Derna Academy Journal for Applied Sciences

E-ISSN: 3006-3159

DAJAS

Subgingival Bacterial Profiles and Antimicrobial Susceptibility in Patients with Fixed Orthodontic Appliances: A Comparative Study

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Volume: 7 **Issue: 1** **Page Number: 101 - 109**

Keywords:
Orthodontic appliances; subgingival bacteria; antimicrobial susceptibility; gingival bleeding; oral biofilm

Received: 10\08\2026
Accepted: 18\08\2026
Published: 19\08\2026

https://doi.org/10.71147/2teaqq90

ABSTRACT

Fixed orthodontic appliances can retain dental plaque and modify the subgingival environment. This comparative cross-sectional study evaluated cultivable subgingival bacteria and antimicrobial susceptibility among patients with fixed orthodontic appliances and non-orthodontic controls in Benghazi, Libya. One hundred participants were enrolled between June and October 2023: 50 had worn fixed metallic appliances for at least 3 months, and 50 had no orthodontic appliance. Subgingival samples were collected with sterile paper points from central incisor, first premolar, and first molar sites, cultured on selective and differential media, and identified by Gram staining, biochemical tests, and the RENDER MA120 automated system. Antimicrobial susceptibility was evaluated by Kirby–Bauer disk diffusion. Sixty-seven isolates representing 11 bacterial species were identified (38 orthodontic and 29 control isolates). In the orthodontic group, *Staphylococcus aureus* was most frequent (26.32%), followed by viridans streptococci (23.68%) and *Staphylococcus simulans* (13.16%). In controls, viridans streptococci predominated (34.48%), followed by *Streptococcus pyogenes* (20.69%) and *Enterobacter* spp. (17.24%). The orthodontic group showed broader species diversity. Doxycycline and amoxicillin/clavulanic acid showed the most consistent activity, whereas metronidazole and, for several orthodontic isolates, oxacillin showed high resistance. Fixed appliances were accompanied by a broader cultivable subgingival bacterial profile, supporting reinforced oral hygiene, periodontal monitoring, and culture-guided antimicrobial selection.

1. INTRODUCTION

The oral cavity contains a complex, site-specific microbial ecosystem that contributes to colonization resistance, immune education, and the maintenance of oral health. Teeth, gingival sulci, mucosal surfaces, saliva, and the tongue provide distinct habitats in which microorganisms organize as structured biofilms. In health, host factors and microbial interactions maintain a dynamic equilibrium; disruption of this balance can favor selected organisms and contribute to caries, gingivitis, and periodontitis (Arweiler & Netuschil, 2016; Deo & Deshmukh, 2019; Sanz et al., 2017; Zaura & Ten

Cate, 2015). Dental plaque is therefore a microbial community whose composition and pathogenic potential are shaped by local oxygen tension, nutrient availability, surface characteristics, and the effectiveness of mechanical plaque control (Marsh, 2006). Fixed orthodontic treatment improves dental alignment, function, facial appearance, and self-confidence; however, brackets, bands, archwires, and ligatures add plaque-retentive surfaces and complicate brushing and interdental cleaning. These components can reduce natural self-cleansing, increase food stagnation, and create protected niches around bracket margins and posterior bands. Consequently, fixed appliances have been associated with quantitative and qualitative changes in the oral microbiota, greater plaque accumulation, gingival inflammation, white-spot lesions, and an increased need for preventive care (Contaldo et al., 2021; Ireland et al., 2014; Santonocito & Polizzi, 2022; Wang et al., 2019). Previous studies have reported temporary increases in subgingival periodontal pathogens after appliance placement and changes in the relative abundance of streptococci, staphylococci, lactobacilli, and potentially pathogenic Gram-negative organisms (Guo et al., 2017; Guo et al., 2019; Kim et al., 2012; Lucchese et al., 2018; Sun et al., 2018). The direction and magnitude of these changes vary because microbial findings are affected by age, appliance design, treatment duration, oral-hygiene behavior, sampling site, culture conditions, and the use of culture-based or molecular identification. Some investigations have shown transient changes that approach pretreatment levels after several months, whereas others have demonstrated persistent differences during active treatment (Guo et al., 2017; Kado et al., 2020).

