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Evaluation of microbial colonisation at the attachment interface in fixed orthodontic treatments

Evaluación de la colonización microbiana en la interfase aditamento diente en tratamientos de ortodoncia fija

María Elizabeth Llivichuzca-Illescas
maría.llivichuzca.37@est.ucacue.edu.ec
Catholic University of Cuenca, Azogues, Azuay, Ecuador
https://orcid.org/0000-0002-1966-3846

Magaly Noemí Jimenez-Romero
mjimenezr@ucacue.edu.ec
Catholic University of Cuenca, Azogues, Azuay, Ecuador
https://orcid.org/0000-0002-0736-6959

Jessica María Sarmiento-Ordóñez
sarmientoo@ucacue.edu.ec
Catholic University of Cuenca, Cuenca, Azuay, Ecuador
https://orcid.org/0000-0003-4159-9286

Carlos Alberto Flores-Cárdenas
odontflores@hotmail.com
Catholic University of Cuenca, Cuenca, Azuay, Ecuador
https://orcid.org/0009-0008-5714-4351

ABSTRACT

Objective: To determine the presence of oral microorganisms in patients with fixed orthodontic appliances, considering that adhesion and colonisation may be influenced by several factors. **Materials and methods:** Observational and cross-sectional study conducted in 67 patients. Samples were collected with Citoswab® swabs from three anatomical sites in the upper right quadrant. **Results:** Fifteen microorganisms were isolated, with *Streptococcus mutans* being the most frequent, followed by *Candida albicans.* No significant association was found with cement type, age or sex; however, statistically significant differences (p = 0.017) were obtained with treatment time, in which the count of *Candida albicans* increased in the treatment ranges of five to twelve months. **Conclusions:** A significant correlation was observed between orthodontic treatment time and increased microbial colonisation. This suggests that prolonged treatment favours the development of biofilms in the attachment-tooth interface.
Keywords: bacteria; dental biofilm; fixed orthodontic appliances. (DeCS).

RESUMEN

Objetivo: Determinar la presencia de microorganismos orales en pacientes con aparatología ortodóntica fija, considerando que la adherencia y colonización pueden estar influenciadas por varios factores. **Materiales y métodos:** Estudio observacional y transversal realizado en 67 pacientes. Se recolectaron muestras con hisopos Citoswab® en tres sitios anatómicos del cuadrante superior derecho. **Resultados:** Se aislaron quince microorganismos, siendo el *Streptococcus mutans* el más frecuente, seguido de *Candida albicans.* No se encontró asociación significativa con el tipo de cemento, edad o sexo; sin embargo, se obtuvieron diferencias estadísticamente significativas (p = 0.017) con el tiempo de tratamiento, en el cual aumento el conteo de *Candida albicans*, en los rangos de tratamiento de cinco a doce meses. **Conclusiones:** Se observó una correlación significativa entre el tiempo del tratamiento ortodóntico y el aumento en la colonización microbiana. Esto sugiere que la prolongación del tratamiento favorece el desarrollo de biopelículas en la interfase aditamento diente.
Descriptores: bacterias; biopelícula dental; aparatos ortodóncicos fijos. (DeCS).

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Original article

INTRODUCTION

Orthodontic treatments have become the treatment of choice when solving dental problems such as malocclusion and facial and dental aesthetics, contributing significantly to the proper functioning of the stomatognathic system.(1) However, this type of treatment involves different attachments such as brackets, arches, elastomeric chains, metal ligatures, (2) etc., which create retention sites and promote the accumulation of dental biofilm, causing alterations in the oral microbiota by promoting bacterial colonisation. (3)

The oral microbiota contains a wide variety of species found in both soft and hard tissues. It is estimated that there are at least 700 species, including bacteria, viruses and fungi,(4) which contribute to the overall health of the host when in a state of equilibrium (eubiosis). Oral hygiene therefore plays a fundamental role in preventing the colonisation and proliferation of microorganisms in the interface between the tooth and fixed orthodontic appliances.(3)

