Original Article

Antimicrobial resistance of bacterial strains in patients undergoing orthodontic treatment with and without fixed appliances

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ABSTRACT

Objectives: To identify microorganisms isolated from patients wearing fixed orthodontic appliances and to evaluate the resistance of isolated bacterial strains to different antimicrobials.

Materials and Methods: Seventeen healthy patients wearing a fixed orthodontic appliance (group 1) and six nonwearers (group 2, control group) were evaluated. The biofilm that formed around the orthodontic brackets was collected, and the samples were then plated in a chromogenic medium (chromIDT, bioMérieux). Colony-forming units (CFUs) were isolated and inoculated in blood-agar medium. Automated biochemical tests (VITEK 2, bioMérieux) were carried out to identify the genus and species of the microorganisms and the resistance provided by 43 drugs (37 antibacterial and 6 antifungal).

Results: The most prevalent microbial genera identified in group 1 were *Streptococcus* (24.0%), *Staphylococcus* (20.0%), *Enterobacter* (12.0%), *Geobacillus* (12.0%), and *Candida* (12.0%), and the most frequent species were *Enterobacter cloacae complex* (13.6%) and *Staphylococcus hominis* (13.6%). In group 2, the most prevalent genera were *Streptococcus* (57.1%), *Staphylococcus* (14.2%), *Sphingomonas* (14.2%), and *Enterobacter* (14.2%). With regard to antimicrobial resistance, 14 of 19 (74%) isolated bacterial strains were found to be resistant to at least 1 of the tested antimicrobials.

Conclusions: The findings of the present study suggest that patients undergoing orthodontic treatment with fixed appliances have a more complex biofilm with a higher level of bacterial resistance. (*Angle Orthod.* 2021;91:672–679.)

KEY WORDS: Orthodontics; Biofilm; Bacterial resistance

INTRODUCTION

Many factors can affect microbial colonization of the oral cavity, and fixed orthodontic appliances are one of the main factors.[1,2] Installed in the oral cavity, these appliances not only favor accumulation and maturation of biofilm[3] but also cause changes in the composition, pH, carbohydrate content, and microbial populations of *Streptococci* and *Lactobacilli*.[4] These changes can usually be observed 1 month after the start of treatment and occur regardless of the type of device. However, fixed appliances have a greater comparative impact on oral bacteria than removable appliances do.[5]

Studies showed that an orthodontic appliance in the oral cavity changed the biofilm quantitatively and qualitatively. This may have negative effects, such as dental caries or periodontal problems, which may have an impact on the patient's quality of life.[6,7] The microorganisms that accumulate around the brackets

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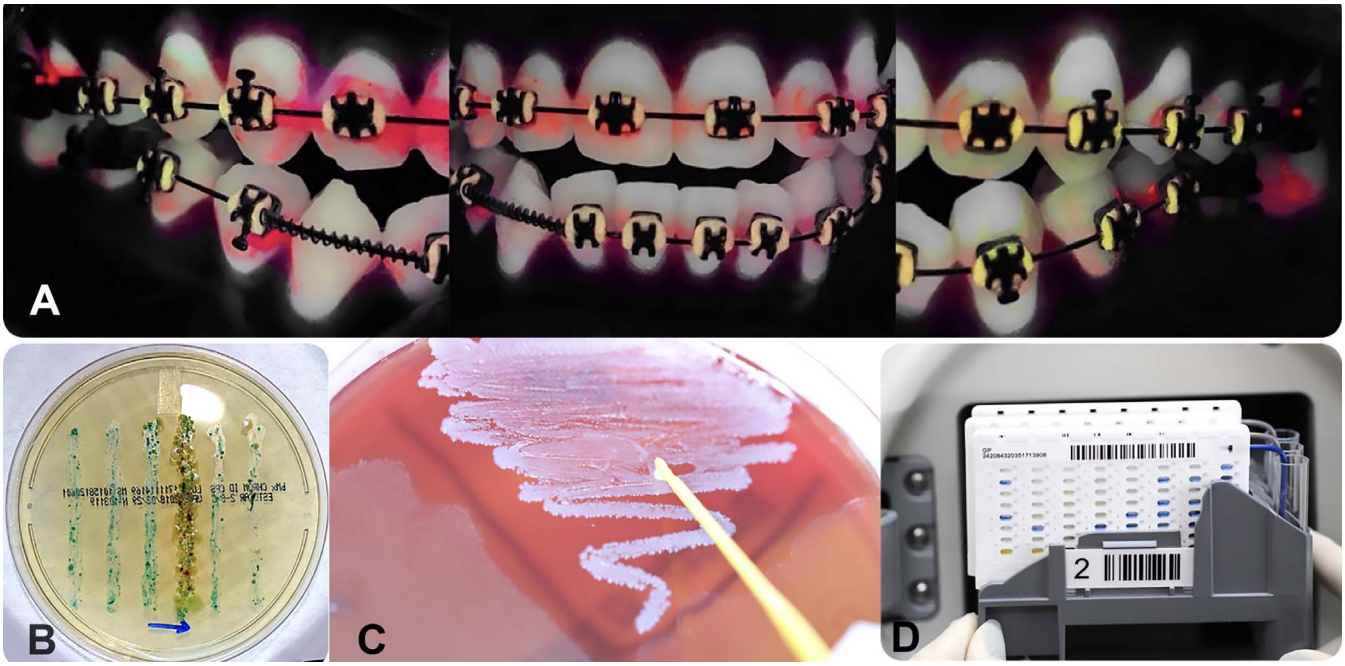

