Systematic Review Article

Orthodontic treatment and oral microbiota changes: a systematic review of oral dysbiosis revealed by *16S* rRNA gene analysis

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ABSTRACT

Objectives: Evaluate the changes in oral microbiota linked to orthodontic treatment by analyzing the *16S* rRNA gene.

Materials and Methods: A total of 22 articles was included in the systematic review. The methodological quality of these studies was assessed using the Newcastle-Ottawa Scale for nonrandomized studies and the Risk of Bias tool for randomized studies.

Results: Orthodontic appliances significantly influenced the composition of oral microbiota. Specifically, fixed orthodontic appliances were linked to an increase in periodontopathogenic bacteria associated with various systemic diseases. In contrast, transparent aligners correlated with an increase in *Streptococcus* species.

Conclusions: In this study, we evaluated the changes in oral microbiota associated with orthodontic treatment by analyzing the *16S* rRNA gene. Results revealed significant alterations in oral microbiota following orthodontic treatment; however, significant variability among studies prevents firm conclusions. Additional research is essential to clarify the effects on oral health. (*Angle Orthod*. 2025;00:000–000.)

KEY WORDS: Oral microbiota; Orthodontic treatment; *16S* rRNA; Periodontopathogenic bacteria

INTRODUCTION

The oral cavity is regarded as the second largest microbiota habitat in the human body—following the gastrointestinal tract—inhabited by over 700 different species of bacteria, viruses, and fungi,[1] playing a crucial role in maintaining stability of the oral ecosystem and contributing to normal tissue development and immune processes.[2] Recent advances in genome sequencing techniques, especially the study of the *16S* rRNA gene and shotgun metagenomics, have allowed for a detailed characterization of the structure, composition, and diversity of oral microbiota.[3,4] The emergence of these technologies has demonstrated that changes in the oral polymicrobial community may significantly influence the development and progression of various diseases.

Oral dysbiosis can disrupt the relationship between host and microbes, making the environment more prone to low-grade inflammatory conditions like gingivitis and periodontitis.

According to the Global Burden of Disease Study, periodontitis is the sixth most common noncommunicable disease worldwide, affecting more than 790 million people and with a significant economic and public health impact on a global scale. Poor oral hygiene and plaque accumulation contribute to the development of gingivitis. Additionally, nonintegrated prosthetic restorations in the mouth are strongly linked to a decrease in salivary flow and pH levels, leading to an increase in fungal and bacterial proliferation.[5]

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Accepted: July 23, 2025. Submitted: November 27, 2024.
Published Online: August 29, 2025

Orthodontic treatment is in high demand in clinical practice, and the appliances used can act as retentive factors for bacterial plaque and contribute to oral dysbiosis with an increased risk of inflammation of the gingival tissues.[6,7] Electron microscopy and fluorescence imaging have confirmed microbial colonization on temporary anchorage devices (TADs) which, though useful for complex tooth movements, may trigger gingival inflammation and support bacterial growth.[8] Clear aligners are thought to improve oral hygiene by lowering plaque, gingival bleeding, and rebalancing the oral microbiome. However, no strong evidence has shown they are superior to self-ligating appliances in these aspects.[9]

The link between orthodontics and oral dysbiosis is increasingly relevant but remains unclear due to varied sampling methods, sequencing approaches, and device types, complicating clinical interpretation.

In this review, we systematically examine microbiota changes related to different orthodontic devices using *16S* rRNA sequencing on saliva, supragingival, and subgingival plaque. The aim was to evaluate dysbiosis-related risks, including potential systemic effects, and promote greater awareness among dental professionals.

MATERIALS AND METHODS

The protocol for this systematic review was registered with PROSPERO (CRD42024612490). The study adhered to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.

Identification of Studies and Search Strategy

The PubMed database was searched on October 7, 2024, for studies in which authors examined the effects of various orthodontic appliances on oral microbiota. The primary search strategy was conducted by one author (S.D.N.). A combination of Medical Subject Heading (MeSH) terms related to orthodontic appliances and oral microbiota was used for the search, as follows:

((“RNA, Ribosomal, 16S” Mesh) AND “Microbiota” Mesh) AND “Orthodontics” Mesh)).

Manual searches were also conducted on Embase, Scopus, and Cochrane, with no restrictions on language.

Eligibility Criteria

Studies were selected based on the following inclusion criteria:

- randomized controlled trials;
- clinical trials;
- observational studies;
- case-control studies;
- cohort studies;
- retrospective studies;
- prospective studies;
- cross-sectional studies;
- participants included of both sexes and all ages undergoing orthodontic treatment with either fixed or removable appliances;
- investigation of the effects of orthodontic appliances on oral microbiota, following the PICO question: In patients undergoing orthodontic treatments, different types of orthodontic appliances really modify the composition of oral microbiota assessed with *16S* rRNA sequencing?

