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Article

Effects of different orthodontic appliances on *Actinomyces* spp.

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Abstract: This study aims to identify the presence of *Actinomyces* species in the oral flora during orthodontic treatments using removable appliances and fixed wire technique, and to track changes throughout the treatment process. In this study, subgingival and supragingival plaque samples obtained from 48 patients undergoing orthodontic treatment at a dental facilities of Ataturk University, Türkiye were utilized as the primary material. The study groups comprised individuals undergoing fixed orthodontic treatment, those using removable appliances, and individuals who had not undergone any orthodontic treatment. Plaque samples were collected from the patients at the initiation of treatment and six months thereafter. After selective culturing in a specific medium, dental plaque samples were purified and subjected to microbial analysis using the VITEK 2 system (Biomerieux). The study aimed to identify and compare the microbial content of plaque samples from different orthodontic treatment groups. *Actinomyces* species were identified in all groups at both the initiation of treatment (T0) and six months later (T1) periods. At the beginning of treatment, *Actinomyces* species were found in 33 samples, while at six months, they were present in 18 samples. The curent study revealed similar microorganisms among the groups at both T0 and T1 periods. The *Actinomyces* species identified in this study include *Actinomyces meyeri*, *Actinomyces naeslundii*, and *Actinomyces odontolyticus*. It is evident that orthodontic treatments significantly impact oral health and that *Actinomyces* microorganisms are always likely to be present in the oral flora.

Keywords: *Actinomyces*; dental plaque; microbiology; orthodontic appliances; VITEK 2

1. Introduction

The use of bands and brackets in orthodontic treatments tends to enhance the adherence of food particles. This situation leads to several dental issues, including a decrease in pH, heightened colonization by certain microorganisms (such as *Streptococcus mutans*), biofilm and plaque formation (Zakrzewski *et al*., 2021). These factors contribute to unhealthy and potentially irreversible damage to tooth enamel, including cavity formation and decalcification. *Actinomyces*, which are gram-positive rods present in the oral flora, are mostly facultative anaerobes, although some are entirely anaerobic (Schaal, 1986). The genus *Actinomyces* was initially identified as a part of the phylum Actinobacteria by Harz and colleagues in 1877. The first species of *Actinomyces* to be isolated was *Actinomyces bovis* (Harz, 1878; Schaal *et al*., 2006). It is highlighted that *Actinomyces* species can colonize in various environments, including soil, animals, and humans, indicating their widespread presence. Numerous *Actinomyces* species are part of the anaerobic, natural microbiota located in the human mucous

membranes of the oropharynx, gastrointestinal tract, and female genital tract (Nyvad and Kilian, 2006; Marsh and Martin, 1984).

Infections caused by these microorganisms are typically chronic, developing slowly after they penetrate deep tissues following trauma to the mucosa where they reside. Pelvic actinomycosis is frequently linked to the use of an intrauterine device. Poor oral hygiene in a patient may increase the risk of developing cervicofacial actinomycosis (Murray *et al*., 2007). How *et al*. (2016) reported that *Actinomyces naeslundii*, *Actinomyces israelii*, and *Actinomyces viscosus* are prominent members of the human oral microbiota. It is noted that *Actinomyces* species are also dominant in the microflora of the gingival groove. *Actinomyces*, particularly *A. naeslundii*, are often isolated from root rot lesions and have been known to cause root rot in experimental animals. However, these organisms are also commonly found on healthy root surfaces, which makes the role of *Actinomyces* in the disease process ambiguous. *Actinomyces* species are reported to play a significant role in both coronal and root caries (Lamont and Jenkinson, 2010).

Actinomyces are commonly found in the subgingival microflora of the oral cavity. *Actinomyces* make up a substantial part of the natural oral microbiota in humans and are among the predominant microorganisms found in both supragingival and subgingival plaque (Ximénez-Fyvie *et al*., 1999). However, there are relatively few studies concerning the specific oral locations and prevalent species of *Actinomyces*. Alongside studies suggesting that *Actinomyces* species may contribute to the development of periodontal diseases (Socransky *et al*., 1970; Ximénez-Fyvie *et al*., 1999), there is also research indicating an increase in periodontal diseases in oral areas where the presence of *Actinomyces* is reduced (Dzink *et al*., 1988).

