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Persistent lesions in endodontics

Lesões endodonticas persistentes

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Abstract
Apical periodontitis is mainly caused by both microrganisms and their virulence factors. This process can be associated with the survival of its causative agents due to coronary microleakage after chemical-mechanical preparation and filling. However, persistent apical periodontitis can be solved through endodontic retreatment or apical surgery. In addition, its association with systemic diseases has been reported in different studies. The aim of the current study is to perform a literature review on studies about apical periodontitis, with emphasis on current microbial profile and treatment modalities. Studies have shown significant heterogeneity in microbial profile, as well as likely associations with some diseases. Moreover, they have mainly focused on investigating persistent apical periodontitis based on molecular biology. It was possible concluding that, although Enterococcus faecalis is the species most often associated with failure in endodontic treatments, the incidence of other microrganisms, such as Candida albicans, Streptococcus spp., Actinobacteria spp., has been observed at the same, or even at higher, level than that of E. faecalis. Retreatment should always be taken into consideration as the first option to treat morbidities, although surgery can be performed if retreatment is not successful.
Keywords: Biofilm; Periapical periodontitis; *Candida albicans*.

Resumo
A periodontite apical é causada principalmente por microrganismos e seus fatores de virulência. Esse processo pode estar associado à sobrevivência de seus agentes causadores devido à microinfiltração coronariana após preparo

químico-mecânico e preenchimento. No entanto, a periodontite apical persistente pode ser resolvida através de retratamento endodôntico ou cirurgia apical. Além disso, sua associação com doenças sistêmicas tem sido relatada em diferentes estudos. O objetivo do presente estudo é realizar uma revisão de literatura sobre periodontite apical, com ênfase no perfil microbiano atual e modalidades de tratamento. Estudos têm demonstrado significativa heterogeneidade no perfil microbiano, bem como prováveis associações com algumas doenças. Além disso, eles se concentraram principalmente na investigação da periodontite apical persistente com base na biologia molecular. Foi possível concluir que, embora Enterococcus faecalis seja a espécie mais frequentemente associada ao insucesso em tratamentos endodônticos, a incidência de outros microrganismos, como Candida albicans, Streptococcus spp., Actinobacteria spp. nível superior ao de E. faecalis. O retratamento deve sempre ser considerado como a primeira opção para tratar as morbidades, embora a cirurgia possa ser realizada se o retratamento não for bem sucedido.
Palavras-chave: Biofilmes; Periodontite periapical; *Candida albicans*.

Resumen
La periodontitis apical es causada principalmente por microorganismos y sus factores de virulencia. Este proceso puede ocurrir a través de la supervivencia de los agentes causales debido a la microinfiltración coronaria luego de la preparación químico-mecánica y la obturación. Sin embargo, la periodontitis apical persistente puede ser solucionada a través del retratamiento endodóntico o la cirugía apical. Este trabajo tuvo como objetivo realizar una revisión de literatura sobre la periodontitis apical destacando el perfil microbiano actual y modalidades de tratamiento. Las investigaciones han demostrado gran diversidad en lo que respecta al perfil microbiano, así como posibles asociaciones con algunas enfermedades sistémicas. Además, los estudios han dado gran enfoque a la periodontitis apical persistente a nivel molecular. Pudimos concluir que a pesar del Enterococcus faecalis ser la especie más comúnmente asociada a la falla del tratamiento endodóntico, se ha observado la presencia de otros tipos de microorganismos, como: Candida albicans, Streptococcus spp., Actinobacteria spp. En relación con las modalidades de tratamiento, el retratamiento debe ser considerado como primera opción y en caso de fracaso, la cirugía puede ser realizada.
Palabras clave: Biofilm; Periodontitis apical; *Candida albicans*.

1. Introduction

Persistent lesion cases are mainly caused by bacteria and by their virulence factors; they may develop and persist without detectable clinical signs. This process can take place due to survival of its causative agents after chemical-mechanical preparation and filling, or due to bacterial infiltration along the restoration margins (Barbosa-Ribeiro *et al.*, 2016).