Studies involving patients diagnosed with periodontitis or peri-implantitis, pregnant females, and those using antibiotics, antiseptics, mouthwash, probiotics, prebiotics, or any other drugs that could alter the microbiota were excluded.

Study Selection and Data Extraction

Two reviewers (S.D.N. and E.O.) independently assessed the eligibility of studies by reviewing titles and abstracts. Full-text articles were then examined for inclusion. Data extraction was performed using a standardized Excel form by both reviewers. Discrepancies were resolved through discussion. Extracted data included study design, participant characteristics, orthodontic appliance type, sampling site, assay type, and microbiota composition before and after treatment (Table 1). Authors were contacted for incomplete data, and studies for which authors did not respond within 4 weeks were excluded.

Risk of Bias and Quality Assessment

Bias risk assessment was conducted using tools for both observational and interventional studies. The Newcastle-Ottawa Scale was used for case-control and cohort studies (Table 2), while the Cochrane Risk of Bias (RoB 2.0) was applied to randomized clinical trials (Figure 1). Two independent reviewers evaluated the studies, with a senior author (A.M.) resolving conflicts. For case-control and cohort studies, scores of 7 indicated *low risk of bias*, 5–6 *moderate*, and 4 or less *high risk*. In this assessment, 14 of the 22 studies had low risk of bias; the rest had high or unclear risk.

RESULTS

Description of the Included Studies

A PRISMA flowchart in Figure 2 illustrates the study selection process. In our search, we identified

Table 1. Description of the Selected Studies[a]

Reference (Year)	Sample (N)	Female (N)	Age, Mean ± SD or Range	Type of Appliances
Zheng et al.[10] (2024)	35 (17 aligner group, 18 brackets group)	35	Aligner group = 26.2 ± 6.0 y; brackets group = 27.9 ± 5.2 y	Aligners and brackets
Wang et al.[11] (2024)	21 (11 brackets, 10 aligners)	18	Brackets group = 13.18 ± 2.13 y; aligners group = 19.30 ± 6.96 y	Aligners and brackets
Zhao et al.[12] (2023)	15	15	22.4 ± 7 y	TADs
Babikow et al.[13] (2024)	24	13	12.8 ± 1.5 y	Fixed appliances
Rouzi et al.[14] (2023)	15	9	19–35 y	Aligners
Catunda et al.[15] (2023)	10	5	23.0 ± 4 y	Fixed appliances
Song et al.[16] (2023)	28	15	13.8 ± 2.1 y	Aligners
Zhao et al.[17] (2023)	Successful TADs: 14, failed TADs: 15	26	27.50 ± 7.66 y	TADs
Hoffsted et al.[18] (2023)	14	NA	16.8 y	Fixed appliances
Shokeen et al.[19] (2022)	24 (12 fixed appliances, 12 aligners)	16	Fixed group: 22 ± 13 y, aligner group: 29 ± 12 y	Aligners and fixed appliances
Chen et al.[20] (2022)	9	5	14 ± 1 y	Fixed appliances
Yan et al.[21] (2021)	8	NA	18–25 y	Aligners
Kado et al.[22] (2020)	17	14	29.3 ± 12.3 y	Fixed appliances
Lombardo et al.[23] (2021)	17 (13 fixed appliances, 14 aligners)	17	Fixed group: 14 ± 0.75 y, aligners group: 21 ± 0.25 y	Fixed appliances
Wang et al.[24] (2020)	19 (7 aligners, 12 fixed appliances)	NA	20–25 y	Fixed appliances and aligners
Vidović et al.[25] (2019)	11	NA	19.85 y	Fixed appliances
Zhao et al.[26] (2020)	25	22	28 y	Aligners
Benic et al.[27] (2019)	32	21	14.9 ± 3.2 y	Fixed appliances
Guo et al.[28] (2019)	10	10	23.3 ± 37 y	Fixed appliances
Sun et al.[29] (2018)	30	NA	22 y	Fixed appliances
Pan et al.[30] (2017)	20	12	14.42 ± 0.86 y	Fixed appliances
Sandic et al.[31] (2014)	16	8	12–36 y	Fixed appliances

[a] MBT indicates McLaughlin-Bennett-Trevisi; NA, not applicable; and TADs, temporary anchorage devices. Assay type for all studies was *16S* rRNA.

38 manuscripts, of which 13 were excluded after title and abstract review, leaving 25 for full-text review. Of these, 22 met the inclusion criteria. Published between 2014 and 2024, they included various study types: prospective, clinical, cohort, case-control, cross-sectional, and randomized trials. Authors of studies analyzed microbiota changes in patients with fixed appliances, clear aligners, and TADs.

