Frequency of Pathogenic Microorganisms in Removable Orthodontic Appliances and Oral Mucosa in Children

Marisela Rodríguez-Rentería* / Raúl Márquez-Preciado** / Marine Ortiz-Magdaleno*** / Josué Bermeo-Escalona**** / Luis Octavio Sánchez-Vargas*****

Objective: *The aim of this study was to evaluate the frequency of Staphylococcus aureus, Pseudomonas aeruginosa, and Candida species in removable orthodontic appliances (ROA) and the support oral mucosa in children.* ***Study design:*** *The study participants comprised 55 patients aged 6-12 years requiring ROA. The samples of biofilm colonization from the support oral mucosa and the ROA were taken prior to the use of the ROA (T0) and 4 weeks (T1) after ROA placement. The biofilm samples were seeded on chromogenic culture plates and incubated for 24-48 h.* ***Results:*** *The microbial species evaluated were not present in either the support oral mucosa nor in the ROA at T0. After 4 weeks, P. aeruginosa was found in the support oral mucosa with a frequency of 60%, Candida spp. with 30.9% and S. aureus with 89.09%; in the ROA, P. aeruginosa with 67.7%, Candida spp. with 32.7%, while S. aureus with 90.9%. In the ROA were found C. glabrata in 15 cases, C. albicans in 14 cases, C. tropicalis in two cases, and C. krusei in one case. In the oral mucosa there were 10 cases of C. glabrata, 14 cases of C. albicans, one case of C. tropicalis, and 0 cases of C. krusei.* ***Conclusions:*** *The frequency of S. aureus, P. aeruginosa, and Candida spp. increased after the orthodontic treatment in either the ROA and or in the support oral mucosa. There is a direct relation between the use of the ROA and the increase of periodontal-pathogenic microorganisms.*

Keywords: *Staphylococcus aureus, Pseudomonas aeruginosa, Candida species, Removable orthodontic appliances.*

*Marisela Rodríguez-Rentería DDS, Pediatric Dentistry Postgraduate Program, Faculty of Dentistry, Autonomous University of San Luis Potosí, San Luis Potosí, Mexico

**Raúl Márquez-Preciado DDS, MSc, PhD, Paediatric Dentistry Postgraduate Program, Faculty of Dentistry, Autonomous University of San Luis Potosí, San Luis Potosí, Mexico.

***Marine Ortiz-Magdaleno DDS, MSc, PhD, Aesthetic, Cosmetic, Restorative, and Implantological Dentistry Postgraduate Program, Faculty of Dentistry, Autonomous University of San Luis Potosí, San Luis Potosí, Mexico

****Josué Bermeo-Escalona DDS, MSc, PhD, Faculty of Dentistry, La Salle Bajío University, León, Guanajuato, México. Av. Dr. Manuel Nava #2, Zona Universitaria, 78290, S.L.P. Mexico.

*****Luis Octavio Sánchez-Vargas DDS, MSc, PhD, Biochemical and Microbiology Laboratory, Faculty of Dentistry, Autonomous University of San Luis Potosí, San Luis Potosí, Mexico.

Send all correspondence to:
Luis Octavio Sánchez-Vargas
Biochemical and Microbiology Laboratory, Faculty of Stomatology, Autonomous University of San Luis Potosí,
Av. Dr. Manuel Nava #2, Zona Universitaria, 78290, S.L.P. México.
Phone:+524448262357.
Fax: +524448139743.
E-mail: octavio.sanchez@uaslp.mx,

INTRODUCTION

The appliances used in orthodontic treatments are indicated for the correction of dental malocclusions and alterations in the pattern of facial growth. Its purpose is to improve the oral function of chewing, swallowing, breathing, and facial esthetics. The removable appliances are designed to apply forces to the teeth and can be taken out by the patient for cleaning.[1-3] The Removable Orthodontic Appliances (ROA) placed in the maxilla completely cover the palatal area to increase the muco-supported anchorage, while the ROA in the lower jaw covers the lingual alveolar ridge. PolyMethylMethAcrylate (PMMA) is the most widely used acrylic in the traditional fabrication of these orthodontic appliances due to its simple and rapid handling.[4,5]