The following microorganisms stand out among the species that can be found in these cases: Enterococcus faecalis, Candida albicans, Streptococcus gordonii, Actinomyces viscosus and Lactobacillus acidophilus (Gao *et al.*, 2016). E. faecalis is the species mostly associated with endodontic treatment failure (Antunes *et al.*, 2015), since it can survive harsh environmental conditions, such as alkalinity or lack of nutrients in the root canal environment (Gao *et al.*, 2016). It is a Gram-positive bacterium equipped with different virulence and resistance mechanisms that turn the process of eliminating it into a hard task to be accomplished (Barbosa-Ribeiro *et al.*, 2016).

Several tooth-related predictive variables can influence endodontic treatment outcome in teeth affected by apical periodontitis. This is the reason why teeth affected by endodontic infection (AP cases) often show low treatment success rate (Restrepo-Restrepo *et al.*, 2019). Understanding the etiological factors involved in this process, mainly the most common bacterial types, can help better and safely defining the best clinical management to be adopted for AP cases. The aim of the current study is to carry out a literature review on research focused on investigating persistent lesions in endodontics.

2. Methodology

Bibliographic reviews, as stated by Pereira *et al.* (2018) should specify the sources of consultation, the period of coverage and the criteria used for the research. The present work is a narrative review of the literature, in which a bibliographic search was carried out in the PubMed database that reported the proposed theme from 2015 to 2021. The keywords used were: Biofilm, Periapical Periodontitis, Enterococcus faecalis, Candida albicans, Streptococcus spp.

The inclusion criteria were articles that addressed the topic of the present literature review, in English, which would provide better evidence for this work.

First, they were selected according to the titles of the works and later analyzed the abstracts. Works that did not present a detailed methodology in the abstract or did not correspond to the proposed theme were excluded, thus, 20 articles were used.

3. Literature Review

Antunes *et al.* (2015) have assessed the total levels and details of likely endodontic pathogens in the apical region of teeth treated for apical periodontitis (AP). Twenty-seven patients referred to periradicular surgery were included in their study. The lesion was curetted during the procedure and acknowledged in histopathological examination. DNA transformation into powder (cryogenic milling) was the method selected to enable data analysis and DNA obtainment from samples extracted from 100 mg of root powder. Total bacterial level quantification in seven bacterial groups or species was carried out based on the polymerase chain reaction (qPCR) of 16S ribosomal RNA. Two tooth samples recorded positive results for bacteria. Streptococcus species were the most prevalent ones (76%); they were followed by Actinobacteria (52%) and Pseudoramibacter alactolyticus (19%). Mean total bacterial load in the apical region recorded 5.7x104 equivalent cells per apex (or 2.1x104 /100 mg root powder). Streptococci accounted for 0.02% - 99.9% of the total bacterial counting; Actinobacteria, for 0.02% - 84.7% of it; and P. alactolyticus, for 67.9% - 99%. Although Enterococcus faecalis was only identified in three (14%) cases, it was the dominant species in two of them. Thus, Streptococcus, Actinobacteria and P. alactolyticus species were the most prevalent and dominant bacterial communities identified in several AP cases.

Jhajharia *et al.* (2015) carried out a literature review on studies focused on investigating biofilm formation mechanisms, and the role played by them in pulp and periapical therapy, different biofilm types, and factors influencing biofilm formation. They confirmed the bacterial etiology of oral diseases such as caries, as well as of periodontal and endodontic infections. Bacteria accounting for causing these diseases are organized in biofilm structures, which comprise complex wide variety microbial communities. The successful treatment of these diseases depends on biofilm removal, as well as on the effective elimination of biofilm-related bacteria. Thus, oral biofilm control is the key to maintain oral health and to prevent dental caries, gingivitis and periodontitis from taking place. Based on these aspects, biofilm formation has special clinical significance, since hosts' defense mechanisms, as well as therapeutic efforts, such as chemical and mechanical treatments applied as antimicrobial measures, accounts for the hardest task to deal with microorganisms accumulated in the biofilm. The aforementioned authors have concluded that the most common endodontic infection is caused by the growth of surface-associated microorganisms. It is important applying the concept of biofilm to endodontic microbiology to help better understanding the pathogenic potential of root canal microbiota, as well as to set the basis for new disinfection approaches. It is essential understanding that biofilm formed by root canal bacteria can resist measures taken in endodontic treatments.