Gingival bleeding is a visible sign of inflammation and may become more frequent when plaque-retentive appliance components increase the microbial challenge at the gingival margin. Brackets and molar bands may also cause mechanical irritation and make posterior cleaning more difficult. Although plaque-induced gingivitis is reversible, persistent biofilm accumulation can contribute to more advanced periodontal changes in susceptible patients (Murakami et al., 2018; Shrestha et al., 2016). The recovery of cultivable bacteria from bleeding and non-bleeding sites provides descriptive information about the local microbial environment, but culture findings alone cannot establish causation. Antimicrobial susceptibility is also clinically relevant. Antibiotics may be prescribed for acute odontogenic or periodontal infections, yet susceptibility differs among organisms, and empirical treatment can select resistant strains. Disk-diffusion testing remains a widely used method for evaluating antimicrobial activity when organism-specific interpretive criteria and laboratory controls are applied (Reller et al., 2009). In a heterogeneous oral community, culture-guided selection is preferable whenever systemic antimicrobial therapy is clinically indicated. The present study was conducted in Benghazi, Libya. It aimed to characterize cultivable subgingival bacteria in patients wearing fixed orthodontic appliances, compare the isolate distribution with non-orthodontic controls, describe bacterial recovery at selected tooth sites in relation to gingival bleeding, and summarize the antimicrobial susceptibility patterns reported for the recovered isolates. The working expectation was that fixed appliances would be accompanied by a broader cultivable bacterial profile than that observed in controls.

2. METHOD

2.1 Study design, setting, and participants

A comparative cross-sectional, culture-based study was conducted in Benghazi between June and October 2023. Participants were recruited from the Libyan Center for Orthodontics and Implantology and the Central Dental Clinic in Al-Salmani Al-Gharbi. The study included 100 participants: 50 patients who had worn fixed orthodontic appliances for at least 3 months and 50 participants without orthodontic appliances who served as controls. Both sexes were represented. A structured questionnaire recorded demographic characteristics, toothbrushing frequency, orthodontic-treatment duration, gingival symptoms, and relevant recent medication history.

2.2 Eligibility criteria and recruitment

The orthodontic group included patients treated with metallic brackets and bands. Patients with ceramic or lingual brackets were excluded, as were individuals undergoing orthognathic surgery because surgery could independently disturb the subgingival environment. Control participants were recruited during routine dental-clinic visits and did not have orthodontic appliances. The source thesis did not provide a complete participant-level exclusion list beyond these criteria; therefore, no additional criteria were introduced into this manuscript.

2.3 Subgingival specimen collection

Subgingival plaque was sampled from three representative tooth positions: the central incisor (C1), first premolar (P4), and first molar (M6). The selected tooth was rinsed with water and isolated from saliva with cotton rolls. Visible supragingival plaque was removed, and a sterile absorbent paper point (#40, 0.04 taper) was inserted vertically into the mesiobuccal gingival sulcus and retained for 20 s. The paper point was then transferred into sterile tryptone soy broth

(TSB) and transported immediately to the microbiology laboratory. This approach followed sampling principles used in previous orthodontic subgingival studies (Guo et al., 2017; Sargolzaie et al., 2014).

2.4 Culture and bacterial identification

Each inoculated TSB tube was incubated at 37°C for 24 h and subcultured on blood agar and MacConkey agar; selected samples were additionally cultured on mannitol salt agar. Plates were incubated aerobically at 37°C overnight and examined for growth, colony morphology, pigmentation, hemolysis, lactose fermentation, and mixed growth. Mixed cultures were purified before identification. Conventional identification included Gram staining, catalase and coagulase tests for Gram-positive cocci, and oxidase, citrate, urease, and triple sugar iron reactions for Gram-negative isolates. Optochin and bacitracin susceptibility were used to support differentiation among selected streptococci (Cheesbrough, 2006; Dahlén et al., 2018; MacFaddin, 2000).

Isolates that were difficult to resolve by manual testing were identified with the RENDER MA120 automated ID/AST system (Render Biotech Co., Ltd., Shenzhen, China). Bacterial suspensions were adjusted to 0.5 McFarland, inoculated into the identification wells, incubated at approximately 36°C for 18–24 h, and read by the automated instrument. No molecular confirmation or re-identification of stored isolates was reported.