Studies have reported that oral bacteria in patients with ROA is different from those healthy individuals, due to increased levels of dentobacterial biofilm.[6-8] Orthodontic appliances increase areas where food remnants can accumulate and increase the number of bacterial niches.[9] The biofilm that forms on the acrylic surface of ROA acts as a reservoir for periodonto-pathogenic microorganisms[10,11] and is difficult to eliminate if the patient has poor oral hygiene and is not able to perform adequate oral hygiene.[12] The combination of factors, such as the formation of an ideal microenvironment for the growth of the biofilm, the hydrophobic surface of

the PMMA, the age of the patient, and poor oral hygiene promotes the colonization of bacteria in individuals using ROA.[14] It has been reported that 60% of all orthodontic patients experience some alteration in biofilm accumulation and in oral microbiota after the use of ROA.[13,14]

Knowledge of the composition of the biofilm and the evolution of oral microbiota in pediatric patients provides an explanation of the cariogenic and perio-pathogenic mechanisms of the microorganisms. A pediatric patient using ROA has a greater probability of developing periodontal diseases, gingivitis, or periodontitis than a patient without orthodontic treatment.[15,16] Some colonizers that favor the formation of oral biofilm are *Streptococcus sanguis*, *Streptococcus oralis*, *Streptococcus mitis*, *Actinomyces naeslundii*, *Streptococcus mutans*, *Streptococcus salivarius*, *Prevotella intermedia*, *Streptococcus gordonii*, *Streptococcus parasanguis*, *Neisseria* spp., *Prevotella loescheii*, *Capnocytophaga* spp., *Fusobacterium nucleatum*, and *Porphyromonas gingivalis*. However, in the bacterial profile, there are other microorganisms that have been less studied, such as *Staphylococcus aureus* and *Pseudomonas aeruginosa* that are highly pathogenic, as well as *Candida* spp. *S. aureus* is responsible for local abscesses, *P. aeruginosa* is related to gingivitis, periodontitis, valvular endocarditis, otitis media, and septicemia, and *Candida* spp. are considered the principal causes of the most common mycoses in the oral cavity.[17,18]

The appearance in a short time of alterations in oral microbiota and the frequency of the formation of a pathogenic biofilm of *S. aureus*, *P. aeruginosa*, and *Candida* spp. on the acrylic surface of ROA and in the support oral mucosa due the use of ROA in children has not been reported. There are no published studies, to our knowledge, concerning which microorganisms have high pathological potential and whether they form part of the oral microbiota or whether their frequency of appearance occurs after the use of ROA. The objective of this study was to evaluate the frequency of *S. aureus*, *P. aeruginosa*, and *Candida* spp. on the acrylic surface of ROA and the colonization of the support oral mucosa in children between 6 and 12 years of age with orthodontic treatment at a short term-of-use.

MATERIALS AND METHOD

This study was conducted at the Specialty in Pediatric Dentistry, Faculty of Stomatology, Autonomous University of San Luis Potosí, S.L.P., Mexico, on patients aged 6-12 years, of both genders and, based on a clinical, photographic, and radiographic study, a ROA was indicated. All appliances were made of PMMA. The study was approved by the University's Ethics Committee (CE-IFE-039-015), and written informed consent was received from all participants and their parents. Patients were excluded if they had a medical history that might have been affected by *Candida* spp., such as systemic disease, immunosuppression, or antibiotic use during the 4 weeks prior to the beginning of the use of the ROA, which could favor changes in the microbiota of the oral cavity. Elimination criteria included patients who used ROA for fewer than 12 h a day, who did not use their ROA in their mouths on the day of the biofilm samples, whose samples were contaminated during the process, or who had used an antiseptic buccal before the sample was taken.

Surface sampling and preparation

To ensure zero baseline frequency in the ROA, prior to their placement in the oral cavity, the ROA were washed by brushing vigorously with enzymatic detergent, rinsing, and disinfected by immersion in 0.12% chlorhexidine (Peroxidin™, Lacer, Barcelona, Spain) for 5 min. This cleaning and disinfection was performed only at this time, to ensure that the ROA were free of microorganisms at the moment of placement. To obtain baseline colonization frequency (T0), two surface samples were taken while the patient sat comfortably in the dental unit, under controlled ambient and room temperature. First, the support oral mucosa of the ROA was rubbed with a sterile cotton-tipped swab. The total acrylic surface of the ROA was rubbed with a second sterile swab. This same procedure was performed 4 weeks after use of the ROA. The swabs with the surface samples were placed in container tubes with Stuart transport medium (Copan, Italy) to be seeded for a period not exceeding 2 h.