However, disruption of this balance (dysbiosis) can contribute to the accumulation of biofilm in this area, facilitating the development of exogenous bacteria and fungi that can colonise the appliances due to their ability to adhere to surfaces by forming resistant biofilm, causing disease and affecting overall health.(5) Studies report an increase in the gram-positive microbial population, especially *Streptococcus mutans (SM), Lactobacillus spp and Actinomicetes spp*, which are responsible for the development of white spot lesions, dental caries and periodontal disease after the start of treatment with fixed orthodontic appliances.(6) There is also an increase in the colonisation of fungi such as *Candida spp*, *Candida albicans (CA)* being a common opportunistic pathogen of the oral cavity, functioning as a reservoir for the spread of microorganisms, with the ability to colonise dental tissues. (7,8)

The adhesion and colonisation of these microorganisms during orthodontic therapy may be influenced by various factors, such as the type of bracket, orthodontic cement, duration of treatment and age of the patient. (2,9) Oral hygiene is also a factor, as the presence of orthodontic appliances can hinder effective cleaning. (10)

To date, scientific evidence supporting the influence of these factors is limited. For this reason, the present study aimed to determine the presence of oral microorganisms in patients with fixed orthodontic appliances, considering variables such as treatment time, type of orthodontic cement, sex, and patient age.

METHOD

A descriptive, observational, cross-sectional, prospective study was conducted on patients treated at the postgraduate orthodontic clinic of a private university in the city of Azogues, Ecuador.

Samples were taken during monthly orthodontic treatment check-ups. Patients were informed of the requirements prior to sampling: no antibiotics in the last month, no food consumption, and no tooth brushing one hour before the appointment.

The inclusion criteria were patients in good general health, aged between 12 and 42 years, and undergoing conventional fixed orthodontic treatment. The exclusion criteria were patients with systemic diseases, pregnant women, and participants undergoing treatment with oral contraceptives or antibiotics.

Participants were selected from the specialist training clinic at a private university in the city of Azogues, where orthodontic treatment was being carried out. Patients undergoing orthodontic follow-up were contacted and invited to participate in the study, and instructions were provided on the importance of brushing their teeth before attending the consultation. All participants signed an informed consent form, and in the case of minors, the corresponding consent was obtained. An intraoral clinical examination was performed to verify the selection criteria of the participants.

Finally, the sample was collected.

The dependent variables included the presence and diversity of microbes in the bracket-tooth interface. The independent variables were the age of the participants, categorised into three groups: 12–18 years, 19–26 years and 27–42 years; the treatment time was divided into 1–4 months, 5–8 months and 9–12 months; and the gender of the participants, which was categorised as female and male. The type of cement used was grouped into five categories: GoTo®, Orthobond®, Orthocem®, Enlight® and Ortholink®. 5–8 months and 9–12 months; female and male gender; and the type of cement used, which was grouped into five categories: GoTo®, Orthobond®, Orthocem®, Enlight® and Ortholink®.

For sample collection, a mouth guard was placed to prevent cross-contamination and facilitate access to the examination areas. Prior to sampling, active orthodontic elements were removed. In accordance with biosafety measures, swabs were taken using Citoswab® swabs applied to the vestibular surface of the bracket-tooth interface at three anatomical sites in the upper right quadrant, as shown in Figure 1.

These samples were stored in transport boxes with cooling gel at 4–8°C, ensuring their preservation until laboratory analysis.

To minimise bias, each participant was informed of the requirements prior to sample collection and the collection protocol was standardised. Microbiological and molecular biology techniques considered to be the gold standard were used to identify microorganisms. The analyses were performed by an independent team, blind to demographic and clinical variables.