Actinomyces species are among the dominant groups of bacteria found in human dental plaque (Marsh and Martin, 1984). Owing to its frequent occurrence and significance in the oral environment, routine identification of *Actinomyces* has been a focus, with various studies conducted for this purpose (Kalfas and Edwardsson, 1990). The timing and characteristics of the initial colonization by certain species significantly impact the composition of both the indigenous and pathogenic oral microbiota. This early colonization forms the foundation for subsequent bacterial growth. The significance of these species lies in their capacity to adhere to debris and to interact with other bacteria (Cisar *et al*., 1989).

Some species of Streptococcus and *Actinomyces* play a key role in early plaque formation. They initially colonize tooth and mucosal surfaces, creating a substrate for adhesion that supports the growth of various microorganisms in dental plaque. This includes bacteria that struggle to grow otherwise, such as gram-negative and anaerobic bacteria, as described by Kolenbrander *et al*. (1993).

Actinomyces can be isolated not only from infections in the oral area but also from various other parts of the human body (Schaal, 1986). Tanner *et al*. (1998) noted the association of *A. naeslundii* with subjects showing active buccal periodontal conditions. *Actinomyces meyeri* levels are significantly higher in active destructive periodontitis cases than in adult gingivitis. Therefore, the existing data indicate that specific *Actinomyces* species may have varying roles in oral health and disease. Consequently, their rapid and precise differentiation in plaque samples is essential. Even though *Actinomyces* species are a part of the normal, resident microbiota on various oral surfaces, they contribute to different plaque-related diseases, such as dental caries and periodontal diseases (Moore and Moore, 1994). *Actinomyces* are frequently present in endodontic infections, typically as part of a polymicrobial infection (Peters *et al*., 2002). While *Actinomyces* species are linked with teeth that have necrotic pulp, they are more commonly associated with cases of unsuccessful root canal treatments (Kalfas and Edwardsson, 1990; Sarkonen, 2007). *Actinomyces* can lead to extraradicular infections, thereby hindering periapical healing through conventional treatment. Members of this species have also been frequently isolated from the infected dentin of active root caries lesions. Although *A. naeslundii*, *A. israelii*, and *A. gerencseriae* are particularly the most frequently isolated species, the specific role of individual *Actinomyces* species in the root caries process remains uncertain (Sarkonen, 2007). One significant change in the microbial composition of dental plaque as gingivitis develops is the increase of *Actinomyces* spp. (Rafatjou *et al*., 2019).

This study aimed to identify *Actinomyces* species naturally present in the oral flora and to determine the specific *Actinomyces* species in the oral flora of patients undergoing orthodontic treatment with removable appliances and fixed technique. This study has made a significant contribution to the identification of *Actinomyces* species in both supragingival and subgingival plaque samples.

2. Materials and Methods

2.1. Ethical approval and informed consent

Ethics committee approval was received for this study from the ethics committee of Dentistry Faculty, Atatürk University, Türkiye (Date: 13.10.2021, Number: 10/2021). Written informed consent was obtained from patients who participated in this study.

2.2. Study area
A total of 48 patients, aged between 10 and 25, voluntarily participated in this study. They were individuals seeking orthodontic treatment at Department of Orthodontics, Faculty of Dentistry, Atatürk University, Türkiye.

2.3. Study group
The group included patients using fixed orthodontic treatments or removable appliances according to their treatment plans, as well as individuals who did not use any appliances. The study involved collecting both subgingival and supragingival dental plaque samples from the patient at two separate times. The research was carried out without consideration of gender. Patients and their parents who voluntarily consented to participate in the research received both verbal and written information about the methodology. Additionally, wet-signed written informed consent forms were obtained from both the patients and their legal guardians to participate in the study. The study incorporated specific criteria for the inclusion and exclusion of patients. Those included in the study were required to fall within the age range of 10 to 25, possess normal root development, and have no prior history of orthodontic treatment. Conversely, individuals were excluded from the study if they exhibited any of the following: missing teeth, periodontal bone loss, a nail-biting habit, systemic or syndromic diseases (hepatic, renal, hematological, cardiovascular), congenital, genetic, or acquired craniofacial deformities resulting from trauma (e.g., cleft palate or lip), smoking habits, or untreated tooth decay in the oral cavity. These criteria aimed to ensure a homogeneous and relevant participant group for the research investigation.

2.3.1. Group treated with fixed orthodontic appliances
This group includes 16 volunteers who received treatment with fixed upper and lower orthodontic techniques. This group consists of patients whose treatment began directly with brackets, without any tooth extractions or the cementing of a fixed appliance. At the start of the treatment, patients received oral hygiene training and were advised to maintain good oral hygiene throughout the treatment period.