Hygiene indications

Once the ROA was placed in the oral cavity, a professional provided standardized oral-hygiene instructions for all parents and patients for carrying out a daily procedure of brushing the oral cavity (modified Bass brushing technique) without sanitizing the ROA. All of the instructions were provided by the same investigator. The subjects were asked to brush three times daily, after meals, and were instructed to not use any hygienic products other than toothpaste and dental floss. Additionally, each patient was supplied with written instructions for oral hygiene and for the cleaning of the ROA, as well as two extra-soft-bristle toothbrushes with different sizes (Colgate™, USA), one for tooth brushing and one for the ROA, and a toothpaste (Colgate, USA). A control appointment was scheduled after 15 days at the clinic with the purpose of reviewing and monitoring that the patient followed the care instructions.

Microbiological processing

The biofilm samples from the oral mucosa and the ROA were seeded on culture plates in CHROMagar™ *Staph aureus*, CHROMagar™ *Pseudomonas*, and CHROMagar™ *Candida* (CHROMagar™ Paris, France) and were subsequently incubated at 36°C ± 1.5°C for 24-48 h. These chromogenic culture media are efficient, simple, and fast with respect to the identification of particular species through the differential development of pigmented colonies due to the enzymatic properties of each colony. Identification of the species was performed following the manufacturer's instructions according to the characteristics and color of the colonies in each medium. CHROMagar™ *Staph aureus* has 95.5% sensitivity and 99.4% specificity, CHROMagar™ *Pseudomonas* has 92% sensitivity and 95% predictability, and CHROMagar™ *Candida* has 99% sensitivity/specificity. Once we identified the species, the colonies were purified and reseeded for their subsequent confirmation by means of conventional microbiological methods. The colonies of *S. aureus* were replated in salty mannitol agar and the coagulase and catalase test was performed. The colonies of *P. aeruginosa* were replated in duplicate on Cetrimide Agar plates. These were incubated at 36°C and 42°C for 24 h and the oxidase test was performed. *Candida* spp. were identified by the carbohydrate assimilation ID 32 C AUX system and the Apiweb™ database (BioMérieux®, Marcy l'Etoile, France). Additionally, all colonies had smears, Gram staining, and microscopic identification.

Statistical analysis

For statistical analysis, the SPSS version 20 statistical software program (IBM, Chicago, IL, USA) was used. Descriptive statistics was conducted and the Nagelkerke coefficients of determination were obtained. Adjustment of the models was carried out by means of the Hosmer and Lemeshow test, and significance levels of $p < 0.05$ were considered significant.

RESULTS

In total, 55 patients participated (34 boys/21 girls), with a mean age, 8.4 years of age), and the types of ROA are presented in Table 1. Quantification of Colony-Forming Units (CFU) in the ROA prior to placement (T0) was nil. The CFU of the identification of *S. aureus, P. aeruginosa*, and *Candida* spp at 4 weeks (T1) were between 1 Log_3 and 4 Log_{10}. Frequencies were 50 cases of *S. aureus*, 37 cases of *P. aeruginosa*, and 18 cases of *Candida* spp. Frequencies of *Candida* spp. were 15 cases of *C. albicans*, 14 cases of *C. glabrata*, two cases of *C. tropicalis*, and one case of *C. krusei* (Figures 1 and 2). There was no statistical difference ($p < 0.001$) in the presence of microorganisms according to the type of ROA.