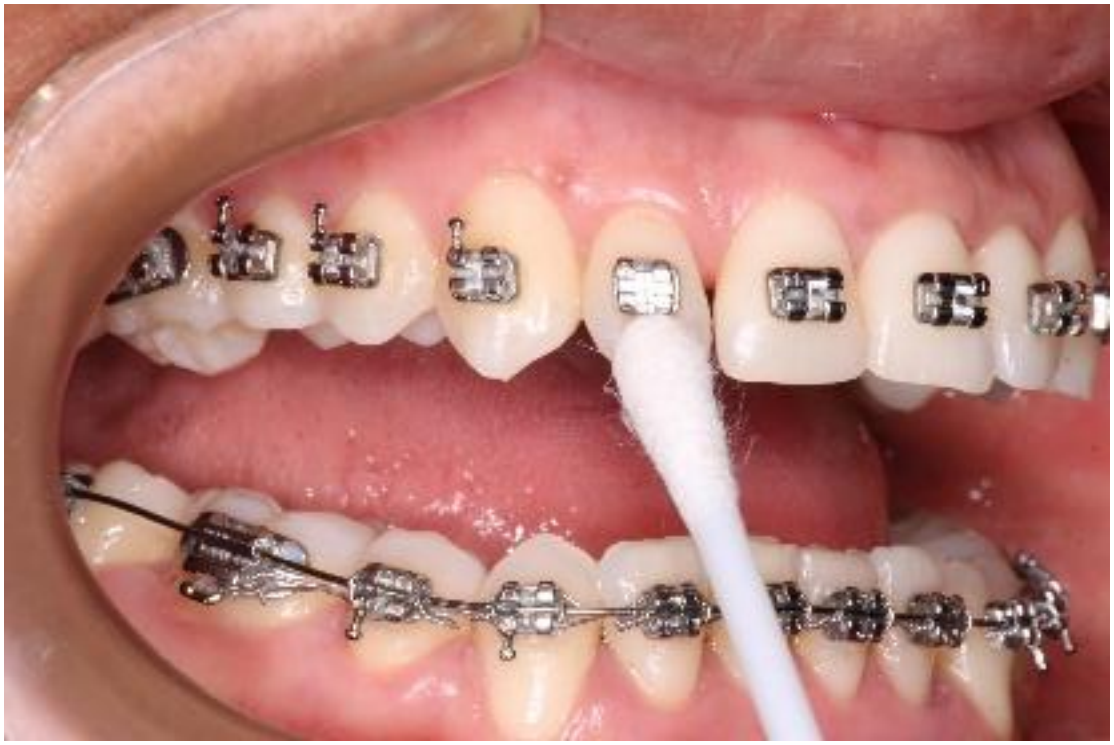

Figure 1. *Smear from the bracket-tooth interface of the right lateral incisor.*

Microbiological analysis

Each sample was cultured on blood agar (BA), Mitis Salivarius Agar (MSA), Eosin-Methylene Blue Agar (EMBA), and Chromoagar for Candida (CC), which were left at 35ºC for 48 hours. The samples seeded in AMS were placed in microaerophilic conditions with 10% carbon dioxide (CO2). After this time, the isolated bacterial colonies were taken and a second isolation was performed, with the aim of growing pure colonies for identification by the Polymerase Chain Reaction (PCR) technique and by Matrix-Assisted Laser Desorption/Ionisation Time of Flight (MALDITOF).(9)

Extraction of deoxyribonucleic acid (DNA) for SM and Aggregatibacter actinomycetemcomitans (Aa)

DNA extraction was performed using the chemical method of 1% sodium dodecyl sulphate (SDS) in 0.5N Na(OH). SM and Aa colonies were placed in a 1.5 mL tube

in 1 mL of distilled water, centrifuged for 10 minutes at 10,000 RPM after homogenisation in a vortex, the supernatant was discarded and 50 uL of SDS in Na(OH) was added, brought to the boil in a thermoblock at 98 °C for 10 minutes, then 450 µL of ultrapure water was added, the tube was centrifuged for 30 seconds at 3000 RPM and stored frozen until use. (11,12)

PCR for SM and Aa

The primers used were GTFB F: 5'-ACTACACTTTCGGGTGGCTTGG-3' and GTFB R: 5'-CAGTATAAGCGCCAGTTTCATC-3' for SM and Aa F: 5'-ATTGGGGTTTAGCCCTGGTG-3' and Con R: 5'-ACGTCATCCCCACCTTCCTC-3' for Aa. PCR was performed using the SimpliAmp thermocycler from Invitrogen. Ten microlitres of GoTaq Green Master Mix 2X from Promega, 6 microlitres of ultrapure water, 1.5 microlitres of each primer and 1 microlitre of DNA were used, giving a final reaction volume of 20 microlitres. The thermocycler protocol used was an initial denaturation at 98 °C, 30 cycles of denaturation at 95 °C, hybridisation at 53 °C, extension at 72 °C for 1 minute and a final extension at 72 °C for 10 minutes. (11)

MALDITOF

The isolated colonies are exposed to laser pulses that generate ions, which are analysed by the spectrometer. The data obtained are compared with a database of protein profiles of known microorganisms, allowing identification in minutes. Each bacterium has a specific "molecular fingerprint" that can be visualised by the formation of a pattern of peaks specific to each species, which are compared with the database. The most accurate match, i.e. 99.9%, corresponds to the bacterial identification.