Participants

Among 22 studies, 439 patients received orthodontic treatment: 258 with fixed appliances (58.77%), 137 with

Table 1. Extended

Type of Treatment	Elapsed Time	Sampling Site	Study Type	Outcome
Brackets: Ni-Ti archwires; clear aligners	6 mo	Supragingival plaque	Cross-sectional study	Increase in Actinobacteriota, (*Streptococcus*) in the aligner group. Enrichment in Bacteroidetes, with an increase in anaerobic and Gram-negative bacteria in fixed appliances group.
Brackets: MBT metallic brackets and metal ligatures; clear aligners	3 mo, 6 mo	Saliva	Clinical trial	Aligners and fixed appliances lead to an increase in the relative abundance of *P. salivae*
Self-drilling titanium TADs	2–3 y	Saliva	Case-control study	Increased abundance of Firmicutes and Bacteroidetes at the phylum level, with a notable presence of *Veillonella* and *Prevotella*
Not specified	12 wk	Supragingival plaque	Cohort study	The predominant genera found were *Prevotella* and *Selenomonas*, particularly *P. salivae*, *S. sputigena*, and *P. melaninogenica*, while *S. mutans* did not show any variation
Not specified	3 mo	Subgingival plaque	Prospective study	Increase in the Pseudomonadota phylum and a reduction in Bacillota. The relative abundance of *Haemophilus* increased over time, both at 1 mo (T1) and 3 mo (T3) after treatment
Fixed self-ligating appliances	12 mo	Saliva	Prospective cohort study	Increase in *Streptococcus*
Not specified	12 mo	Saliva	Cross-sectional study	*Streptococcus* comprised 34% of the entire oral microbiota, together with *Veillonella*, *Neisseria*, *Prevotella*, *Haemophilus*, *Aggregatibacter*, and *Actinomyces*
	NA	Saliva	Clinical study	The microbiota associated with failed TADs was enriched with taxa linked to periodontal disease, such as *F. nucleatum*, *F. alocis*, *P. gingivalis*, and *P. nigrescenis*
MBT fixed orthodontic appliances, extraction of 2–4 premolars	3–6 mo	Saliva	Randomized controlled trial	*Streptococcus* also showed an increase, approximating 20.5%
Not specified		Supragingival plaque	Comparative study	No significant differences were found in the supragingival microbial composition between the two groups
Arch-wires, metal brackets and bands cemented on the molars	12 wk	Subgingival plaque	Prospective study	42 new bacterial species at 6 wk after the start of treatment
Aligners without attachment or interdental proximal reduction	24 h after aligners used	Saliva	Prospective study	Increase in the phylum Firmicutes (65%), along with Bacteroidetes and Lactobacillus. At genus level, *Streptococcus* increased, while *Actinomyces* and *Rothia* decreased
Standard edgewire system (0.018-inch slot brackets and archwire)	2 y	Supragingival plaque	Clinical study	Increase in the relative abundance of periodontopathogenic and anaerobic bacteria increased (including *Prevotella*, *Porphyromonas*, *Capnocytophaga*, *Parvimonas*, and *Selenomonas*), while aerobic or facultative anaerobic bacteria (such as *Actinomyces*, *Corynebacterium*, *Rothia*, and Proteobacteria) decreased
Fixed appliances: metal brackets and metal ligatures with archwire; clear aligners with vestibular grip points	6 mo	Subgingival plaque	Prospective study	Relative abundance of red complex bacteria, including *C. rectus* and *F. nucleatum,* at both the third and sixth month of treatment in fixed group
Not specified	6 mo	Saliva	Randomized clinical study	Firmicutes and TM7 at the phyla level and *Neisseria* at the genus level displayed statistically significant differences between the two orthodontic groups.
Not specified	3 mo	Subgingival plaque	Randomized clinical trial	*Prevotella* is the most enriched genus in patients treated with fixed orthodontics
Not specified	6 mo	Saliva	Prospective clinical study	The levels of Firmicutes increased, with *Streptococcus* being the most represented genus, along with *Prevotella*, *Neisseria*, *Haemophilus*, *Veillonella*, *Fusobacterium*, *Rothia*, Leptotrichia, and *Bacillus*
Stainless steel brackets in two arches	3 mo	Supragingival plaque	Prospective, randomized, triple-blind, placebo-controlled trial	Both alpha and beta diversity were reduced
Roth brackets technique	3 mo	Subgingival plaque	Clinical study	*P. intermedia* showed a temporary increase 1 mo after the placement of fixed orthodontic appliances, returning to pretreatment levels after 3 mo
Straight archwire	10–12 mo	Saliva	Cohort study	Reduction in *Streptococcus* from 16% to 11.73% after 2 y of treatment, along with *Neisseria* decreasing from 9% to 3.6%
Premolars extractions + fixed appliances	3 mo	Subgingival plaque	Case-control study	Significant increase of *P. intermedia* during treatment (94.73%) and after appliance removal (47%)
Fixed appliances in the upper arch (maxilla)	1 mo and 3 mo	Subgingival plaque	Clinical study	Significant reduction in *P. gingivalis* both 1 mo and 3 mo after orthodontic treatment, decreasing from 28.6% on central incisors to 0% after 3 mo. *A. actinomycetemcomitans* and *T. forsythia* increased by 7% on the central incisors and 15% on molars

clear aligners (31.21%), and 44 with TADs (10.02%; Table 3; Figure 3A). Women were more likely to seek care in all groups (Figure 3B). The fixed appliance group mainly used McLaughlin-Bennett-Trevisi (MBT) metallic brackets, edgewise systems, straight archwires, and Ni-Ti archwires. Only two studies involved premolar extractions. For clear aligners, authors of two studies specified attachment use: in one study, no attachments or enamel reduction were reported, while in another, grip points on buccal surfaces were described (Table 1).