In the bony supported oral mucosa, microorganisms were isolated from the 55 patients before the use of the ROA. This quantified the CFU between 2 Log_3 and 3 Log_{10}; nevertheless, the studied species,

Figure 1. Frequency of *Stap aureus*, *P. aeruginosa*, and *Candida spp.* at 4 weeks after ROA placement, on the support oral mucosa and the surface of the ROA

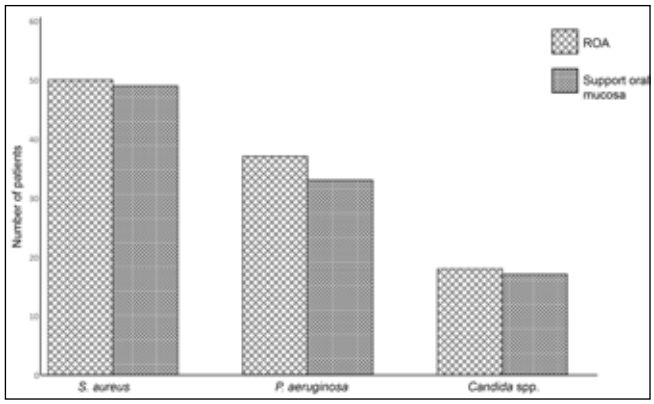

Figure 2. Frequency of *C. albicans*, *C. glabrata*, *C. tropicalis*, and *C. krusei* at 4 weeks after ROA placement, on the support oral mucosa and the surface of the ROA

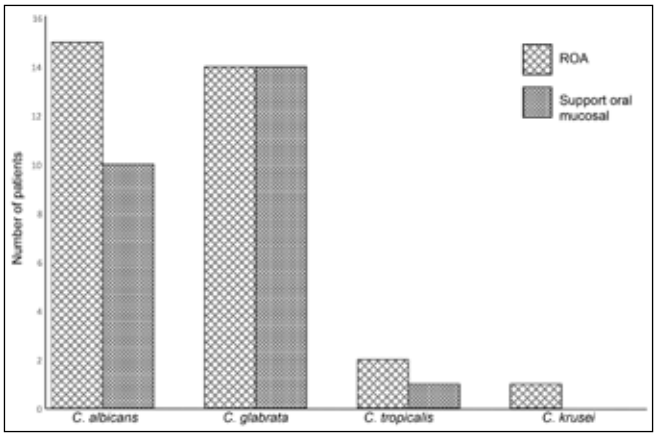

Table 1 Frequency of type of removable orthodontic appliance placed in the patients

Appliance	Frequency	
	Number of patients	%
Hawley	28	50.91
Schwartz	16	29.09
Bimler	4	7.27
Thurrow	3	5.45
SN3	3	5.45
SN7	1	1.82
Total	55	100

including *S. aureus*, *P. aeruginosa*, and *Candida* spp., were not present in any of the 55 patients. In the sampling at 4 weeks (T1), the frequency of *S. aureus* support oral mucosa was 49 cases, while there were 33 cases of *P. aeruginosa* and 17 cases of *Candida* spp. The frequency data obtained from *Candida* spp. comprised *C. albicans* in 10 cases, *C. glabrata* in 14 cases, and *C. tropicalis* in one case. No case presented *Candida krusei* after 4 weeks of ROA use.

P. aeruginosa was found in the bony supported oral mucosa with a frequency of 60% and in the ROA with a frequency of 67.7%. *Candida* spp. exhibited a frequency in the ROA of 32.7% and in the support oral mucosa of 30.9%, while *S. aureus* exhibited a frequency of 90.9% in the ROA and of 89.09% in the support oral mucosa. There was a statistical correlation ($p < 0.001$) with the use of the ROA over 4 weeks among the presence of *S. aureus*, *P. aeruginosa*, and *Candida* spp. in the support oral mucosa with regard to the time of use of the ROA during the first 4 weeks between the presence of *S. aureus* and *P. aeruginosa*.

DISCUSSION

The prevalence of malocclusions and orthodontic treatments has increased in children and adolescents; the use of ROA makes renders the pediatric population more exposed and susceptible to the formation of a pathogenic biofilm.[19-21] In this study, the relation and frequency between the use of ROA by children aged 6 to 12 years and the presence of microorganisms with high pathogenic potential, such as *S. aureus*, *P. aeruginosa*, and *Candida* spp., has been demonstrated both on the surface of the ROA and as colonizing the support oral mucosa.

The frequency of the Hawley-type palatal plate in this study was 50.91% and of the Schwartz-type plate, 29.09%. Our results are in agreement with Pérez *et al* who studied a population of 1,760 patients and the Hawley-type palatal plate was the most common ROA employed.[22] We observed that, regardless of the type and design of ROA, either with a larger acrylic surface or with a greater number of retainers or metal appliances, colonization occurred in the support oral mucosa and the frequency of pathogens increased in the ROA.