The data were obtained through direct collection of microbiological samples and analysis in the laboratory belonging to the Centre for Research, Innovation and Technology Transfer (CITT). The study protocol was developed in accordance with

the Helsinki guidelines and was approved by the Human Research Ethics Committee of the Catholic University of Cuenca (CEISH- UCACUE- 2024-065-15/07/2024).

Statistical methods

Frequency and contingency tables were presented. Pearson's chi-square test was used to analyse the association between microorganisms and demographic and clinical variables, and Cramer's V was used to establish the association between microorganisms and cement type. Kendall's tau-c test was used to establish the association between the presence of microorganisms and treatment time. The significance level was 0.05%, and the Statistical Package for Social Sciences (SPSS) version 25 was used.

RESULTS

Of a total of 158 patients treated at the postgraduate orthodontic clinic, 91 were excluded because they did not meet the inclusion criteria. The study evaluated the presence of oral microorganisms in 67 patients with fixed orthodontic appliances. Most participants were between 12 and 18 years old (55.2%), with a predominance of females (65.7%). The most common treatment time was 1 to 4 months (50.7%), and the most commonly used cement was GoTo® (58.2%). A higher presence of microorganisms was observed in these groups, which can be attributed to their predominant representation in the sample (Table 1).

Table 1 *Demographic distribution of the sample*

	Presencia		Ausencia		p	Total	
	n	%	n	%		n	%
Edad							
12-18 años	14	20,9	23	34,3		37	55,2
19-26 años	6	9	10	14,9	0,990	16	23,9
27-42 años	5	7,5	9	13,4		14	20,9
Sexo							
Masculino	8	11,9	15	22,4	0,96	23	34,3
Femenino	17	25,4	27	40,3		44	65,7
Tiempo de tratamiento							
1-4 Meses	13	19,4	21	31,3		34	50,7
5-8 Meses	6	9	16	23,9	0,308	22	32,8
9-12 Meses	6	9	5	7,5		11	16,4
Tipo de cemento							
Orthocem®	2	3	1	1,5		3	4,5
GoTo®	16	23,9	23	34,3		39	58,2
Enlight®	5	7,5	7	10,4	0,326	12	17,9
Orthobond®	1	1,5	9	13,4		10	14,9
Ortholink®	1	1,5	2	3		3	4,5

Se usó la prueba Chi-cuadrado de Pearson, nivel de significancia 0,05.

No statistically significant association was observed between the type of cement and the presence of microorganisms, as the absence of these was high for all cements, reflecting a low level of colonisation in general, which indicates that the type of cement does not have a relevant impact on the presence of microorganisms in this study; however, GoTo® cement had slightly higher percentages compared to the other cements: Gram-positive 12%, Gram-negative 11.9% and yeast 6% (Table 2).

Table 2. *Association between microorganisms and cement type.*

Microorganismo		Tipo de cemento												
		Orthocem®		GoTo®		Enlight®		Orthobond®		Ortholink®		Total		
		n	%	n	%	n	%	n	%	n	%	n	%	p
Grampositivos	Ausencia	1	1,5	31	46,3	10	14,9	9	13,4	2	3	53	79,1	0,313
	Presencia	2	3	8	12	2	3	1	1,5	1	1,5	14	20,9	
Gramnegativos	Ausencia	2	3	31	46,3	9	13,4	9	13,4	3	4,5	54	80,6	0,748
	Presencia	1	1,5	8	11,9	3	4,5	1	1,5	0	0,0	13	19,4	
Levaduras	Ausencia	2	3	35	52,2	12	17,9	10	14,9	3	4,5	62	92,5	0,127
	Presencia	1	1,5	4	6	0	0,0	0	0,0	0	0,0	5	7,5	

Se usó la prueba Chi-cuadrado de Pearson, nivel de significancia 0,05.

Table 3 reveals a statistically significant association between the duration of fixed orthodontic treatment and the presence of yeasts, specifically *CA* (p = 0.017). An increase in *CA* colonisation was observed in the treatment ranges of 5 to 8 months and 9 to 12 months, reaching a maximum of 3%.