2.3.2. Group treated with removable appliances
This group comprises 16 patients who used removable appliances for a single jaw, such as removable space maintainers, screw or spring protrusion appliances, and removable maxillary expansion screws. At the start of the treatment, patients received oral hygiene training and were advised to maintain good oral hygiene throughout the treatment.

2.3.3. Group not using any apparatus
No treatment was planned for individuals in this group. They received oral hygiene training at the study's outset and were advised to maintain good oral hygiene throughout the study. This group consists of 16 volunteers.

2.4. Records collected from patients for the study
Throughout the study, subgingival and supragingival dental plaque samples were gathered from a cohort of 48 volunteer participants. The collection of samples occurred at two distinct time points: T0, which signified the moment just prior to the initiation of orthodontic appliance application in accordance with the planned treatment, and T1, representing a period six months after the appliance had been installed in the oral cavity.

2.5. Collecting a subgingival dental plaque sample
Patients were instructed to avoid eating, drinking any beverages, or brushing their teeth for at least one hour before their appointment. The subgingival dental plaque sample was obtained through two methods; initially, it was carefully collected from below the gumline with a thin-tipped periodontal probe, taking care to avoid damaging the gum. The subgingival plaque was gathered from the vestibular surfaces of a total of 24 teeth. This particular number of teeth, 24, was established through trials. Preliminary studies showed that a weight of 0.001 g from the plaque samples of these teeth is necessary for accurate identification and quantification. The vestibular surface was selected because it is where brackets and clasps make direct contact with the tooth. The sample, gathered using a periodontal probe, was then transferred to tubes filled with Amies Transport Medium and swiftly dispatched to the microbiology laboratory. Additionally, subgingival dental plaque samples were collected using 8 sterile paper points, which were applied to the deep pocket areas of the teeth. In this procedure, the chosen areas were isolated using cotton rolls, and the paper point was held at the base of the pocket for 20 seconds. Then, the samples were immediately placed into tubes with 2 ml of thioglycolate broth and rapidly sent to the microbiology laboratory.

2.6. Collecting a dental plaque sample from teeth

A scaler was used to remove the dental plaque from the tooth. Supragingival dental plaque samples were taken from the vestibule surfaces of 24 teeth. Samples collected using a scaler were placed in tubes with tryptic soy broth and promptly sent to the microbiology laboratory. Samples from selected volunteers at Atatürk University's Faculty of Dentistry Orthodontics Clinic were quickly delivered to the Microbiology Laboratory during the laboratory phase. In this study, 96 samples were analyzed: 48 in the T0 period and 48 in the T1 period.

2.7. Identification of anaerobic bacteria

In the case of total anaerobic bacteria count, we vortexed samples containing 5 ml of a medium suitable for anaerobic bacteria. Then, we took 200 µL of this mixture and inoculated it onto Brain Heart Infusion Agar, supplemented with vitamin K (0.1 µg/mL) and hemin (1 µg/mL). The samples were incubated anaerobically for 2-7 days. To determine if the environment was anaerobic, we used Anaerotest (Merck) strips (Rafatjou *et al*., 2019).

Samples of anaerobic bacteria, collected in thioglycolate broth and amies transport medium, were inoculated into appropriate media. To isolate anaerobic bacteria, reinforced clostridial medium with 5% sheep blood agar and brain heart infusion agar, supplemented with vitamin K (0.1 µg/mL) and haemin (1 µg/mL), were used. Anaerobic incubation took place in a jar where oxygen was removed and replaced with a mixture of 90% hydrogen and 10% carbon dioxide. Anaerobic conditions were established using Anaerocult A (Merck) placed in the jar. The anaerobic environment was then verified with Anaerotest (Merck) strips. Incubation was carried out at 37°C for 72 hours. Different colonies growing on the media were repeatedly cultured to achieve purification. Catalase, oxidase, and Gram staining tests were conducted on the microorganisms that were incubated on blood agar for 24-48 hours after transfer. Bacterial suspensions, derived from young breeding colonies, were prepared at concentrations of 2.70-3.30 McFarland Standard in tubes filled with sterile physiological saline. The bacterial suspensions were then placed in the device's cassettes, and VITEK 2 ANC cards were assigned for each tube. The cassettes were inserted into the VITEK 2 device for identification.