Figure 1. Biofilm collection. (A) Biofilm was assessed using Qscan Plus (AIOBIO) equipment, which reveals mature biofilm in red by using microbial autofluorescence. (B) A microbrush applied the biofilm that was found in each sextant directly to the CPS Elite agar plate (chromID, bioMérieux) by streaking, resulting in immediate inoculation. (C) Isolated strains plated on blood-agar. (D) The isolated strains were tested in the VITEK 2 Compact System.

of a fixed orthodontic appliance can enter the patient's bloodstream after procedures in which the oral tissues are manipulated and cause transient bacteremia.[8] Procedures such as removal of a Haas expander appliance, placement of orthodontic mini-implants, installation and removal of orthodontic bands, and even toothbrushing with the appliance are highly related to transient bacteremia.[9]

Bacterial resistance to antimicrobials is currently one of the most relevant global public health problems and can lead to clinical and economic consequences of great, ongoing concern. It is mostly associated with the inappropriate use of antimicrobials.[10]

Several previous studies have been conducted to evaluate the influence of orthodontic treatment on the composition of dental biofilm. However, studies evaluating the impact of wearing fixed orthodontic appliances on the antimicrobial resistance pattern of the biofilm are still scarce. Hence, the objective of this comparative study was to isolate and identify microorganisms from the biofilm of patients wearing fixed orthodontic appliances and assess the susceptibility of the identified bacterial strains to antimicrobials.

MATERIALS AND METHODS

This research was approved by the Ethics and Research Committee of Faculdade São Leopoldo Mandic, Campinas, SP, Brazil (80022517.7.0000.5374).

Selection of Patients

Twenty-three patients were selected. They comprised both genders, between 18 and 41 years of age, with good general conditions and oral health, without active caries or periodontal disease, enrolled in the Post-graduation of Orthodontics at Faculdade São Leopoldo Mandic, Campinas, São Paulo, Brazil.

The participants were divided into two groups: group 1 consisted of patients undergoing orthodontic treatment with fixed braces, including brackets, straight wire arches, and modular elastomeric ties in both arches ($n = 17$; 7 men, 10 women; mean age 20.7 $\pm$ 8.7 years) for 3 to 6 months of treatment; group 2 consisted of nonwearers of orthodontic appliances (control; $n = 6$; 2 men, 4 women; mean age 19.6 $\pm$ 1.3 years).

Biofilm Collection

Biofilm collection was performed using full personal protective equipment and a dry microbrush applicator (KG Brush Fine, São Paulo, Brazil). Before collection, the biofilm in each sextant was assessed using Qscan Plus (AIOBIO, Seoul, Republic of Korea) equipment, LED equipment that reveals mature biofilm (3 days) in red by using microbial autofluorescence (Figure 1A).