Studies confirm that the use of ROA alters the microbiological homeostasis of the oral cavity due to various factors, such as the presence of new retention surfaces, the ROA design, and the time of use of the ROA, which favors bacterial adhesion and biofilm formation.[23,24] An orthodontic appliance in the mouth hinders conditions for autolysis, complicates the cleaning of the teeth, and creates a favorable environment for the accumulation of food and dentobacterial plaque.[25] In our study, we identified that this microbiological alteration can enable the significant colonization of microorganisms

with pathogenic potential in the support oral mucosa. Studies have reported that the growth of microorganisms in ROA increases the risk of their spread to other tissues or organs, eventually relating to other systemic diseases and hospital infections and complicating the state of health if the child presents any predisposing condition.[26]

In a previous work by our research group, we found a high frequency of microorganisms with high pathogenic potential, such as *S. aureus*, *Enterobacteriaceae*, and *Candida* in orthodontic appliances placed in a pediatric population used for at least 3 months before the identification of the microorganisms.[27] Thus, we decided to evaluated whether this frequency was associated with the use of the orthodontic appliances and their improper handling, or whether the children were already carriers of these bacteria significantly, even without the use of ROA. Therefore, in this study, we took a biofilm sample prior to the use of the placement of the ROA. Our study revealed that the pediatric population was not a carrier of the studied microorganisms, but that, beginning orthodontic treatment was the cause for bacterial colonization in the ROA and the support oral mucosa.

Zharmagambetova *et al* evaluated the influence of orthodontic treatment with ROA in 12-year-old patients with dentoalveolar anormalities in the oral microbiota.[28] These authors reported a decrease in the normal level of the microbiota and an increase in the frequency of *C. albicans*, *S. aureus*, and *S. mutans*. Lara *et al* noted that, from month 1 of orthodontic treatment, there was a significant increase in the growth of *S. mutans* and *Lactobacillus* in the oral cavity.[29] Arendorf *et al* demonstrated a direct relation between the use of ROA and the presence of *Candida*.[30] The results obtained in this study reported the presence of *S. aureus*, *P. aeruginosa*, and *Candida* spp. in the ROA and in the support oral mucosa after 4 weeks of using the ROA. The number of patient-carriers of these microorganisms was nearly similar in the ROA/support oral mucosa: *S. aureus*, 50/49 cases; *P. aeruginosa*, 37/33 cases, and *Candida* spp., 18/17 cases. This study confirmed that the ROA exerts a direct influence on the prevalence and frequency of pathogenic microorganisms in the oral cavity in short-term appliance use.

Differences in the oral microbiota of patients with and without orthodontic treatment have demonstrated higher microbial diversity in the orthodontic treatment group.[31] Studies have reported the increase in subgingival pathogens in patients with orthodontic appliances, but this may be temporary and the microbiota could return to a balance several months later. The reason for the increase of the microorganisms can be explained by the imbalance of host–microorganism interaction due to the orthodontic appliance and its force. However, after a few months, the host–microorganism balance was re-established and the level of periodontopathogens returned to pretreatment levels, with improved host immunity.[24]

According to our results, *S. aureus* was the most frequent bacterium found, in the support oral mucosa with 89.09% and in the ROA, with 90.9%. This bacterium is related to respiratory tract infections and has a high mortality rate. Its presence is an important factor that predisposes colonized individuals to infection at a surgical site. Although there is little evidence of the colonization and isolation of this bacterium in patients with orthodontic treatments, studies have reported that they represent a high risk for immunosuppressed or hospitalized children.[32,33]

Pseudomonas spp. dominate the oral microbiome of orthodontic patients, which cannot be detected in comparison with that of healthy individuals.[34,35] *P. aeruginosa* is recognized as one of the most important pulmonary pathogens and the predominant cause of morbidity and mortality in cystic fibrosis.[36] In our results, *P. aeruginosa* was the second-most-frequent microorganism in the ROA (67.27%) and in the support oral mucosa (60%).