Intervention Methods

Microbiota analysis was performed on various samples: authors of seven studies analyzed subgingival

Table 2. New Ottawa Scale for Assessing Risk of Bias for Cohort and Case-Control Studies[a]

	Selection			Comparability			Exposure			
Non-RCT Studies	A	B	C	D	E	F	G	H	I	Overall
Zheng et al.[10] (2024)	*	*	*	*	*	*	*	*		8
Babikow et al.[13] (2023)	*						*	*	*	4
Zhao et al.[12] (2023)	*	*	*	*	*	*	*	*	*	9
Rouzi et al.[14] (2023)	*	*	*	*	*	*	*	*	*	9
Catunda et al.[15] (2023)	*	*	*	*	*	*	*	*		8
Song et al.[16] (2023)	*	*	*	*	*	*	*	*	*	9
Zhao et al.[17] (2023)	*	*	*	*	*	*	*	*		8
Shokeen et al.[19] (2022)	*	*	*	*	*	*	*	*	*	9
Chen et al.[20] (2021)	*	*	*	*	*	*	*	*	*	9
Yan et al.[21] (2021)	*	*			*	*		*	*	6
Kado et al.[22] (2020)	*	*	*	*	*	*	*	*		8
Lombardo et al.[23] (2020)	*	*	*		*	*	*	*		7
Wang et al.[24] (2020)	*		*	*	*	*		*	*	7
Zhao et al.[26] (2020)	*		*		*	*	*	*	*	7
Sun et al.[29] (2018)	*	*	*	*	*		*	*		7
Guo et al.[28] (2019)	*				*	*	*	*	*	6
Pan et al.[30] (2017)	*	*	*	*	*	*	*	*		8
Sandić et al.[31] (2014)	*	*			*	*	*	*		6

[a] RCT indicates randomized controlled trial; A = case definition is adequate with independent validation; B = consecutive or obviously representative series of cases; C = community controls; D = controls with no history of disease (endpoint); E = cases and controls with comparable ages; F = cases and controls with comparability on any other factors; G = ascertainment of exposure using secure records (eg surgical records) or structured interviews with blinding to case/control statuses; H = ascertainment of exposure using the same method for cases and controls; I = ascertainment of exposure with nonresponse rate for both groups.

plaque, authors of three focused on supragingival plaque, and authors of two collected both saliva and supragingival plaque. Saliva was most analyzed in clear aligner patients (52.57%; Figure 4). In fixed treatment, all samples were collected in similar proportions. DNA was extracted, and *16S* rRNA genes were amplified by real-time plaque control record (PCR), targeting regions V1-V2, V1-V3, and V3-V4.

Changes in Oral Microbiota in Patients Treated with Fixed Orthodontic Appliances

Authors of 12 studies evaluated oral microbiota changes in patients with orthodontic brackets. Babikow et al.[13] found significant changes in beta diversity but no changes in alpha diversity after 12 weeks, with *Prevotella* and *Selenomonas* as predominant genera, and no variation in *Streptococcus mutans*. Kado et al.[22] observed an increase in periodontopathogenic and anaerobic bacteria after 6 months, with both alpha- and beta-diversity increases. In their trial, Benic et al.[27] showed a reduction in both diversities. *Prevotella* was common in subgingival plaque studies. Vidovic et al.[25] and Guo et al.[28] found *Prevotella* enriched, with *Prevotella intermedia* temporarily increasing after 1 month in the Guo et al.[28] study. Pan et al.[30] noted an increase in *P. intermedia*, *Prevotella gingivalis*, and *Tannerella forsythia*. Authors of other studies reported increased *Streptococcus* in plaque and saliva,[15,18,20,31] while