2.5 Antimicrobial susceptibility testing and statistical analysis

Antimicrobial susceptibility was assessed by the Kirby–Bauer disk-diffusion method on Mueller–Hinton agar. Approximately three to five colonies from each pure culture were used to prepare the inoculum. Antibiotic disks were placed at standardized distances, plates were incubated at 37°C for 24 h, and inhibition zones were measured to the nearest millimeter. The tested agents were amoxicillin/clavulanic acid (AMC), azithromycin (AZM), doxycycline (DO), erythromycin (E), metronidazole (MET), oxacillin (OX), tetracycline (TE), and trimethoprim/sulfamethoxazole (SXT). Susceptibility testing principles were consistent with the general disk-diffusion approach described by Reller et al. (2009). Data were entered and analyzed using the Statistical Package for the Social Sciences (SPSS). The thesis specified t tests, one-way analysis of variance, and two-way analysis of variance with a significance threshold of $P \leq 0.05$. Because the available result tables contained counts and percentages without complete test statistics, the comparisons in this manuscript are presented descriptively and no unsupported inferential claims are made.

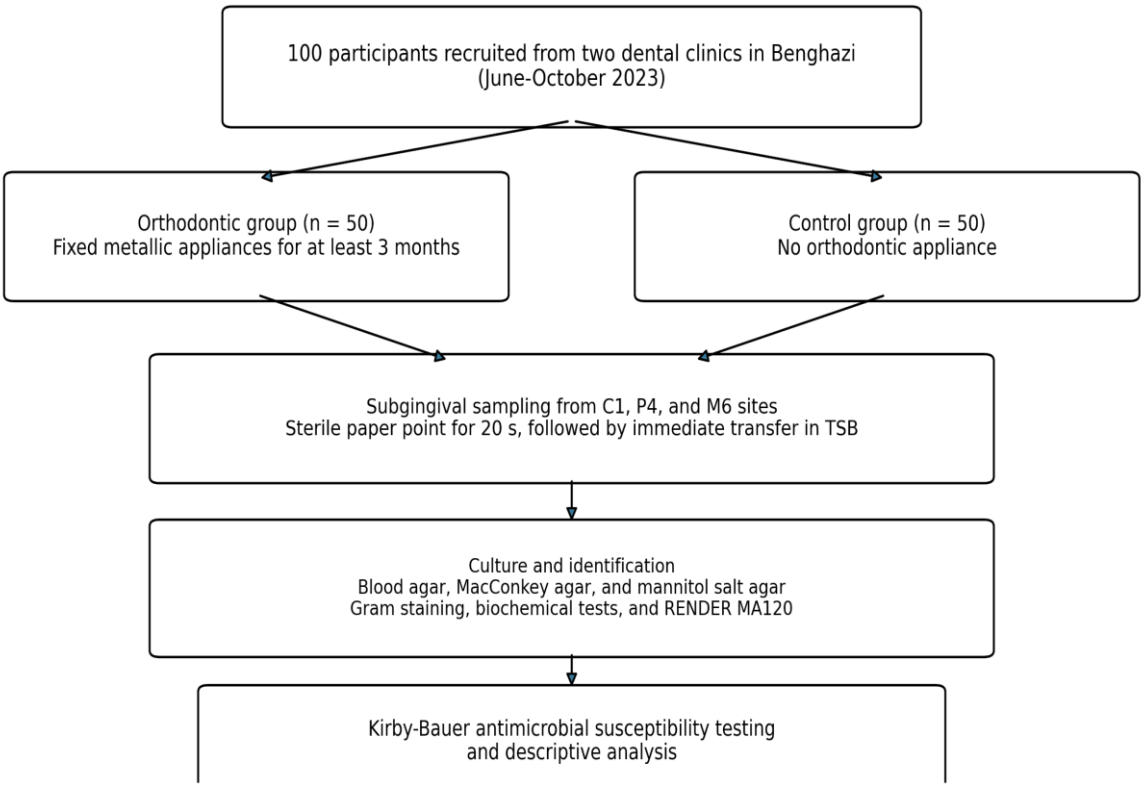

Fig. 1. Study workflow for participant grouping, subgingival sampling, bacterial identification, and antimicrobial susceptibility testing.

3. ETHICAL APPROVAL

Ethical approval was obtained before commencement of the study, and all participants who met the participation criteria provided written informed consent permitting the use of their data for research purposes. The source thesis did not state the formal name of the approving research ethics committee or the approval number; both details must be inserted from the original approval document before journal submission.

4. RESULT

4.1 Participant characteristics and oral-hygiene profile

The study included equal numbers of orthodontic and control participants (50 in each group). Women constituted 70% of the orthodontic group and 78% of the control group, producing an overall female proportion of 74%. The largest age category reported in the results was 26–35 years (30% of the sample), followed by 19–25 years and 36–45 years (27% each).