In this study, the frequency of *Candida* spp. was in the ROA with 32.72% and with 30.9% in the support oral mucosa. This coincides with the report by Budtz *et al* who reported that the surfaces of the ROA that are in contact with the palate support mucosa work as deposits for the adherence of microorganisms.[37] This yeast possesses a large number of virulence factors and is one of the main causative organisms of different types of candidiasis.[38] According to the results from other studies, the most common spp. of *Candida* includes *C. albicans*, *C. glabrata*, and *C. tropicalis*.[39,40] Studies have reported that the main predisposition to *Candida* spp. in orthodontic appliances is due to the deficiency of oral hygiene.[41] The results obtained in this study indicate that *C. albicans* and *C. glabrata* are the most common spp. after 4 weeks of use of ROA in children betwwen 6 and 12 years of age. The importance of *C. glabrata* should be considered, as it entertains an intrinsic resistance to azole antifungals.[42]

In this study, 38 of 55 patients who presented *Candida* in their appliances contained screws made of metal alloys. This allows us to propose that the adherence of different species of microorganisms increases not only in ROA with a PMMA surface, but also when they are in combination with other materials, possibly due to the greater retentive space between the screw and the acrylic—an area that is difficult to clean and that has previously been cited as a cause of the increase in microbial frequency.[37] The most used material in the manufacture of ROA is PMMA; it is a highly porous material that allows for the proliferation of microorganisms. However, the design and components of ROA are factors that will determine the formation and retention of the biofilm.[43]

Instructions regarding the brushing technique and the method for cleaning the appliance are essential to prevent the ROA from starting to become a reservoir of pathogenic microorganisms. However, as we observed in this study, strict monitoring and a change of awareness in care are essential to oral health and hygiene, especially when children use orthodontic appliances. The control of dental biofilm can prevent the appearance of highly pathogenic microorganisms that can eventually infect the patient or impair the patient's general health.[44]

The results obtained in this study explain the value of prevention in the dental area, instructions and hygiene habits, the monitoring of each case, and the necessary change in the awareness of children under orthodontic treatment and in their parents, in that this not only guarantees the success of treatment and oral health, but can also decrease the risk of acquiring systemic and/or disseminated diseases.[45] It is necessary to propose guidelines for the better management and proper care of ROA, in addition to the evaluation of antiseptic agents to aid in preventing the appearance of thus the spread—of highly pathogenic microorganism infections.

CONCLUSION

We found that the use of ROA in children aged 6-12 years predisposes these appliances to becoming reservoirs of potentially pathogenic microorganisms such as *S. aureus*, *P. aeruginosa*, and *Candida* spp., giving rise to the colonization of the supporting oral mucosa. Orthodontic appliances are shown to be the reservoirs of these pathogenic microorganisms.

Source of support

None.

Conflict of interest

The authors declare that they have no conflict of interest.