Table 3. *Association between microorganisms and time.*

Microorganismo		1--4 meses		5--8 meses		9--12 meses		Total		p
		n	%	n	%	n	%	n	%	
Grampositivos	Ausencia	28	41,8	17	25,4	8	11,9	53	79,1	0,730
	Presencia	6	9	5	7,5	3	4,5	14	16,4	
Gramnegativos	Ausencia	26	38,8	20	29,9	8	11,9	54	80,6	0,316
	Presencia	8	11,9	2	3	3	4,5	13	19,4	
Levaduras	Ausencia	33	49,3	20	29,9	9	13,4	62	92,5	0,017*
	Presencia	1	1,5	2	3	2	3	5	7,5	

Se usó la prueba Tau-c de Kendall para establecer la asociación entre la presencia de microorganismos y el tiempo de tratamiento, nivel de significancia 0,05.

Figure 2 shows the distribution of microorganisms identified in the oral cavity of patients with fixed orthodontic appliances. *SM* was the most prevalent microorganism, isolated in 8 patients, representing its high cariogenic capacity and affinity for retentive surfaces such as the bracket-tooth interface. This finding reinforces its central role in the development of white spot lesions and caries in patients with orthodontic appliances.

Next was *CA*, detected in 5 patients. This opportunistic yeast has been associated with imbalances in the oral microbiota, especially in conditions of poor hygiene or the presence of foreign bodies, such as brackets, which favour its adhesion and persistence.

Thirdly, *Pseudomonas fluorescens* and *Pseudomonas fragi* stand out, each present in 4 patients. Although they are not typical oral pathogens, their ability to form biofilms and their antimicrobial resistance may represent a potential risk in patients with gingival inflammation or pre-existing periodontitis.

The remaining microorganisms identified, *Achromobacter sp.*, *Staphylococcus aureus*, *Pantoea agglomerans*, and *Enterobacter cloacae*, were found in a single

patient each. Although rare in the oral cavity, these bacteria can act as secondary colonisers, especially in modified environments such as those induced by fixed appliances.

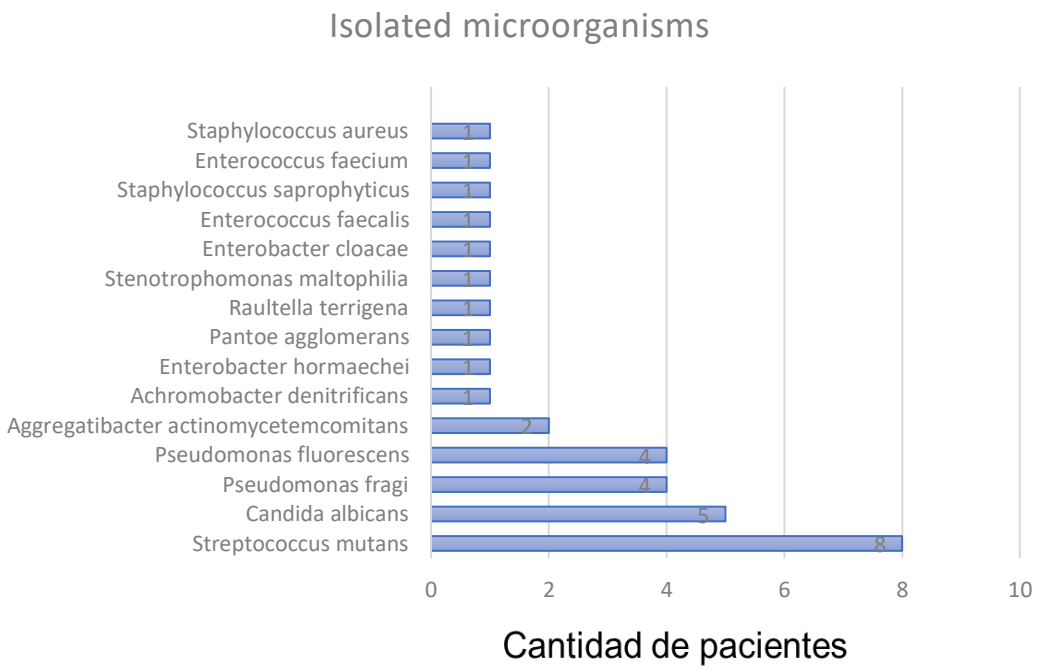

Figure 2. *Distribution of patients according to the presence of the microorganism.*

DISCUSSION

The results obtained show that the highest proportion of colonisation by microorganisms occurs in patients with orthodontic treatment lasting 1 to 4 months, compared to lower proportions in the other groups. More than half of the study participants were in the 1 to 4 month group, which could have directly influenced the higher frequency of cases with the presence of microorganisms in this interval.