Toothbrushing frequency differed descriptively between groups. In the orthodontic group, 34% reported brushing twice daily, 30% three times daily, 22% four times daily or after meals, and 14% once daily. In the control group, 52% reported brushing once daily and 36% twice daily. Among orthodontic participants, 84% had worn the appliance for 6 months or longer and 16% for approximately 3 months.

Table 1. Selected participant and oral-hygiene characteristics.

Characteristic	Orthodontic group, n (%)	Control group, n (%)
Female	35 (70.0)	39 (78.0)
Male	15 (30.0)	11 (22.0)
Brush once daily	7 (14.0)	26 (52.0)
Brush twice daily	17 (34.0)	18 (36.0)
Brush three times daily	15 (30.0)	5 (10.0)
Brush four times daily / after meals	11 (22.0)	1 (2.0)
Fixed-appliance duration: about 3 months	8 (16.0)	Not applicable
Fixed-appliance duration: 6 months or longer	42 (84.0)	Not applicable

Values are descriptive counts and percentages reported for the study groups.

4.2 Culture yield and distribution of bacterial isolates

A total of 67 bacterial isolates were identified from the 100 subgingival samples collected: 38 from the orthodontic group and 29 from controls. Eleven bacterial species or species groups were recovered overall—gram-positive organisms predominated in both groups. Ten taxa were represented in the orthodontic isolate set compared with six in the control isolate set.

Among orthodontic isolates, *Staphylococcus aureus* was most frequent (10/38; 26.32%), followed by viridans streptococci (9/38; 23.68%), *Staphylococcus simulans* (5/38; 13.16%), and *Streptococcus pneumoniae* (4/38; 10.53%). *Pseudomonas* spp. accounted for 7.89%; *Acinetobacter* spp. and an organism reported in the source thesis as *Streptococcus orissratti* each represented 5.26%. *Moraxella* spp., *Proteus* spp., and *Streptococcus pyogenes* each accounted for 2.63%.

In controls, viridans streptococci predominated (10/29; 34.48%), followed by *Streptococcus pyogenes* (6/29; 20.69%), *Enterobacter* spp. (5/29; 17.24%), *Staphylococcus aureus* (4/29; 13.79%), *Streptococcus pneumoniae* (3/29; 10.34%), and *Pseudomonas* spp. (1/29; 3.45%). *Staphylococcus simulans*, *Acinetobacter* spp., the source-reported *S. orissratti*, *Moraxella* spp., and *Proteus* spp. were reported only in orthodontic isolates, whereas *Enterobacter* spp. was reported only among controls. Percentages were calculated from recovered isolates rather than all enrolled participants and therefore do not represent participant-level prevalence.

Table 2. Distribution of bacterial isolates in orthodontic and control groups.

Bacterial species/group	Control, n (%)	Orthodontic, n (%)	Total, n (%)
Viridans streptococci	10 (34.48)	9 (23.68)	19 (28.36)
Staphylococcus aureus	4 (13.79)	10 (26.32)	14 (20.89)
Streptococcus pneumoniae	3 (10.34)	4 (10.53)	7 (10.45)
Streptococcus pyogenes	6 (20.69)	1 (2.63)	7 (10.45)
Staphylococcus simulans	—	5 (13.16)	5 (7.46)
Enterobacter spp.	5 (17.24)	—	5 (7.46)
Pseudomonas spp.	1 (3.45)	3 (7.89)	4 (5.97)
Acinetobacter spp.	—	2 (5.26)	2 (2.99)
*Streptococcus orissratti**	—	2 (5.26)	2 (2.99)
Moraxella spp.	—	1 (2.63)	1 (1.49)
Proteus spp.	—	1 (2.63)	1 (1.49)
Total isolates	29 (43.28)	38 (56.72)	67 (100.0)

*Species name reproduced from the source thesis and should be verified against the original RENDER report. Percentages are within each group-specific isolate total.

