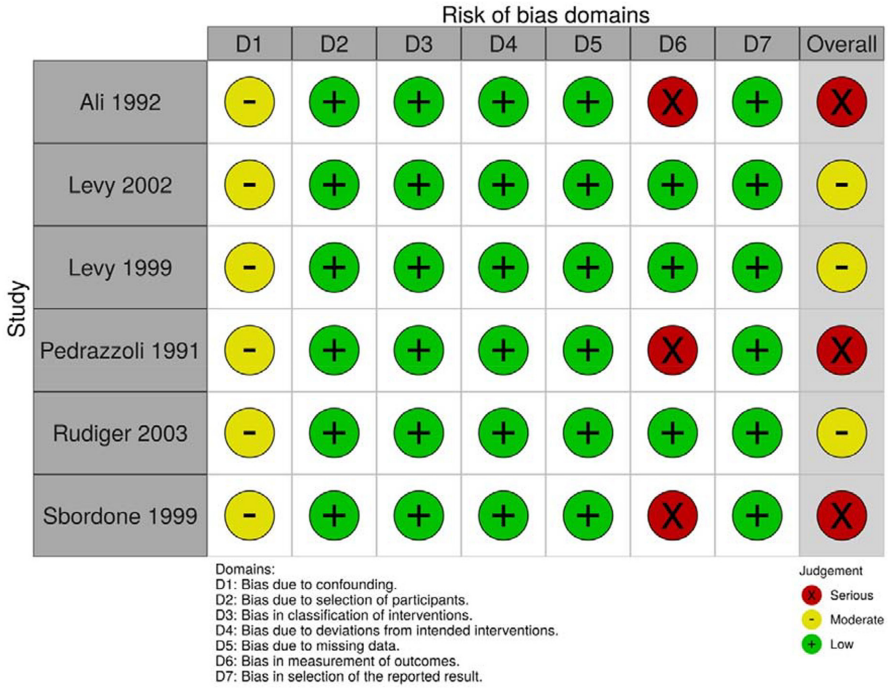


**FIGURE 9** Risk of bias assessment of longitudinal studies.

and species detected in the samples. It has been previously reported that sampling from sites with different PPD may lead to different microbial profiles.[58] In addition, samples collected from the deepest site/pocket, may underestimate the effect of periodontal surgery on the reduction of disease-associated periodontal pathogens, as they may represent sites with poor treatment response.[28,40,44–46,51] Similarly, pooled samples may be unable to show small changes caused by periodontal surgery in the affected sites.[59] Furthermore, subgingival bacterial profile can display major intra-individual differences.[60] These findings underline the importance of collecting site specific samples.

Time points for sampling after periodontal surgery was the least heterogeneous aspect in the methodologies of the included studies. Most studies (75%)[25,28,30,32,34,36–51] collected plaque samples 3 months after periodontal surgery, and therefore this time point was the predominantly used time-point for our analyses. Earlier time points (1.5 months[26,27] and 2 months,[34,52] 7%) were seldom. This may have been due to practical considerations to avoid sampling (especially with a curette) soon after periodontal surgery during the initial healing phase of periodontal tissues. Various studies collected plaque samples at later time points ranging from 4 to 12 months.[8,25,27,30,34–36,38,39,42,44–46,50,51] However, these later time points may be too late to identify the initial post-surgical changes in the microbiome, which may be important for evaluating the course of the post-surgical healing. Future studies should consider earlier time points for sampling, possibly with a paper point to avoid trauma of the surgical site.

Relevant clinical aspects that may explain heterogeneity of the microbiological findings are amongst others the plaque scores. Included studies that used a dichotomous PI[25,27,28,32,33,35–37,39,41,42,44,47,48,51,52] reported a mean PI of 43.47% ranging from 11%[51] to 100%[41] at baseline. After surgery the average PI stayed high (mean 43.83%) ranging from 15.63%[28] to 79%.[37] Early studies reported that periodontal surgery in patients with high plaque scores, leads to further destruction of periodontal tissues[61] and current guidelines[16] recommend oral hygiene instructions as the initial stage of periodontitis treatment to establish low plaque scores with a PI of <20%.[62]