Kang *et al.* (2015) have evaluated the clinical and radiographic results of non-surgical endodontic retreatment and of endodontic microsurgery, based on meta-analysis. Searches were carried out on databases such as PubMed, Embase, Medline and Cochrane Library to identify all clinical studies focused on evaluating clinical and radiographic results after retreatment or microsurgery, in compliance with inclusion and exclusion criteria. Results have shown that endodontic microsurgery and non-surgical retreatment reached 92% and 80% success rate, respectively. With respect to post-treatment and follow-up periods, the microsurgery group recorded significantly higher success rate in less than four years, whereas there was no significant difference between treatments in the long-term follow-up (more than four years). The aforementioned authors have concluded that endodontic microsurgery can be confirmed as reliable treatment option due to initial favorable healing and predictable outcome, although both investigated treatments are effective, depending on patients' clinical condition.

Ricucci *et al.* (2015) described three cases of patients who presented persistent symptoms after they were subjected to proper endodontic retreatment (cases 1 and 2) or treatment (case 3). Histopathological and histobacteriological analyses were carried out to determine the cause of symptoms' persistence. Periapical surgery was recommended and performed in all three cases. Case 1 presented apical cyst with necrotic debris, which was strongly colonized by bacteria accumulated in the lumen. Bacteria were not found in the apical third of the root canal. Case 2 presented granuloma with numerous bacterial aggregations through inflammatory tissue. The infection was also identified in dentinal tubules in the apical root canal. Case 3 presented cyst with bacterial colonies loose in its lumen; bacterial biofilm was also seen on the surface of the external apical root, filling a large lateral canal and other apical ramifications, and between cementum layers detached from the surface of the source. Bacteria were not detected in the main root canal. The aforementioned authors have concluded that different extra radicular infection forms were associated with symptomatology in these cases; thus, the short-term endodontic failure was only solved through periapical surgery.

Gao *et al.* (2016) have investigated the starvation resistance of paired-species models comprising E. faecalis and Lactobacillus acidophilus, Actinomyces viscosus, Streptococcus gordonii or C. albicans, as well as analyzed biofilm formation through scanning electron microscopy and confocal laser scanning microscopy. Bovine incisors were used to measure species formation in pairs within the root canal. Roots were longitudinally divided into two parts, and the canal was exposed to bacteria for biofilm formation purposes. Moreover, 100 mg/L of cefuroxime sodium was added to BHI agar to differentiate E. faecalis from A. viscosus, L. acidophilus, and S. gordonii; whereas 100 mg/L of vancomycin was added to BHI agar in order to differentiate E. faecalis from C. albicans. The following pairs were used to establish four double-species models for starvation rehearsal purposes: E. faecalis and C. albicans (EC), E. faecalis and S. Gordonii (ES), E. faecalis and A. viscosus (EA) and E. faecalis and L. acidophilus (EL). The total bacterial counting was performed at the first, second, fourth, seventh, fourteenth and twenty-eighth starvation day. Bacterial competition during infection was observed under abundant nutrient condition, based on confocal laser scanning microscopy. Results have shown that E. faecalis was more resistant to starvation in coexistence with C. albicans, S. gordonii, A. viscosus, or L. acidophilus; S. gordonii was fully inhibited in coexistence with E. faecalis. The double-species biofilm has shown that E. faecalis coexisting with S. gordonii and A. viscosus has formed biofilms thicker and denser than those formed when it coexisted with C. albicans and L. acidophilus. The aforementioned authors concluded that E. faecalis was more resistant to starvation in coexistence with four other bacteria; A. viscosus and S. gordonii promoted E. faecalis survival due to abundant nutrition.

Ricucci *et al.* (2016) described 2 cases that have presented persistent intra-canal exudation (wet canal), even after several endodontic treatment sessions. Histological and histo-bacterial investigations were carried out to determine their causes. The 2 cases concerned teeth with apical periodontitis lesions presenting persistent exudation and resistance to treatment after several attempts. It was not possible finding the dry canal in case 1, and it forced the need of surgery. The attempts to dry out the canal were successful in case 2, and the canal was blocked; however, the follow-up exam showed enlarged apical periodontitis lesion, and it allowed the extraction procedure. Biopsy specimens consisted of root apex and apical periodontitis lesion in case 1, and of whole root in case 2; they were subjected to histological and histo-bacterial analyses. Both cases have shown complex bacterial infection in the apical third, which affected either the intra-radicular space or the external root surface. Case 1 showed branched bacterial biofilms on untouched walls; they extended themselves up to root external surface in order to form a thick and partially mineralized structure presenting high bacterial density. Different bacterial morphotypes were evidenced. Case 2 presented a bulge in the wall of the apical canal, which emerged after instrumentation; this bulge was full of necrotic debris, filler material and bacteria. The walls of the apical portion of the canal were covered with bacterial biofilm, which was continuous and had thick extra-radicular biofilm covering the cement and the dentin in resorption defects. The aforementioned authors have concluded that the extra-radicular biofilm presented mineralization areas; it was dominated by filamentous bacteria. The two cases with humid canals and treatment failure were associated with complex persistent infection in the apical part of the root canal system; it extended itself to form thick and partially mineralized biofilm structures on the root's external apical surface.