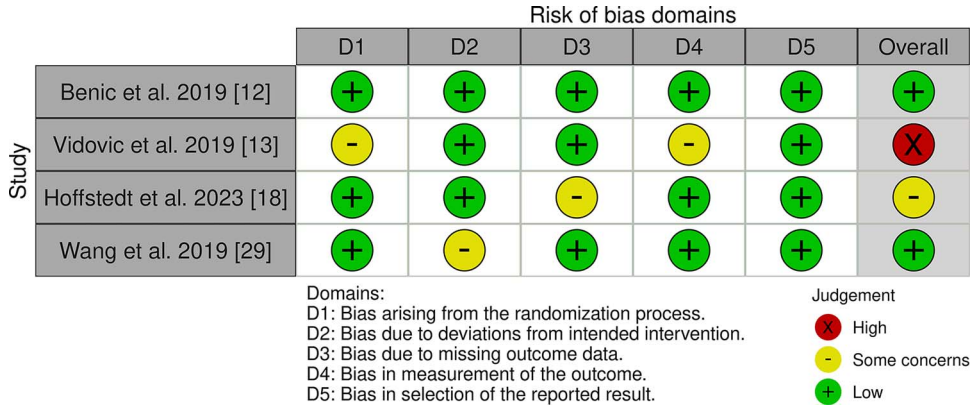

Figure 1. Cochrane Risk of Bias template (RoB 2.0) for assessing the risk of bias in randomized trials.

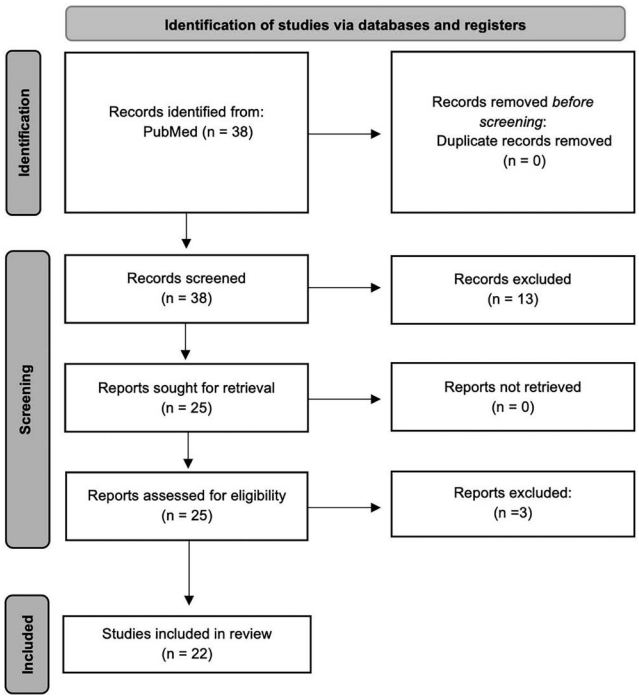

Figure 2. Flowchart based on Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines, illustrating the process for including studies.

Sun et al.[29] observed a reduction in *Streptococcus* and *Neisseria* after 2 years.

Changes in Oral Microbiota in Patients Treated with Clear Aligners

Authors of four studies examined oral microbiota changes in patients with clear aligners. Yan et al.[21] showed reduced alpha- and beta-diversity in saliva, with an increase in Firmicutes (65%) and changes in *Streptococcus*, *Actinomyces*, and *Rothia* after 24 hours. Zhao et al.[26] found no diversity changes after 6 months but an increase in Firmicutes and *Streptococcus*, along with other genera like *Prevotella* and *Neisseria*. Song et al.[16] reported that *Streptococcus* made up 34% of the microbiota after 12 months, alongside *Veillonella*, *Neisseria*, and *Prevotella*. A subgingival plaque analysis[14] showed increased Pseudomonadota and decreased Bacillota after 3 months, with *Haemophilus* steadily increasing and *Fusobacterium*, *Neisseria*, and others decreasing.

Changes in Oral Microbiota in Patients Treated with TADs

Authors of two studies evaluated the microbiota of patients treated with TADs. Authors of one found increased Firmicutes and Bacteroidetes at the phylum level, with *Veillonella* and *Prevotella* at the genus level.[12] Authors of the other compared successful and failed TAD cases, showing higher levels of *Fusobacterium nucleatum*, *P. gingivalis*, and *P. intermedia* in the failed group.[17]

Table 3. Characteristics of the Population[a]

	Device			
	Aligners	Fixed	TADs	Overall (n)
Sex				
F (n)	90	159	41	290
M (n)	47	99	3	149
Overall (n)	137	258	44	439

[a] TADs indicates temporary anchorage devices.

Changes in Oral Microbiota in Patients Treated with Aligners vs Fixed Orthodontic Appliances

Authors of four studies examined the oral microbiota in patients treated with aligners vs fixed orthodontic appliances. Shokeen et al.[19] found no significant differences in supragingival microbial composition between the groups. However, Zheng et al.[10] and Wang et al.[24] reported an increase in Actinobacteriota and *Streptococcus* in the aligner group, while the fixed appliance group showed enrichment in Bacteroidetes and *Prevotella*. Authors of one study analyzed subgingival plaque,[23] finding an increase in red complex bacteria in the fixed appliance group. Saliva samples indicated increased *Prevotella salivae* with both treatments, more so after 6 months with aligners and fixed appliances.[11]

Evaluation of Oral Hygiene Practices and Monitoring in Included Studies

Of the 22 studies included, authors of most explicitly reported providing standardized oral hygiene instructions, typically by dental hygienists, orthodontists, or via educational materials (Table 4). Commonly recommended techniques included the Bass brushing method, interdental flossing, and cleaning of appliances or aligners after meals. Most authors detailed protocols reinforced at every follow-up.[11,14,23,26] Hoffstedt et al.[18] also recommended a standardized enzyme-free toothpaste with 1450 ppm sodium fluoride, used twice daily.