Table 1. List of Isolated Bacterial Strains Identified in Patients With (Group 1) and Without (Group 2) Orthodontic Appliances, and Resistance to the Antimicrobials[a]

Genus	Species	Ampicillin	Ampicillin/ Sulbactam	Benzylpenicillin	Cefoxitin	Cefuroxime	Cefuroxime Axetil	Clindamycin
Group 1 with orthodontic appliances								
Streptococcus	*anginosus* (99%)	S	-	S	-	-	-	S
	anginosus (94%)	S	-	S	-	-	-	S
	oralis (99%)	S	-	S	-	-	-	S
	oralis (99%)	S	-	-	-	-	-	R
	parasanguinis (97%)	S	-	S	-	-	-	S
	suis II (96%)	R	-	R	-	-	-	S
Staphylococcus	*aureus* (99%)	-	-	R	-	-	-	R
	hominis (99%)	-	-	R	-	-	-	S
	hominis (96%)	-	-	R	-	-	-	S
	hominis (94%)	-	-	R	-	-	-	S
	warneri (95%)	-	-	R	-	-	-	S
Enterobacter	*cloacae* complex (99%)	R	R	-	R	R	R	-
	cloacae complex (97%)	R	R	-	R	R	R	-
	cloacae complex (91%)	R	R	-	R	R	R	-
Geobacillus	*stearothermophilus* (92%)	-	-	-	-	-	-	-
	stearothermophilus (89%)	-	-	-	-	-	-	-
	Thermoleovorans (95%)	-	-	-	-	-	-	-
Klebsiella	*oxytoca* (99%)	R	S	-	S	S	S	-
	oxytoca (95%)	R	S	-	S	S	S	-
Escherichia	*coli* (99%)	S	S	-	S	S	S	-
Granulicatella	*adiacens* (91%)	-	-	-	-	-	-	-
Pseudomonas	*aeruginosa* (91%)	-	-	-	-	-	-	-
	intermedius (98%)	S	-	S	-	-	-	S
Group 2 without orthodontic appliances								
Streptococcus	*oralis* (88%)	S	-	S	-	-	-	S
	salivarius (91%)	S	-	S	-	-	-	S
	vestibularis (92%)	R	-	R	-	-	-	R
Enterobacter	*aerogenes*	R	R	-	R	R	R	-
Sphingomonas	*paucimobilis* (97%)	-	-	-	-	-	-	-
Staphylococcus	*hominis* (95%)	-	-	S	-	-	-	-
	Resistant	8	4	7	4	4	4	3
	Sensitive	9	3	8	3	3	3	12
	% Resistant	47.1	57.1	38.9	57.1	57.1	57.1	20.0
	% Sensitive	52.9	42.9	44.4	42.9	42.9	42.9	80.0

[a] S indictes sensitive; R, resistant; -, not detected.

The microbrush was rubbed around the brackets in areas where there was biofilm, without removing the orthodontic wire. A microbrush applied the biofilm that was found in each sextant directly to the CPS Elite agar plate (chromID, bioMérieux, Marcy l'Etoile, France) by streaking, resulting in immediate inoculation (Figure 1B).

Isolation and Growth of Microorganisms

The plates were stored in microaerophilic incubators at 37°C for up to 48 hours. After growth, the strains were isolated using a platinum needle, and the isolated strain was smeared on glass slides, later stained with Gram stain, and evaluated microscopically. All isolated strains were plated on blood-agar (bioMérieux) with a calibrated 10-μL platinum loop in the culture media and kept in a microaerophilic incubator at 37°C for 48 hours (Figure 1C).