He *et al.* (2017), have determined successful cases of molar retreatment based on contemporary endodontic techniques. Their study included 63 patients referred to retreatment in first molars; 27 of them were followed-up for 6, 12 and 24 months. Results were categorized based on clinical and radiographic criteria. Fifty-two (52) of the 63 patients participated in the final analysis; 5 (9.6%) of them did not require any repair until the last follow-up; 37 (71.2%) presented full BP recovery and the last 10 (19.2%) remained asymptomatic and showed evidence of radiographically lesion repair. The aforementioned authors have concluded that endodontic retreatment achieved success rate of 90.4% after two years, as well as that it has significantly improved patients' quality of life and chewing ability, over-time.

Mohammadi *et al.* (2017) carried out a literature review to find effective anti-microbial strategies to reduce anti-microbial resistance and to control infectious diseases. The treatment applied to infected canal systems may not be capable of removing all the bacteria; therefore, it is likely finding bacterial persistence after the treatment. The application of anti-bacterial nanoparticles can be a potential strategy to improve the process to rule out bacteria from the canal. Furthermore, the action mechanism and application of photodynamic therapy, as well as of both photon-induced photoacoustic transmission (PIPS) and GentleWave System, were reviewed. They have concluded that recent advancements in root canal disinfection based on using new technologies and on recent studies can improve the disinfection ability of the root canal system. However, conventional methods remain useful to find good prognostic.

Persoon *et al.* (2017) conducted a systematic review and meta-analysis on fungal prevalence and diversity in root canal infections. Extensive literature research was carried out in databases such as Cochrane, EMBASE, MEDLINE, LILACS, SciELO and Web of Science. Additional studies were identified in six endodontic journals, four main endodontic textbooks and relevant article references. The selected clinical studies included procedures such as sampling necrotic pulps in permanent teeth and subjecting these samples to microbial analysis. In total, 54 studies were selected after screening 1,041 titles and abstracts, and reading full texts. The overall fungal prevalence in root canal infections reached 7.5%. Candida albicans was the species most often isolated. Significant fungal species heterogeneity was observed. Subgroup analyses based on geographic location, publication time, infection type, overall health condition, communication with the oral cavity, sample type and identification method did not evidence any factor influencing prevalence. The aforementioned authors have concluded that, despite the heterogeneity in study protocols and the increased risk of bias, fungi accounted for 7.5% of root canal infections.

Mergoni *et al.* (2018) conducted a systematic literature review on the prevalence of Candida species in root canal infections. An extensive bibliographic search was carried out in the most important electronic biomedical databases; additional studies were identified based on references in relevant articles. In total, 2,118 of the 2,225 records were excluded from the review, based on their titles and abstracts. Of the 107 remaining studies, 50 were excluded from the review after full text review; 57 studies were included in it for qualitative and quantitative analyses. The general prevalence of Candida spp. in root canal infection reached 8.20%. Candida albicans was the most often isolated species. There was significant heterogeneity among studies. The analysis of subgroups showed the highest prevalence of Candida spp. (of African samples). The aforementioned authors have concluded that Candida spp. occurred in a small portion of root canal infections.