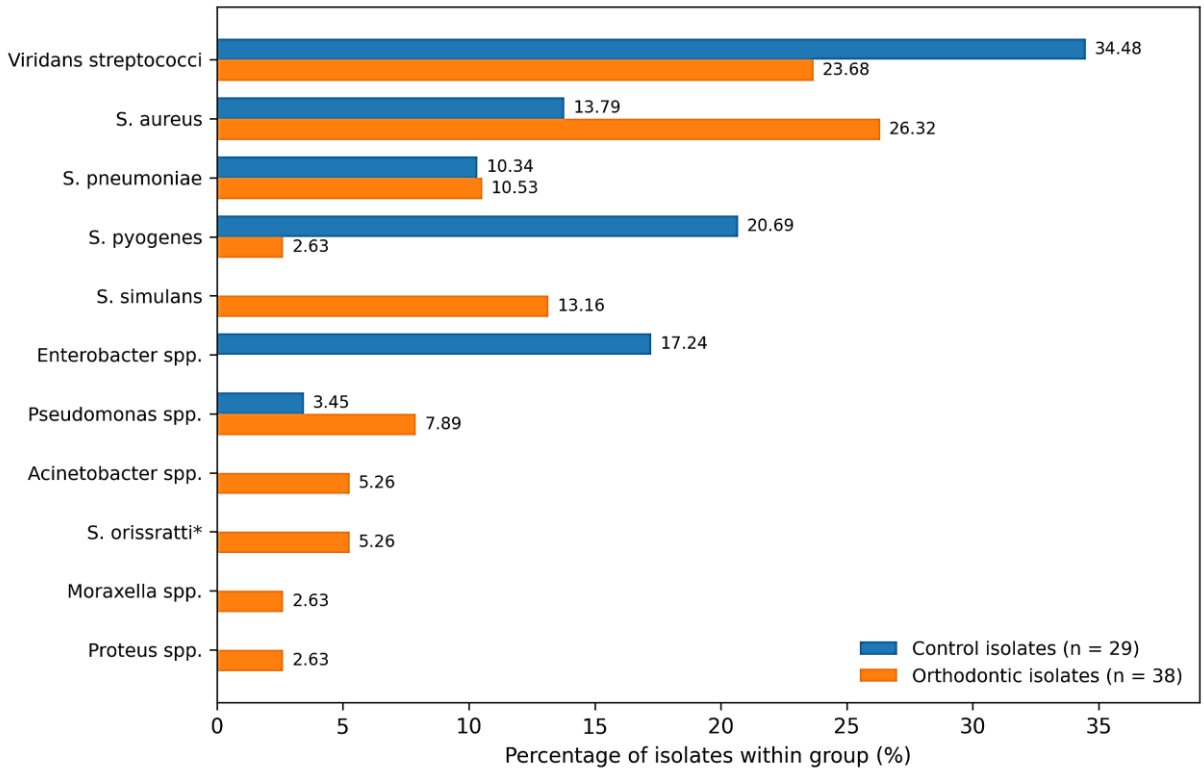

Fig. 2. Distribution of cultivable bacterial species among orthodontic and control isolates. Percentages are calculated within each isolate group. *Species label reproduced from the source thesis.

4.3 Distribution by sex and gingival bleeding at sampled tooth sites

More isolates were reported from women than men in both groups: 27 versus 11 isolates in the orthodontic group and 23 versus 6 isolates in controls. This pattern paralleled the female predominance among enrolled participants. Within the orthodontic female isolate set, viridans streptococci were most frequent, followed by *Staphylococcus simulans*, *Staphylococcus aureus*, and *Streptococcus pneumoniae*. In control women, viridans streptococci and *Streptococcus pyogenes* were the principal organisms.

Bleeding-associated isolates were reported at all three sampled tooth positions. In controls, *Streptococcus pyogenes* and *Staphylococcus aureus* were recovered at bleeding central-incisor sites, while *Streptococcus pneumoniae* was recovered at bleeding first-molar sites. In orthodontic cases, bleeding-site isolates included *Streptococcus pneumoniae* at first molars, *Pseudomonas* spp. at central incisors, and *Moraxella* spp., *Proteus* spp., and *Streptococcus pyogenes* at first premolars. The detailed source table also recorded *Staphylococcus aureus*, viridans streptococci, *Staphylococcus simulans*, and *Acinetobacter* spp. at selected bleeding sites. These are descriptive co-occurrences and do not demonstrate organism-specific causation of gingival bleeding.