Microbial Identification and Antimicrobial Susceptibility Testing (AST) (VITEK 2)

The isolated strains were tested in the VITEK 2 Compact System (bioMérieux) for microbial identification and antimicrobial susceptibility, according to the manufacturer's protocol and other studies.[11,12] The cards used to identify the microorganisms were the VITEK 2 GP ID Card (bioMérieux) for gram-positive bacteria; VITEK 2 GN ID Card, for gram-negative bacteria; BCL ID, for identification of gram-positive bacilli; and VITEK 2 YST ID Card, for yeasts. The cards used in the susceptibility test were AST-P637 for nonstreptococcal gram-positive bacteria, AST-ST03 for streptococcal gram-positive bacteria, AST-N239 for gram-negative bacteria, and AST-YS08 for yeast.

For the identification tests, only the strains with levels of reliability greater than 91% were accepted for use in the present study. For the AST tests, the results ranged in sensitivity (S) and resistance (R).

Table 1. Extended

Erythromycin	Tigecycline	Tetracycline	Total Tested	Total Resistant	Total Sensitive	% Resistant	% Sensitive
-	S	S	13	0	13	0.0	100.0
S	S	S	13	0	13	0.0	100.0
R	S	S	13	1	12	7.7	92.3
R	S	R	13	3	10	23.1	76.9
S	S	S	13	0	13	0.0	100.0
R	S	S	13	3	10	23.1	76.9
R	S	-	15	3	12	20.0	80.0
S	S	-	14	1	13	7.1	92.9
R	S	-	14	2	12	14.3	85.7
R	S	-	14	2	12	14.3	85.7
S	S	-	14	1	13	7.1	92.9
-	S	-	17	5	12	29.4	70.6
-	S	-	17	5	12	29.4	70.6
-	S	-	17	5	12	29.4	70.6
-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-
-	S	-	17	1	16	5.9	94.1
-	S	-	17	1	16	5.9	94.1
-	S	-	17	0	17	0.0	100.0
-	-	-	-	-	-	-	-
-	R	-	10	1	9	10.0	90.0
S	S	S	13	0	13	0.0	100.0
R	S	S	13	1	12	7.7	92.3
R	S	S	13	1	12	7.7	92.3
R	S	S	13	4	9	30.8	69.2
-	S	-	17	5	12	29.4	70.6
-	-	-	-	-	-	-	-
-	S	-	14	0	14	0.0	100.0
9	1	1		45	273	-	-
5	22	9		77	-	-	-
64.3	4.3	10.0		-	-	-	-
35.7	95.7	90.0		-	-	-	-

Data Analysis

The data were tabulated for further descriptive analysis and application of the Fisher's exact test, with statistical significance less than 5%.

RESULTS

A total of 32 strains were isolated and identified, 25 strains from 17 patients in group 1 (wearers of orthodontic appliances) and 7 strains from 6 patients in group 2 (nonwearers of orthodontic appliances). Of the 25 strains in group 1, 12 gram-positive cocci, 7 gram-negative bacilli, 3 gram-positive bacilli, and 3 yeasts were identified. Of the 7 strains in group 2, 5 were gram-positive cocci and 2 were gram-negative bacilli.

In group 1, the most prevalent strains were *Streptococcus* (24.0%), *Staphylococcus* (20.0%), *Enterobacter* (12.0%), *Geobacillus* (12.0%), and *Candida* (12.0%) (Tables 1 and 2). In group 2, the most prevalent genera were *Streptococcus* (57.1%), *Staphylococcus* (14.2%), *Sphingomonas* (14.2%), and *Enterobacter* (14.2%).

For assessment of the number of susceptible and antimicrobial-resistant strains (AST), only 19 of the 22 bacterial strains in group 1 were tested because the card manufacturer did not produce AST cards specifically for the group of gram-positive bacilli researched ($n = 3$). In group 1, 14 of the 19 strains (74%) were resistant to at least 1 of the tested antimicrobials, and four strains (26%) were sensitive to all of the tested antimicrobials. For group 2 patients, results were obtained for six of seven strains, four of which (67%) were resistant to at least one of the tested antimicrobials, and only two (33%) were sensitive to all of the tested antimicrobials. There was no statistically significant difference ($P = 1.00$) between the two variables (groups and resistance pattern).