2.8. Using the VITEK 2 (Biomerieux) system for bacterial ıdentification

In the VITEK 2 (Biomerieux) system, gram-positive and gram-negative microorganisms have different identification and sensitivity cards. The VITEK 2 system uses GP and GN cards, each with 64 wells containing 19-20 antimicrobial agents. This allows for the simultaneous identification and antibiotic sensitivity testing of up to 60 isolates. The system includes 43 biochemical tests that assess carbon source utilization, enzymatic activity, and resistance. In the VITEK 2 system, cards are first filled and then automatically moved to the reader incubator system. They undergo periodic optical readings, and upon completion of the process, the cards are automatically discarded. Identification results can be determined in approximately 8 hours (Garcia-Garrote *et al*., 2000; Ligozzi *et al*., 2002).

3. Results

The participants consisted of 62.5% females and 37.5% males. The average age of the participants was 15.75 ± 0.93 in the fixed treatment group, 15.37 ± 0.88 in the mobile appliance group, and 15.50 ± 0.74 in the control group. The *Actinomyces* species and their frequencies identified in the T0 period are presented in Table 1.

Table 1. Types of microorganisms defined in the VITEK 2 System and their detection numbers in the T0 period.

Control group	Removable appliance group	Fixed appliance group
Actinomyces meyeri (4)	*A. meyeri* (4)	*A. meyeri* (3)
A. naeslundii (4)	*A. naeslundii* (3)	*A. naeslundii* (3)
A. odontolyticus (4)	*A. odontolyticus* (4)	*A. odontolyticus* (4)

The *Actinomyces* species and their frequencies identified in the T1 period are presented in Table 2.

Table 2. Types of microorganisms defined in the VITEK 2 System and their detection numbers in the T1 period.

Control group	Removable appliance group	Fixed appliance group
Actinomyces meyeri (1)	*A. meyeri* (2)	*A. meyeri* (1)
	A. naeslundii (3)	*A. naeslundii* (3)
A. odontolyticus (3)	*A. odontolyticus* (1)	*A. odontolyticus* (4)

Actinomyces microorganisms were detected in 33 samples in the T0 period, and this number was 18 in the T1 period (Table 3).

Table 3. Types of microorganisms defined in the VITEK 2 System and their detection numbers in the T0 and T1 periods.

T0	T1
Actinomyces meyeri (11)	*A. meyeri* (4)
A. naeslundii (10)	*A. naeslundii* (6)
A. odontolyticus (12)	*A. odontolyticus* (8)

4. Discussion

The growing aesthetic expectations and concerns in society, along with the significance placed on facial and smile aesthetics, have significantly heightened interest and desire for orthodontic treatment. Maintaining good oral hygiene is crucial during orthodontic treatments. Effectively removing dental plaque is vital for oral hygiene and periodontal tissue protection. It also helps prevent the formation of white spot lesions, as noted by Shils *et al*. (2006). However, the use of attachments and appliances in orthodontics may alter the microbiological balance of the oral flora, as indicated by Marsh (2003).

The formation of new retention areas around bands and brackets is a primary cause of the inflammatory response and increased dental plaque accumulation. This has necessitated examining the effects of orthodontic appliances on oral hygiene and gum health. In this context, there is an emerging need to investigate how orthodontic appliances affect oral hygiene and periodontal health. Numerous studies (Alotaibi, 2023; Rouzi *et al*., 2023) have investigated the potential microbiological effects of orthodontic treatment on oral flora and changes in periodontal tissues.

In this study, the experimental group comprised patients treated with the fixed technique and those using removable appliances. However, as these two patient groups lack alternative treatment options, the primary goal was to observe changes between the T0 and T1 periods. The control group in the study was established for standardization purposes. This approach enhanced the research's reliability and validity, thereby strengthening the study's overall scientific contribution.

For this study, the protocol included individuals who had not received any antibiotic treatment in the past month. The literature documents that antibiotic use can significantly alter the microbiological composition of oral flora, as shown by Cheng *et al*. (2022) and Kopra *et al*. (2021) the study excluded individuals who have a habit of nail biting (onychophagia). This habit, involving frequent hand-to-mouth contact, can spread bacteria and other microorganisms around the mouth and nails. This may disrupt the balance of oral flora and potentially cause tooth and gum diseases.