Objective clinical indices, such as plaque index (PI), gingival index (GI), bleeding index (BI), Turesky Modified Quigley Hein Plaque Index (TQHPI), and PCR, were commonly used to assess hygiene. Yan et al.[21] and Kado et al.[22] required baseline PI $<$ 20%, while Catunda et al.[15] included patients with Oral Hygiene Index- Simplified (OHI-S) 0.1–1.2. Shokeen et al.[19] applied disclosing solutions and clinical scoring at every visit. Authors of some studies used self-reported tools, like hygiene logs[21,31] or patient questionnaires.[26] However, authors of several studies lacked clear hygiene adherence evaluation methods.[10,13,24,25,29–31]

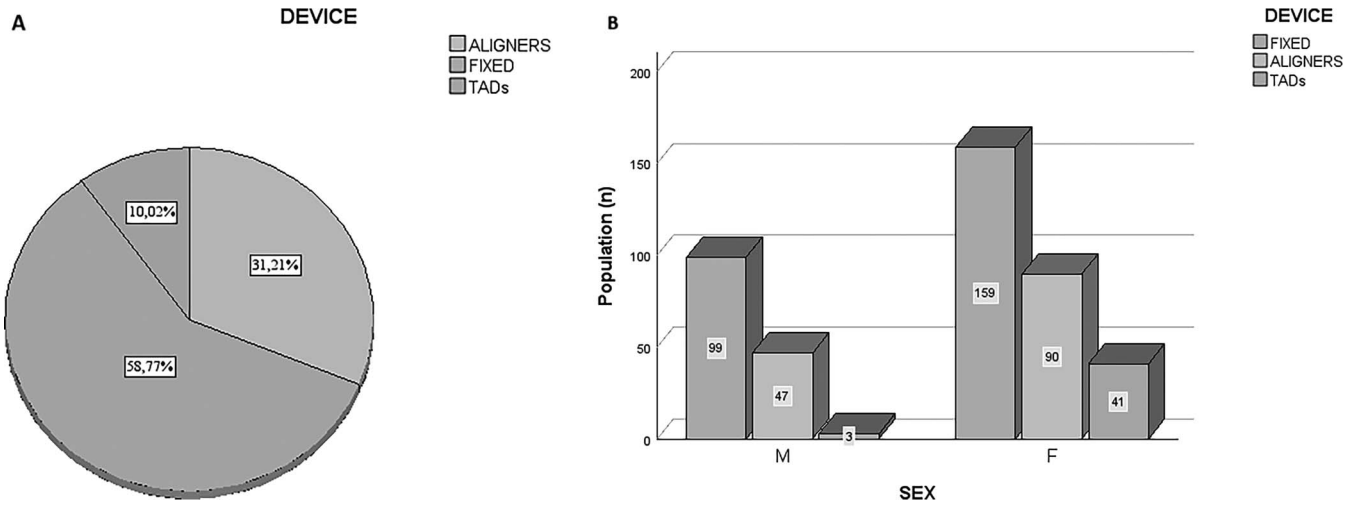

Figure 3. (A) Percentage of patients undergoing different orthodontic treatments. 58.77% of the population were treated with fixed orthodontic appliances, 31.21% wore clear aligners, and 10.02% were treated with temporary anchorage devices (TADs). (B) Bar graph of the devices used according to sex. 439 people were included in the review. Most were female.

DISCUSSION

In this systematic review, we demonstrated that orthodontic treatment affects the oral microbiota, with compositional changes depending on the treatment type. *Streptococcus*, absent in fixed orthodontics, was predominant in saliva and supragingival plaque of patients using clear aligners. *Veillonella* and *Prevotella* levels increased in those treated with TADs.