Another relevant clinical factor that may have influenced the microbiological findings of the studies included in this systematic review, is the overall treatment approach described in each study. Recent guidance for the treatment of periodontal disease is the current EFP S3 step wise approach to periodontitis therapy. Periodontal surgery (Step 3) is implemented only after successful completion of Step 1 and Step 2 periodontal therapy.[16,17] Not all of the included studies, followed this clinical approach. Instead, some studies, performed periodontal surgeries (Step 3) after initial treatment (Step 1).[8,35,41,42,49,50,52] This review evaluated whether this influenced the overall microbiological findings of the studies. Studies that followed the clinical step wise approach[17] were more likely not to find a reduction of subgingival microbiota after periodontal surgeries (8 out of 21, 38%)[27,28,31,33,34,39,44,46] than studies which proceeded to periodontal surgeries directly after initial treatment (Step 1; 5 out of 7, 72%).[8,41,49,50,52] Therefore, it can be suggested that periodontal surgery does not always result in further reduction in mean counts of periodontal pathogens, in particular if the overall subgingival bacterial levels were already reduced through the Step 2 of the periodontal treatment. Another explanation could be, that microbiological techniques that have been used, may not be sensitive enough to detect these changes.

Furthermore, we investigated the potential influence of split-mouth study design to the microbiological findings after periodontal surgery. Studies with different treatment arms were more likely to report a decrease in subgingival pathogens after periodontal surgery than studies which applied a split-mouth design (60% vs.

38%) and were also less likely to have inconclusive results (0% vs. 23%). It has been reported that applying a split-mouth design in clinical studies can influence clinical outcomes.[63] This might be due to the so-called carry-across effect as treatment in one side of the mouth may influence treatment outcomes on the other side.[64] Lowering the overall intra-oral bacterial load with the periodontal surgery in one side of the mouth may have influenced the microbiological parameters in the other side (assessed in the split-mouth design) making it less likely to show changes. Another important factor is the need for participants with symmetrical disease patterns requiring surgical interventions, leading to potential recruitment bias.[65]

A strength of this systematic review is the fact that we performed meta-analysis of clinical studies which used the same microbiological detection technique (checkerboard) in addition to the use of original data obtained from the authors of the studies,[28,47] when needed. Furthermore, for the meta-analysis purposes, only studies that followed the EFP S3 step wise periodontal treatment approach[17] were included. The results were overall heterogeneous. However, different surgical modalities were applied in these studies. We could not establish any clear association between surgical techniques and microbiological findings, which might also be due to the small sample size of each surgical technique. Due to the limited number of studies with split-mouth design included in the meta-analysis it was not possible to perform a separate analysis for this type of study design.

Lastly, to be able to show a significant effect, studies need to be powered appropriately.[66] Out of 28 studies included, only eight studies (8 out of 28, 29%) reported sample size and power calculations. In studies with sample size calculation, most considered changes in either PPD[28,32,47] or CAL.[42] It has been shown that periodontitis, in particular deeper pockets of more than 6 mm, is associated with a diverse subgingival microbiome.[5,67] Therefore, changes in PPD may be a helpful tool to predict variations to the microbiome. In addition, in studies with microbiological responses as a primary endpoint, sample calculation based on microbiological outcomes should be considered.[68] This might be an important point for future studies, which use 16S gene sequencing technologies, where even more data points and large variations between individuals are present.[69]

Overall, microbiological findings in the included studies have shown to be heterogenic which could have been influenced by the lack of statistical power. In conclusion, there is a need for well-designed, adequately powered studies to clarify how periodontal surgery influences the subgingival microbiome and how the individual's microbiome affects the treatment outcomes after periodontal surgery.

## FUNDING INFORMATION

No external funding was obtained for this systematic review.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

## DATA AVAILABILITY STATEMENT

The raw/processed data required to reproduce the above findings cannot be shared at this time due to time limitations.

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**How to cite this article:** Krajewski A, Perussolo J, Gkranias N, Donos N. Influence of periodontal surgery on the subgingival microbiome—A systematic review and meta-analysis. *J Periodont Res*. 2023;58:308-324. doi:10.1111/jre.13092