REFERENCES

1. Shah N. Compliance with removable orthodontic appliances. Evid Based Dent;18(4):105-106. 2017.
2. Khanpayeh E, Jafari AA, Tabatabaei Z. Comparison of salivary *Candida* profile in patients with fixed and removable orthodontic appliances therapy. Iran J Microbiol;6(4):263-268. 2014.
3. Al-Moghrabi D, Salazar FC, Pandis N, Fleming PS. Compliance with removable orthodontic appliances and adjuncts: A systematic review and meta-analysis. Am J Orthod Dentofacial Orthop;152(1):17-32. 2017.
4. Pathak AK, Sharma DS. Biofilm associated microorganisms on removable oral orthodontic appliances in children in the mixed dentition. J Clin Pediatr Dent;37(3):335-339. 2013.
5. Gautam R, Singh RD, Sharma VP, Siddhartha R, Chand P, Kumar R. Biocompatibility of polymethylmethacrylate resins used in dentistry. J Biomed Mater Res B Appl Biomater;100(5):1444-1450. 2012.
6. Alexander SA. Effects of orthodontic attachments on the gingival health of permanent second molars. Am J Orthod Dentofacial Orthop;100(4):337-340. 1991.
7. Choi DS, Cha BK, Jost-Brinkman PG. Microbiologic changes in subgingival plaque after removal of fixed orthodontic appliances. Angle Orthod;79(6):1149-1155. 2009.
8. Kirshenblatt S, Chen H, Dieltjens M, Pliska B, Almeida FR. Adherence to treatment with removable oral ppliances: the Past and the future. J Can Dent Assoc;84:i3. 2018.
9. Alves PV, Alviano WS, Bolognese AM, Nojima LI. Treatment protocol to control *Streptococcus mutans* level in an orthodontic patient with high caries risk. Am J Orthod Dentofacial Orthop;133(1):91-94. 2009.
10. Lee SM, Yoo SY, Kim HS, Kim KW, Yoon YJ, Lim SH, et al. Prevalence of putative periodontopathogens in subgingival dental plaques from gingivitis lesions in Korean orthodontic patients. J Microbiol;43(3):260-265. 2005.
11. Rego RO, Oliveira CA, dos Santos-Pinto A, Jordan SF, Zambon JJ, Cirelli JA, Haraszthy VI. Clinical and microbiological studies of children and adolescents receiving orthodontic treatment. Am J Dent;23(6):317-323. 2010.
12. Harradine NWT, Day C, Al-Anezi S, Jenkinson HF, Sherriff M, Dymock D, et al. The effects of different orthodontic appliances upon microbial communities. Orthod Craniofac Res;17(2):115-123. 2014.
13. Verrusio C, Iorio-Siciliano V, Blasi A, Leuci S, Adamo D, Nicolò M. The effect of orthodontic treatment on periodontal tissue inflammation: A systematic review. Quintessence Int;49(1):69-77. 2018.
14. Sawhney R, Sharma R, Sharma K. Microbial Colonization on elastomeric ligatures during orthodontic therapeutics: An Overview. Turk J Orthod;31(1):21-25. 2018.
15. Mariotti A. Dental plaque-induced gingival diseases. Ann Periodontol;4(1):7-19. 1999.
16. Tatakis DN, Trombelli L. Modulation of clinical expression of plaque-induced gingivitis. Background review and rationale. J Clin Periodontol;31(4):229-38. 2004.
17. Beighton D. The complex oral microflora of high-risk individuals and groups and its role in the caries process. Community Dent Oral Epidemiol;33:248-55. 2005.
18. Costalonga M, Herzberg MC. The oral microbiome and the immunobiology of periodontal disease and caries. Immunol Lett;162:2PtA:22-38. 2014.
19. Krey KF, Hirsch C. Frequency of orthodontic treatment in German children and adolescents: influence of age, gender, and socio-economic status. Eur J Orthod;34(2):152–157. 612. 2012.
20. Chestnutt IG, Burden DJ, Steele JG, Pitts NB, Nuttall NM, Morris AJ. The orthodontic condition of children in the United Kingdom. Br Dent J;200(11):609-638. 2006.
21. Gutiérrez N, Sánchez T, López A. Frequency of appliances used in interceptive orthodontic treatments. Rev Cient Odontol;13(2). 2017.
22. Pérez RR, Villegas RP, Castillo UA. Orthopedic appliances that were used in the Center for Dental Specialties in the year 2008. Arch Inv Mat Inf;2(1):15-18. 2010.
23. Freitas AOA, Marquezan M, Nojima M da CG, Alviano DS, Maia LC. The influence of orthodontic fixed appliances on the oral microbiota: a systematic review. Dent Press J Orthod;19(2):46-55. 2014.