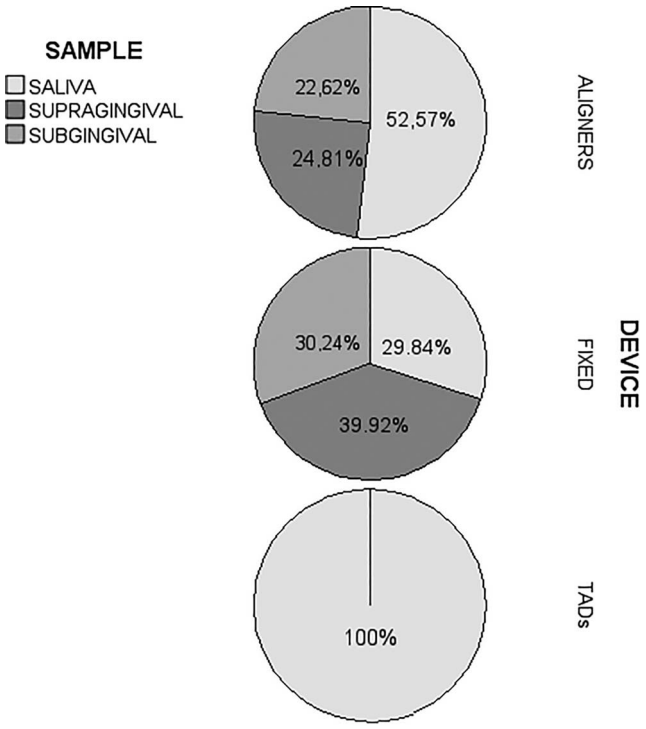

Figure 4. Samples collected according to different devices. The oral microbiota analyzed on three samples: saliva, supragingival, and subgingival plaque.

Prevotella abundance, notably in saliva and both supragingival and subgingival plaque, was strongly associated with fixed appliances and correlated with increased anaerobic Gram-negative periodontopathogens in the subgingival region.

Since oral hygiene represents an important modulating factor, authors of most of the included studies provided standardized hygiene instructions, adopting clinical indices to assess their effectiveness. However, few maintained consistent, objective monitoring over time. This heterogeneity limited the ability to attribute microbial changes solely to orthodontic appliances.

The presence of *Streptococcus* in the oral cavity has long been associated with dental caries. However, shotgun metagenomic and PCR analysis have revealed that *Streptococcus* is just one component of a more complex system, in which other bacterial species, such as *Veillonella* and *Corynebacterium*, also play significant roles.[32] These microorganisms can migrate from the oral cavity to other parts of the body, releasing toxins and contributing to systemic inflammation and disease.

In fact, authors of in vitro studies have associated the presence of *Prevotella* in the oral cavity with increased production of inflammatory mediators, ie, interleukin-6/8 and tumor necrosis factor-α, and neutrophil infiltration, which not only promotes periodontal disease but may also play a role in the progression of oral squamous cell carcinoma by inhibiting the expression of tumor suppressors and altering the tumor microenvironment.[33]

F. nucleatum is strongly associated with the development of periodontitis and alveolar bone resorption. It is considered the primary oral pathobiont linked to Crohn's disease and ulcerative colitis in patients with periodontal issues.[34] Additionally, it is associated with

Table 4. Evaluation of Oral Hygiene Practices and Monitoring in Included Studies[a]

Reference	Oral Hygiene Instructions	Evaluation Method	Brushing Frequency	Follow-Up
Zheng et al.[10]	Essential oral hygiene practice	No objective assessment reported	Not specified	Not specified
Wang et al.[11]	Standardized instructions, Bass brushing method, appliance cleaning after meals	No objective assessment reported	After each meal/snack	Yes, at each visit
Zhao et al.[17]	All patients received oral hygiene instructions and how to clean TADs and the surrounding tissue	No CAL, PD $<$ 3 mm, PI $<$ 30%, and GI $<$ 30%	Not specified	Not specified
Babikow et al.[13]	Not specified	Not specified	Not specified	Not specified
Rouzi et al.[14]	Bass brushing method, appliance cleaning instructions	PI, BI, frequency of brushing recorded	Not specified	Yes, at each visit
Catunda et al.[15]	Soft-bristled toothbrush, fluoride toothpaste, flossing. All patients also received standard dietary advice (no hard/sticky/crunchy foods and sugary drinks)	OHI-S index (0.1–1.2)	2×/d	Regular instructions were provided within 3 mo before brackets placement
Song et al.[16]	Not specified	All patients were given a questionnaire about their oral hygiene routine (tooth brushing frequency, use of fluoride, use of mouthwash, use of dental floss)	Not specified	At the beginning of the study
Zhao et al.[12]	All patients received oral hygiene instructions and how to clean TADs	Not specified	2×/d	Oral hygiene condition was assessed regularly during the study
Hoffsted et al.[18]	All patients were instructed to use a standardized enzyme-free toothpaste with 1450 ppm sodium fluoride	Dental biofilm was detected at baseline and after 8 d, teeth were illuminated with a violet UV light in detection mode	2×/d	Oral hygiene condition was assessed regularly during the study
Shokeen et al.[19]	Essential oral hygiene practice	Patients were rinsed with TRACE Disclosing Solution to visualize the extent of plaque accumulation on their teeth. Plaque levels were scored for both anterior and posterior teeth with the TQHPI. The GI score was calculated at each time point.	Not specified	At each visit
Chen et al.[20]	Oral hygiene instructions, instructional videos	No objective assessment reported	Not specified	Yes, from the initial consult
Yan et al.[21]	Unified oral hygiene treatment, uniform toothpaste and toothbrush	Plaque index $<$ 20% before study start	Not specified	Yes, at each visit
Kado et al.[22]	Tooth brushing instructions from dental hygienists	PCR $<$ 20%	Not specified	Yes, at each visit
Lombardo et al.[23]	Brushing 3×/d, no mouthwash, no antimicrobial agents	No objective assessment reported	3×/d	Yes, reinforced at each appointment
Wang et al.[24]	All participants were instructed regarding oral hygiene procedures at the beginning of the study	No objective assessment reported	No objective assessment reported	No objective assessment reported
Vidovic et al.[25]	All the patients were instructed regarding oral hygiene procedures (Bass technique) and precidiums, and they all followed a dietary protocol.	No objective assessment reported	Not specified	At each visit