For the antimicrobial groups tested, the greatest resistance was to the beta-lactam group (74%),

Table 2. List of Isolated Fungal Strains Identified in Patients With Orthodontic Appliances (Group 1) and Resistance to the Antifungals[a]

Genus	Species	Amphotericin B	Caspofungin	Fluconazole	Fluocytosine	Micafungin	Voriconazole	Total Tested	Total Resistant	Total Sensitive	% Resistant	% Sensitive
Group 1 with orthodontic appliance												
Candida	*albicans* (99%)	S	S	S	S	S	S	6	0	6	0.0	100.0
	albicans (99%)	S	S	S	S	S	S	6	0	6	0.0	100.0
	krusei (99%)	S	S	-	R	S	S	5	1	4	20.0	80.0

[a] S indicates sensitive; R, resistant; -, not detected. No fungal strains were identified in patients without orthodontic appliances.

followed by macrolides (19%), lincosamides (5%), and glycylcyclines (2%). Among the strains found in patients wearing orthodontic appliances, 24 were resistant to the group of beta-lactams (71%), six to macrolides (18%), two to lincosamides (6%), one to glycylcycline (3%), and one to tetracycline (3%). For the strains found in the patients who did not wear orthodontic braces, seven were resistant to the group of beta-lactams (64%), three to macrolides (27%), and one to lincosamides (9%).

DISCUSSION

In patients not wearing orthodontic appliances, the *Streptococcus* genus was the most prevalent (57.1%). This was in agreement with previous findings, as this microorganism is commonly found on the enamel surface.[13] The following *Streptococcus* species were identified: a strain of *S salivarius*, considered one of the most important and predominant pioneer species in the oral cavity,[14] it demonstrated resistance to erythromycin; a species of *S vestibularis*, rarely associated with human diseases despite reported association of the microorganism with infective endocarditis,[15] it demonstrated resistance to four different types of antimicrobials (ampicillin, benzylpenicillin, clindamycin, and erythromycin); a strain of *S mitis*, a microorganism that makes up the oral microbiota and that is highly related to infectious endocarditis,[16] it demonstrated resistance to erythromycin; and a strain of *S intermedius*, a microorganism present in the oral cavity and the upper respiratory, gastrointestinal, and female urogenital tracts.[17]

Other species isolated in group 2 included a strain of *Staphylococcus hominis*. There are reports associating this microorganism with nosocomial diseases associated with immunologically compromised patients.[18] A second species was a strain of *Enterobacter aerogenes*, an important opportunistic bacterial pathogen that is related to nosocomial infections.[19] This microorganism showed resistance to ampicillin, ampicillin/sulbactam, cefoxitin, cefuroxime, and cefuroxime axetil. This raises a concern, because these antimicrobials are only used in a hospital environment, and this strain was isolated from a healthy patient's dental biofilm. A third species was a strain of *Sphingomonas paucimobilis*, an opportunistic pathogen widely identified in water, soil, and also hospital environments.[20]

Overall, patients not wearing orthodontic appliances had microorganisms commonly found in the oral microbiota of healthy patients, except for *S hominis* and *E aerogene*. Gram-positive microorganisms were more prevalent and thus potentially less pathogenic.

The most prevalent genus in patients wearing orthodontic appliances was also the *Streptococcus*

group (24%), bearing in mind that it had a smaller prevalence in patients not wearing orthodontic braces. Two strains of *S anginosus* were identified; this a commensal species, but one strain has pathogenic potential. It has been identified in abscesses and plays a pathogenic role in cystic fibrosis. However, very little is known about the molecular basis of the pathogenicity of this bacterial species.[21] These two strains were sensitive to all tested antimicrobials. There was a strain of *S parasanguinis*, a member of the viridans group, one of the most common colonizers of the mouth, particularly identified on dental surfaces and associated with a variety of infections such as valve endocarditis and aortoenteric fistula.[22] This strain was isolated from a patient wearing orthodontic appliances. Next, there was a strain of *S suis II*, a zoonotic pathogen that can cause serious diseases, especially meningitis, in pigs and humans who have occupational contact with pigs, such as farmers, slaughterhouse workers, and butchers.[23] This strain was found in a patient wearing orthodontic appliances and showed resistance to ampicillin, benzylpenicillin, and erythromycin. Lastly, there were two strains of *S mitis*. One strain was resistant to erythromycin, and the other to erythromycin, clindamycin, and tetracycline.