The results showed that, although some cements such as GoTo® were used more frequently, their greater representation also led to a higher number of cases with both the presence (23.9%) and absence (34.3%) of microorganisms, which could be explained by the distribution of the sample rather than by a differential action of the material itself. Furthermore, the presence or absence of microorganisms is not significantly associated with the type of orthodontic cement used (p-value = 0.326). This indicates that bacterial colonisation is statistically independent of the brand of cement used in the study patients.

From a clinical and microbiological perspective, several studies have shown that, although the chemical composition of orthodontic materials can influence bacterial plaque retention, this influence is limited compared to other factors such as the patient's oral hygiene, the surface of the bracket or the cementation technique used. In general, current orthodontic cements are designed with basic or neutral antimicrobial properties, but do not show significant differences in their ability to prevent or promote microbial growth under real clinical conditions (13,14).

This result coincides with recent studies indicating that oral microbiota tends to adapt quickly to the materials present, and that the greatest influence on its development is associated with exposure time, plaque accumulation and the patient's hygiene habits, rather than the specific type of adhesive used (15). Several studies have shown that the use of fixed appliances can temporarily alter the oral microbiota, affecting both its quantity and quality (16) ,especially if not accompanied by adequate oral hygiene (1,3,9). This imbalance favours the proliferation of microorganisms such as *SM* and *CA,* both identified as predominant in the present study (17,18).

In this study, a statistically significant relationship was observed between orthodontic treatment time and the presence of *CA* (p = 0.017), showing a progressive increase in colonisation as treatment prolonged. This finding is in line with that reported by Perkowski et al.,(19) who detected a higher presence of bacteria and fungi, including

CA, in patients with fixed appliances compared to those with removable appliances or without orthodontic treatment.

However, some studies have shown different results. Sanz-Orrio-Soler et al.(8) and Torlakovic et al.(20) found no significant association between fixed appliances and increased *CA*. It is important to note that these studies differ in their methodological design; for example, Torlakovic et al. (20) took samples only from central incisors, which are easy to clean, while this study also included posterior teeth, where access to cleaning is more limited and therefore more prone to biofilm accumulation.

Similarly, studies such as those by Saloom et al. (21) and Brusca et al. (22) have evaluated the adhesion of *CA* to different types of brackets. Although their conclusions differ, some indicate greater adhesion to metal brackets and others to ceramic or composite brackets, both agree that the materials used in orthodontics influence the colonisation pattern. Given that all patients in our study wore conventional metal brackets, it is possible that this type of surface, combined with poor oral hygiene, facilitated the growth of *CA* over time.

SM is one of the most prevalent cariogenic bacteria in the oral cavity and plays a key role in the formation of white spot lesions during fixed orthodontic treatment. These lesions represent the first clinical manifestation of enamel demineralisation and can progress to caries if not properly treated (23).

The presence of fixed orthodontic appliances creates retentive niches that favour the accumulation of bacterial plaque, especially around brackets. This accumulation hinders oral hygiene and promotes an acidic environment due to the fermentation of carbohydrates by bacteria such as *SM*, which produces organic acids, mainly lactic acid, lowering the local pH and causing enamel demineralisation (24).

Enamel demineralisation weakens the tooth structure, which can compromise bracket adhesion and increase the risk of fractures or detachment during the

application of orthodontic forces (24). Rigorous oral hygiene, the use of high-fluoride toothpastes and antibacterial mouthwashes are recommended to reduce the SM load. In addition, patient education on proper brushing techniques and reduction of fermentable sugar consumption is recommended.