Table 4. Continued

Reference	Oral Hygiene Instructions	Evaluation Method	Brushing Frequency	Follow-Up
Zhao et al.[26]	All patients received standardized oral hygiene instructions by the same dental hygienist and an information brochure on oral hygiene maintenance. Patients were instructed to brush and floss teeth after each meal or snack and to clean aligners using a soft-bristle toothbrush with water and a small amount of toothpaste prior to reinserting their aligners.	Patients received a table to record their oral hygiene habits of brushing and dessert consumption frequency	Every day, after each meal or snacks	Yes, at each visit
Benic et al.[27]	Standardized oral hygiene instructions with the use of a new toothbrush (Colgate® Ortho) and toothpaste (Colgate® Cavity Protection Toothpaste)	PI and GI were detected at each time point	Twice a day	At the beginning of the study, after 1 mo and 3 mo
Guo et al.[28]	Standardized oral hygiene instructions	GBI and PI were detected at each time point	Not specified	At each visit
Sun et al.[29]	Standardized oral hygiene instructions	No objective assessment reported	Not specified	Not specified
Pan et al.[30]	Before the study, all participants received standardized oral hygiene instruction by the same orthodontist	No objective assessment reported	Not specified	Not specified
Sandic et al.[31]	Patients were instructed to take care of oral hygiene by brushing their teeth more often and longer. No antimicrobial mouthwashes	No objective assessment reported	Increased brushing frequency recommended (not quantified)	Not specified

[a] BI indicates bleeding index; CAL, clinical attachment loss; GBI, gingival bleeding index; GI,gingival index; OHI-S, oral hygiene index-simplified; PCR, plaque control record; PD, probing depth; PI, plaque index; TADs, temporary anchorage devices; TQHPI, Turesky Modified Quigley Hein Plaque Index; and UV, ultraviolet.

tumor tissue proliferation, drug resistance, and poor therapeutic outcomes in patients with colorectal adenocarcinoma.[35]

Aggregatibacter actinomycetemcomitans is the main bacterium responsible for aggressive periodontitis; however, lipopolysaccharide accelerates aortic inflammation, lipid peroxidation, and atherosclerotic plaque formation.[36] Increases in both *A. actinomycetemcomitans* and *F. nucleatum* have also been detected in lung aspirates from patients with pneumonia.[37]

Given these findings, it is crucial for dentists to understand the relationship between the treatments administered to patients and oral bacterial colonization, as the latter may play a role in other systemic diseases. Currently, only about 20% of dentists are well informed about this connection.[38] The use of therapeutic aids, such as probiotics and prebiotics, should become standard in clinical practice. The probiotic *Streptococcus salivarius* has been shown to have antibacterial effects against several oral pathogens, including *A. Actinomycetemcomitans*; it also appeared to effectively reduce dental plaque formation and is safe to administer to children.[39]

Despite these findings, the current body of research has limitations. Randomized studies were lacking, and sample collection sites varied (saliva, supragingival, and subgingival plaque), complicating comparisons. Significant heterogeneity existed in orthodontic methods, including bracket type (conventional or self-ligating), archwire systems, bonding protocols, and inclusion of extraction cases, factors that could alter the oral microbiome. Additionally, aligner characteristics were often underreported; authors of only a few studies mentioned the presence of attachments or use of interproximal reduction, which could influence microbial colonization.

Other confounding variables (smoking and systemic conditions) were inconsistently addressed. Importantly, variability in hygiene assessment calls for standardized oral hygiene evaluation in future studies to prevent misattribution of microbial changes to appliances rather than to hygiene differences.

In the current review, we focused exclusively on *16S* rRNA gene sequencing which, while widely used, has limitations in identifying all bacterial species. Additionally, the focus was solely on the oral microbiota, excluding the oral virome and mycobiome, due to the lack of available studies in the literature. However, directing research toward changes in these areas could yield additional insights into the interactions between microorganisms and the host within the oral cavity.

CONCLUSIONS

- Orthodontic treatment alters the oral microbiota, but study variability limits definitive conclusions.
- Further research is needed to better understand its impact on oral health.

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