Of the *Staphylococcus* genus, a strain of *S aureus* was isolated. This human pathogen is associated with serious hospital and community infections such as pneumonia, meningitis, endocarditis, and sepsis, among others.[24] In Brazil today, more than 80% of *S aureus* isolated from hospitalized patients, and about 70% isolated from patients in the community, are resistant to natural penicillin and therefore also to ampicillin and amoxicillin.[25]

In the present study, the isolated strain of *S aureus* showed resistance to benzylpenicillin, clindamycin, and erythromycin. A strain of *S warneri*, a pathogen commonly present in the microbiota of the human epithelium and mucous membranes, capable of causing serious infections,[26] and three strains of *S hominis* were resistant to benzylpenicillin and erythromycin.

A strain of *Granulicatella adiacens* was also isolated, commonly found in the mouth and associated with some cases of endocarditis.[27] *Klebsiella oxytoca* is an important bacterial isolate related to the cause of hospital-acquired infection in adults, causing bacterial endocarditis and having multiple resistance to commonly used antimicrobials[28]; two strains were found to be resistant to ampicillin. A strain of *Pseudomonas aeruginosa* was isolated, known as the main cause of morbidity and mortality in patients with cystic fibrosis and one of the main causes of nosocomial infections. Because of their mechanisms of adaptation, survival, and resistance to multiple classes of antimicrobials, strains of *P. aeruginosa* can cause fatal infections.[29] Three strains of *Enterobacter cloacae* were found and are highly related to nosocomial infections. The pathogenicity of this microorganism stems from it its ability to form biofilms and secrete various cytotoxins (enterotoxins, hemolysins, pore-forming toxins).[19] The three strains were resistant to ampicillin, ampicillin/sulbactam, cefoxitin, cefuroxime, and cefuroxime axetil. A strain of *Escherichia coli*, a bacterium typical of the intestinal tract, was found in the oral cavity and is associated especially with individuals who live in conditions of poor sanitation.[30] This microorganism was the only gram-negative bacillus isolated from a patient wearing an orthodontic appliance.

Overall, the prevalence of gram-negative microorganisms was higher in the oral microbiota of patients wearing orthodontic appliances, thereby making these pathogens more potentially deleterious than those found in patients who were not wearing orthodontic appliances. However, the results obtained did not indicate that an orthodontic appliance per se can promote an increase in resistance to antimicrobials. This was because the prevalence of microorganisms resistant to the tested antimicrobials was surprisingly high in both groups of patients, regardless of whether they were wearing orthodontic appliances (74%) or not (67%). In addition, the mechanisms of resistance to antimicrobials in biofilms seem to depend on multicellular factors, unlike the mechanisms already known such as plasmids, transposons, and mutations, which confer innate resistance to individual bacterial cells.[31] In the present study, the greatest resistance was to the class of beta-lactams, which are the first-choice antimicrobials for dentistry.[32] This is an important result because the indiscriminate use of antimicrobials has increased steadily, and many dentists still prescribe them arbitrarily and unnecessarily, thus aggravating this situation.[33]

It can be inferred that the presence of an orthodontic appliance may indeed favor the retention, maturation, and development of a more complex biofilm. Although this microbiota is more pathogenic, it does not represent a risk to healthy orthodontic patients, who can quickly fight these microorganisms in a possible bacteremia.[34] However, several of these microorganisms are associated with nosocomial infections and are highly resistant. Special attention should be given to patients who wear orthodontic appliances. Because of the changes in biofilm and the complexity of maturation and resistance to antimicrobials, it is essential that patient awareness be raised and that the frequency of professional prophylaxis be increased. This strategy would help disorganize the mature biofilm formed around the appliances and thus decrease its complexity and potential pathogenicity.

CONCLUSIONS

- The results of this study suggest that wearing fixed orthodontic appliances may favor the development of a more complex microbiota compared to controls.
- The microorganisms identified showed a high rate of resistance to antimicrobials.

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