4.4 Antimicrobial susceptibility patterns

Across control isolates, doxycycline and amoxicillin/clavulanic acid showed the most consistent in-vitro activity. Doxycycline sensitivity was reported as 100% for the listed *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, viridans streptococci, *Enterobacter* spp., and *Pseudomonas* spp. isolates. Amoxicillin/clavulanic acid was reported as 100% active against all listed control organisms except *Staphylococcus aureus*, for which 75% sensitivity was reported. Metronidazole resistance reached 100% in the tested control *Staphylococcus aureus*, *Streptococcus pyogenes*, viridans streptococci, *Enterobacter*, and *Pseudomonas* isolates. High resistance was also reported to erythromycin among viridans streptococci (90%) and *Staphylococcus aureus* (75%).

The orthodontic isolate set showed greater heterogeneity. Amoxicillin/clavulanic acid and doxycycline were reported as 100% active against *Streptococcus pyogenes*, viridans streptococci, *Streptococcus pneumoniae*, *Staphylococcus simulans*, *Acinetobacter* spp., and *Pseudomonas* spp.; both agents showed 87.5% sensitivity among *Staphylococcus aureus* isolates. Orthodontic *Staphylococcus aureus* showed 100% resistance to AZM and metronidazole and 87.5% resistance to oxacillin, tetracycline, and SXT. *Staphylococcus simulans* showed 100% resistance to metronidazole, oxacillin, tetracycline, and SXT. Species-level percentages were derived from small isolate counts and require cautious interpretation.

Table 3. Principal antimicrobial susceptibility patterns among control isolates.

Organism	Highest reported susceptibility	Major reported resistance
S. aureus	DO 100%; AMC and TE 75%	MET 100%; E 75%
S. pneumoniae	AMC, DO, and SXT 100%	E, OX, and TE 33.33%
S. pyogenes	AMC and DO 100%; TE and SXT 83.33%	MET 100%
Viridans streptococci	AMC and DO 100%; OX 70%	MET 100%; E 90%; AZM 87.5%
Enterobacter spp.	AMC, DO, and OX 100%; TE 80%	AZM, MET, and SXT 100%
Pseudomonas spp.	AMC, DO, TE, and SXT 100%	AZM and MET 100%

AMC, amoxicillin/clavulanic acid; AZM, azithromycin; DO, doxycycline; E, erythromycin; MET, metronidazole; OX, oxacillin; TE, tetracycline; SXT, trimethoprim/sulfamethoxazole.

Table 4. Principal antimicrobial susceptibility patterns among orthodontic isolates.

Organism	Highest reported susceptibility	Major reported resistance
S. aureus	AMC and DO 87.5%	AZM and MET 100%; OX, TE, and SXT 87.5%
S. pyogenes	AMC, DO, E, TE, and SXT 100%	OX 100%
Viridans streptococci	AMC and DO 100%; SXT 83.33%	OX 83.33%; E 66.67%
S. pneumoniae	AMC, DO, E, OX, and SXT 100%	TE 100%
S. simulans	AMC, DO, and E 100%	MET, OX, TE, and SXT 100%
Acinetobacter spp.	AMC, DO, OX, TE, and SXT 100%	E and MET 100%
Pseudomonas spp.	AMC, DO, MET, TE, and SXT 100%	E and OX 100%

Percentages are summarized from the thesis susceptibility tables and are based on the isolates tested for each organism–antibiotic combination.

5. DISCUSSION

The central finding was the broader cultivable bacterial spectrum reported among patients with fixed orthodontic appliances. Ten taxa were represented in orthodontic isolates compared with six in controls, and the orthodontic group contained several opportunistic Gram-negative organisms not recovered from controls. This pattern is biologically plausible because brackets, ligatures, bands, and archwires increase surface complexity, create stagnation zones, and hinder complete plaque removal (Contaldo et al., 2021; Guo et al., 2017; Santonocito & Polizzi, 2022).

The cross-sectional design does not prove that the appliance caused the difference, but the result is consistent with appliance-related ecological alteration.

Staphylococcus aureus was approximately twice as frequent among orthodontic isolates as among control isolates (26.32% versus 13.79%). Abbas et al. (2017) similarly reported a higher frequency of *S. aureus* in gingivitis samples from patients with orthodontic wires than in healthy controls. The organism may benefit from plaque retention and minor soft-tissue irritation around fixed components, although the present study did not quantify bacterial load or distinguish transient carriage from persistent colonization. Subgingival staphylococci have also been reported at periodontally diseased sites (Santos et al., 2014).