This study did not include individuals with poor oral hygiene or untreated decayed teeth. Literature indicates that individuals with active caries may have dominant microorganisms in their oral cavity (Espinoza *et al*., 2018; Jiang *et al*., 2018; Zhang *et al*., 2022). Therefore, to more accurately assess the microbial changes in the oral flora due to orthodontic applications, individuals with these characteristics were excluded from the study. The study group was composed of individuals who had never received orthodontic treatment before. Literature reviews (Khorsand *et al*., 2013; Polson *et al*., 1988) indicate that individuals who have had orthodontic

treatment in the past may show variations, particularly in periodontal parameters. While liver, kidney, hematological, or cardiovascular system diseases don't limit the applicability of orthodontic treatment protocols, individuals with these conditions were excluded from the study due to their potential systemic impact on oral mucosal health. Existing studies, such as Baboni *et al*. (2010), demonstrate that cigarette smoke can inhibit biofilm development on brackets and bands. Studies, including those by Gomes *et al*. (2006) and Teixeira *et al*. (2009), have also examined species diversity and microbial density in the oral cavities of smokers. Based on this information, smokers were excluded from the study. Individuals with a cleft lip and/or palate were not included in the study.

Maintaining periodontal health is crucial in orthodontic treatment planning for both adult and pediatric patients. Orthodontists prioritize maintaining the integrity of periodontal tissues, which has led to the establishment of specific hygiene protocols for orthodontic patients. Numerous studies in the literatüre (Abbate *et al*., 2015; ElNaghy *et al*., 2023; Jiang *et al*., 2018; Konermann *et al*., 2017) focus on examining changes in periodontal parameters among individuals undergoing orthodontic treatment. In periodontal diseases, there are no specific bacteria identified as indicators of active disease. However, the more frequent detection of certain microorganisms in these pathologies compared to others has led to their acceptance as risk indicators of the disease.

In the curent study, similar microorganisms were identified among the groups in both the T0 and T1 periods. The detected *Actinomyces* species included *A. meyeri, A. naeslundii,* and *A. odontolyticus*. Most microorganisms found in the flora belong to the opportunistic pathogen group. Their risk of becoming pathogenic increases in individuals with weakened immune systems. The isolation of *A. meyeri, A. naeslundii, and A. odontolyticus* species can be significant in causing specific infections in various body parts, as noted by Könönen and Wade (2015). Identifying these bacteria suggests that individuals might have had a condition like oral aphthosis when the samples were taken. As a result, the examination of patients and volunteers undergoing orthodontic treatment shows that there is always a possibility of presence of *Actinomyces* species in the oral flora.

5. Conclusions

Current study results showed that *Actinomyces* species were present in the oral flora during orthodontic treatments, both with removable appliances and fixed techniques. These findings could be crucial for understanding dental hygiene and microbiota dynamics during orthodontic treatment. They may also guide future research to more deeply explore the effects of orthodontic appliances on microbial flora. In our study, we identified several *Actinomyces* species in the mouth (*A. meyeri*, *A. naeslundii*, and *A. odontolyticus*). However, we suspect that additional *Actinomyces* species might also exist. To explore this possibility, further research could be carried out in diverse geographical and cultural regions across different seasons. The success of orthodontic treatments extends beyond achieving ideal occlusion, facial aesthetics, and stability; it also integrally involves protecting and improving periodontal health. In this complex treatment process, collaboration between orthodontists and periodontologists is critical, requiring a multidisciplinary approach. Optimizing patient outcomes and achieving more satisfactory results in both orthodontic and periodontal health heavily relies on this multidisciplinary approach. Hence, in modern dentistry, close collaboration between orthodontists and periodontologists is regarded as best practice.

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Data availability

The dataset that arose and were used in the current study is available from the corresponding author on reasonable request.

Conflict of interest

None to declare.

Authors' contribution

Conceptualization – Abdulvahit Erdem; Design – Aybüke Asena Atasever İşler and Abdulvahit Erdem; Supervision – Abdulvahit Erdem; Resources – Aybüke Asena Atasever İşler and Abdulvahit Erdem; Materials – Aybüke Asena Atasever İşler and Abdulvahit Erdem; Data collection and/or processing – Aybüke Asena Atasever İşler; Analysis and/or interpretation – Aybüke Asena Atasever İşler and Abdulvahit Erdem; Literature

search – Aybüke Asena Atasever İşler and Abdulvahit Erdem; Writing manuscript – Aybüke Asena Atasever İşler; Critical review – Abdulvahit Erdem. All authors have read and approved the final manuscript.

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