24. Guo R, Lin Y, Zheng Y, Li W. The microbial changes in subgingival plaques of orthodontic patients: a systematic review and meta-analysis of clinical trials. BMC Oral Health;17(1):90. 2017.
25. Romanova IM, Didenko LV, Tolordava ÉR, Gintsburg AL. Biofilms of pathogenic bacteria and their role in chronization of infectious process. The search for the means to control biofilms. Vestn Ross Akad Med Nauk;(10):31-39. 2011.
26. Perkowski K, Baltaza W, Conn DB, Marczyńska-Stolarek M, Chomicz L. Examination of oral biofilm microbiota in patients using fixed orthodontic appliances in order to prevent risk factors for health complications. Ann Agric Environ Med;26(2):231-235. 2019.
27. Rodríguez-Rentería M, Márquez-Preciado R, Sánchez-Vargas LO, Mariel-Cárdenas J, Ruíz-Rodríguez MS, Romo-Aranda S, et al. Identificación de *Staphylococcus aureus* en pacientes pediátricos portadores de aparatología ortodóntica removible. Odont Pediatr Act;3(10):18-21. 2014.
28. Zharmagambetova A, Tuleutayeva S, Akhmetova S, Zharmagambetov A. microbiological aspects of the orthodontic treatment. Georgian Med News;264:39-43. 2017.
29. Lara E, Montiel NM, Sánchez L, Alanís J. Effect of orthodontic treatment on saliva, plaque and the levels of *Streptococcus mutans* and *Lactobacillus*. Med Oral Patol Oral Cir Bucal;15(6):924-9. 2010.
30. Arendorf T, Addy M. Candidal carriage and plaque distribution before, during and after removable orthodontic appliance therapy. J Clin Periodontol;12(5):360-368. 1985.
31. Sun F, Ahmed A, Wang L, Dong M, Niu W. Comparison of oral microbiota in orthodontic patients and healthy individuals. Microb Pathog;123:473-477. 2018.
32. Kitada K, de Toledo A, Oho T. Increase in detectable opportunistic bacteria in the oral cavity of orthodontic patients. Int J Dent Hyg;7(2):121-125. 2009.
33. Merghni A, Ben Nejma M, Dallel I, Tobji S, Ben Amor A, Janel S, Lafont F, Aouni M, Mastouri M. High potential of adhesion to biotic and abiotic surfaces by opportunistic Staphylococcus aureus strains isolated from orthodontic appliances. Microb Pathog;91:61-67. 2016.
34. Addy M, Shaw WC, Hansford P, Hopkins M. The effect of orthodontic appliances on the distribution of *Candida* and plaque in adolescents. Br J Orthod;9(3):158-163. 1982.
35. Bhagirath AY, Li Y, Somayajula D, Dadashi M, Badr S, Duan K. Cystic fibrosis lung environment and *Pseudomonas aeruginosa* infection. BMC Pulm Med;16(1):174. 2016.
36. Bradshaw D, JBrown JS, Porter SR, Spratt D, Bozec L. Early adhesion of *Candida albicans* onto dental acrylic surfaces. J Dent Res;96(8):917-923. 2017.
37. Budtz E. The significance of *Candida albicans* in denture stomatitis. Scand J Dental Research;8(2):151-90. 2004.
38. Bergamo AZN, de Oliveira KMH, Matsumoto MAN, do Nascimento C, Romano FL, da Silva RAB, da Silva LAB, Nelson-Filho P. Orthodontic appliances did not increase risk of dental caries and periodontal disease under preventive protocol. Angle Orthod;89(1):25-32. 2019.
39. Williams D, Lewis MAO. Pathogenesis and treatment of oral candidosis. J Oral Microbiol;3:5771-5782. 2011.
40. Nowak M, Kurnatowski P. Biofilm caused by fungi—structure, quorum sensing, morphogenetic changes, resistance to drugs. Wiad Parazytol;55(1):19-25. 2009.
41. Lewis MAO, Williams DW. Diagnosis and management of oral candidosis. Br Dent J;223(9):675-681. 2017.
42. Vermitsky JP, Edlind TD. Azole resistance in *Candida glabrata*: coordinate upregulation of multidrug transporters and evidence for a Pdr1-like transcription factor. Antimicrob Agents Chemother;48(10):3773-3781. 2004.
43. Sivakumar I, Arunachalam KS, Sajjan S, Ramaraju AV, Rao B, Kamaraj B. Incorporation of antimicrobial macromolecules in acrylic denture base resins: a research composition and update. J Prosthodont;23(4):284-290. 2014.
44. Gleiznys A, Zdanavičienė E, Žilinskas J. *Candida albicans* importance to denture wearers. A literature review. Stomatologija;17(2):5466. 2015.
45. Hernández-Solís SE, Rueda-Gordillo F, Flota-Alcocer AD, Agullar-Ayala FJ, Rodríguez-Fernández M del SC, Lama-González EM. Influence of orthodontic appliances on the occurrence of *Candida* spp. in the oral cavity. Rev Chil Infectol;33(3):293-297. 2016.