In the present study, species of the genus *Pseudomonas* were also identified, specifically *Pseudomonas fragi* and *Pseudomonas fluorescens*. Although these are not traditionally considered oral pathogens, their presence in the oral environment has been documented, especially in conditions that alter the homeostasis of the oral microbiota during the use of fixed orthodontic appliances (25).

Pseudomonas fluorescens is an environmental bacterium commonly found in soil, water, and moist surfaces. Although rarely associated with human infections, it has a remarkable ability to form biofilms and exhibit intrinsic resistance to multiple classes of antibiotics, making it a clinically relevant microorganism (26). Studies have shown that this species can adhere to non-dental surfaces and even colonise the oral cavity when there are devices that interrupt regular cleaning, such as fixed orthodontic appliances (27).

The antimicrobial resistance of *Pseudomonas spp*. represents a potential risk in the periodontal field. If these bacteria integrate into an existing subgingival biofilm, for example in patients with periodontitis, they can aggravate the disease by hindering the action of conventional antibiotic treatments and enhancing inflammation (27,28). Its presence in the oral cavity of patients with fixed orthodontic appliances indicates the importance of enhanced hygiene strategies, regular microbiological monitoring and preventive measures during treatment.

The presence of *Aa* in this study is particularly important given its ability to adhere to dental surfaces and orthodontic materials, forming resistant biofilms that can exacerbate gingival inflammation and compromise periodontal stability during

orthodontic treatment. The surface of brackets, especially if there are microdefects or excess cement, can act as an ecological niche for *Aa,* facilitating its colonisation and persistence over time. This is especially critical in patients with poor oral hygiene, as subgingival biofilm can harbour this microorganism in hard-to-reach areas, such as the cement-bracket-tooth junction and gingival pockets induced by chronic inflammation (29).

Studies have also shown that *Aa* has the ability to induce a hypoxic and inflammatory environment that can alter the bone balance necessary for proper tooth movement, leading to complications such as root resorption, loss of attachment, and persistent gingival bleeding (30). This poses a risk to the efficacy and normal course of orthodontic treatment in patients colonised with this microorganism.

The clinical relevance of this research lies in the information provided on changes in the oral microbiota during orthodontic treatment, allowing us to prevent complications. The accumulation of microorganisms could favour the development of caries and periodontal diseases, so early detection would help to minimise these risks.

Likewise, knowing the diversity and presence of microorganisms enables the establishment of personalised oral hygiene protocols, thus optimising treatment results. By identifying the factors that can cause changes in the microbiota, specific preventive strategies can be developed, such as strengthening oral education and raising patient awareness of the importance of maintaining proper hygiene before, during and after treatment, as well as incorporating the use of antimicrobial agents to promote a healthy oral environment throughout the process.

CONCLUSION

Fifteen types of microorganisms were identified in patients with fixed orthodontic appliances. The most common were *Streptococcus mutans*, *Candida albicans*,

Pseudomonas fragi, Pseudomonas fluorescens and *Aggregatibacter actinomycetemcomitans.* The rest of the microorganisms were found at a very low frequency.

No statistically significant association was found between the presence of microorganisms and variables such as cement type, age or gender. However, significant differences were observed in relation to treatment time for yeasts, specifically *Candida Albicans* in the 5 to 12 month treatment ranges, showing an increase in count, which suggests that their prevalence increases as treatment time progresses.

These results highlight the importance of providing clear guidelines on oral hygiene and preventive strategies during fixed orthodontic treatment in order to minimise the risk of complications and optimise treatment outcomes.

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CONFLICT OF INTEREST

There is no conflict of interest with individuals or institutions linked to the research.

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Contributions of the authors:

Conceptualisation: Elizabeth Llivichuzca, Magaly Jiménez.

Formal analysis: Elizabeth Llivichuzca, Magaly Jiménez, Carlos Flores.

Research: Elizabeth Llivichuzca, Magaly Jiménez, Carlos Flores, Jessica Sarmiento.

Methodology: Elizabeth Llivichuzca, Magaly Jiménez, Carlos Flores, Jessica Sarmiento.

Statistical analysis: Elizabeth Llivichuzca, Carlos Flores, Jessica Sarmiento.

Supervision: Magaly Jiménez, Jessica Sarmiento.

Writing, review, and editing: Elizabeth Llivichuzca, Magaly Jiménez, Carlos Flores, Jessica Sarmiento.

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