Viridans streptococci were common in both groups but were proportionally higher among controls. This differs from investigations reporting increased cariogenic streptococci after appliance placement (Lucchese et al., 2018; Topaloglu-Ak et al., 2011). The contrast may reflect use of an aggregate viridans category rather than species-specific quantification, percentages calculated among recovered isolates, aerobic culture conditions, appliance duration, and the larger number of other taxa in the orthodontic group. *Pseudomonas* spp. was also more frequent in orthodontic isolates, consistent with the directional finding reported by Sun et al. (2018).

Bleeding-site observations reinforce the clinical importance of plaque control but require cautious interpretation. Multiple organisms were recovered from bleeding sites, and no single species was consistently associated with all tooth positions. Previous orthodontic studies have shown that microbial and clinical changes can occur soon after appliance placement (Guo et al., 2019; Kim et al., 2012). Posterior bleeding-associated isolates in the present dataset are compatible with the greater cleaning difficulty around molar bands and posterior archwire segments described by Shrestha et al. (2016).

The higher number of isolates reported from women should not be interpreted as an independent sex effect. Women represented nearly three-quarters of the sample, so a larger female isolate count is expected even when the probability of recovery is identical. Research has identified sex-related differences in oral microbial composition (Liu et al., 2023), but the present dataset lacks balanced sampling and adjusted statistical analysis. Future studies should report participant-level positivity and use multivariable models that account for age, hygiene behavior, treatment duration, and appliance-related variables.

The antimicrobial findings support laboratory-guided prescribing. Doxycycline and amoxicillin/clavulanic acid showed the broadest reported activity across both groups, whereas metronidazole resistance was widespread in control isolates and in several orthodontic Gram-positive isolates. Oxacillin, tetracycline, erythromycin, and SXT resistance varied substantially by organism. These data demonstrate why one empirical regimen cannot be assumed to cover all cultivable bacteria recovered from orthodontic patients. At the same time, the small isolate numbers and the absence of fully documented interpretive standards mean that the percentages should not be converted directly into treatment recommendations (Reller et al., 2009).

Oral-hygiene education remains the primary preventive implication. Mechanical disruption of biofilm, careful brushing around brackets and gingival margins, interdental cleaning, dietary counseling, and regular professional review can reduce plaque accumulation without exposing the microbiota to unnecessary antibiotics. Most orthodontic participants reported brushing at least twice daily, yet a broad bacterial profile was still recovered. Frequency alone may therefore be insufficient; technique, access around appliance components, duration, and professional reinforcement are also important (Ireland et al., 2014; Santonocito & Polizzi, 2022).

5.1 Strengths and limitations

The study provides culture-based local data from two dental clinics and combines conventional biochemical testing with automated identification and susceptibility assessment. Sampling the same three tooth positions in both groups also provided a consistent clinical framework. Nevertheless, the cross-sectional design did not measure each patient before and after appliance placement, so group differences cannot be attributed causally to orthodontic treatment. Results were reported as percentages of isolates rather than participant-level prevalence or bacterial load, and complete inferential statistics were not supplied in the thesis result tables.

The aerobic culture protocol likely under-represented obligate anaerobic periodontal organisms, and culture cannot characterize the full oral microbiome as comprehensively as targeted molecular methods or sequencing (Guo et al., 2016; Guo et al., 2017). Several species-specific susceptibility percentages were derived from very small isolate counts. In addition, the source documents contain items that require verification before submission: the formal ethics-approval details, the source-reported spelling of *Streptococcus orissratti*, and minor inconsistencies in the stated study period. Future studies should use longitudinal sampling, molecular profiling, calibrated periodontal indices, and fully reported participant-level statistical analyses.

6. CONCLUSION

Patients wearing fixed orthodontic appliances showed a broader cultivable subgingival bacterial profile than non-orthodontic controls in this Benghazi sample. *Staphylococcus aureus* was the most frequent orthodontic isolate, whereas viridans streptococci predominated in controls. Several taxa were recovered only from the orthodontic isolate set, and bleeding-associated isolates occurred at anterior and posterior sites, supporting periodontal surveillance throughout fixed-appliance therapy.

Doxycycline and amoxicillin/clavulanic acid showed the most consistent in-vitro activity in the reported dataset, while metronidazole and several other agents displayed substantial organism-specific resistance. These findings do not justify routine antibiotic use; rather, they support technique-focused oral-hygiene instruction, regular professional monitoring, and microbiological susceptibility testing when systemic antimicrobial therapy is genuinely indicated. This approach may reduce preventable gingival complications and improve the safety of antimicrobial selection during orthodontic care.

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