Rodrigues *et al.* (2018) assessed the anti-microbial action of an irrigant with silver nanoparticles, in aqueous vehicle (AgNp), sodium hypochlorite and chlorhexidine. They performed two evaluation types against the Enterococcus faecalis biofilm and against infected tooth tubules. In order to do so, they used bovine dentine blocks to develop the E. faecalis biofilm for 21 days; they were irrigated with 94ppm AgNp solution, 2.5% NaOCl and 2% chlorhexidine for 5, 15 and 30 minutes. Dentine samples of bovine incisors were subjected to contamination protocol for 5 days to assess infection in tooth tubules caused by E. faecalis; the samples were irrigated with the same solutions, for the same time intervals used for the biofilm. The samples were stained through the Live/Dead technique and assessed in confocal laser scanning microscope (CLSM). Bioimage L software was used to measure total bio-volume in um3; viable bacteria rate (green cells) in the biofilm and tooth tubules were found after irrigation. The AgNp solution ruled out fewer bacteria, but it got to dissolve more biofilm than chlorhexidine. NaOCl recorded the highest anti-microbial activity and the highest biofilm dissolution ability. The AgNp solution presented less anti-microbial action in infected tooth tubules than NaOCI. The AgNp solution was the most effective to rule out planktonic bacteria from tooth tubules after 5 minutes than from the biofilm; however, at 30 minutes, less viable bacteria were observed in the biofilm in comparison to the intra-tubular dentin. The aforementioned authors have concluded that irrigant AgNp was not effective against E. faecalis in comparison to solutions oftentimes used to treat root canal. NaOCI is an appropriate irrigant, because it was effective to interrupt the biofilm and to rule out bacteria from the biofilm and from tooth tubules.

Siqueira Junior *et al.* (2018) have investigated the reasons why some root canal areas remain unprepared by instruments, as well as addressed strategies to circumvent this issue and to improve infection control during endodontic treatment/retreatment application to teeth affected by apical periodontitis. The aforementioned study used both clinical and in-vitro studies. Root canal preparation using 2.5% NaOCl as irrigant helped reducing bacterial levels by 102-105 times, bacterial reduction rates ranged from 95% to 99%. The best antimicrobial effects of NaOCl were observed when this substance was changed on a regular basis and used at large volumes. Chlorhexidine (CHX) was also used as alternative to NaOCl for irrigation purposes; it presented antimicrobial results similar to those of NaOCl. Despite the significant bacterial reduction promoted by using NaOCl or CHX in association with mechanical instrumentation, bacteria could still be detected in approximately 30% to 60% of canals in teeth affected by apical periodontitis, after preparation based on these solutions. It is important emphasizing that samples collected in most studies of this nature are removed by placing paper points in the main root canal. Although bacteria located in areas around the main root canal can be sampled, results in these studies are mainly associated with bacteriological conditions observed in the lumen and walls of the main canal. According to the aforementioned authors, it is important encouraging the development of systems, techniques and strategies focused on improving the process to clean and disinfect pristine canal walls and hard-to-reach sites to help improving treatment outcomes.

Dioguardi *et al.* (2019) conducted a literature review to identify microbiological issues associated with persistent root apical lesions capable of causing therapeutic failure and loss of dental elements. The goal of endodontic therapy is to create a biologically acceptable environment within the root canal system to enable healing and maintain periradicular tissue health. Bacteria are one of the main causes of pulp issues; they use different routes to penetrate and invade the endodontic treatment space, such as carious lesions, traumatic pulp exposure and fractures. Enterococcus faecalis is one of the main causes of recurrent apical periodontal lesions after endodontic treatment; they can lead to persistent lesion symptoms even after retreatment. The reviewed analyzed articles were identified through electronic databases, such as PubMed and Scopus, and their bibliographic references were thoroughly analyzed; experts in the field were consulted to help identifying new articles. All clinical studies associated with both endodontic issues and their clinical manifestations were analyzed after they met the inclusion criteria. Results have shown that Enterococcus faecalis, Actinomycetes and Propionibacterium propionicum were the species most often involved in persistent root and extracellular infections. Thus, the aforementioned authors have concluded that several microorganisms can be identified depending on the health status of the assessed tooth (cavities, pulpitis, necrotic tooth, acute/chronic parodontitis, extra/intra-persistent root canals). The bacterial species most often involved in endodontic failures comprise facultative anaerobes showing prevalence in recurrent forms of apical periodontitis caused by Enterococcus faecalis, whereas the bacterial species mostly involved in persistent extraradicular infections comprise A. israeli and Propionibacterium propionicum. On the other hand, food contaminated with enterococci accounts for intestinal microbiome incidence in the oral cavity and, consequently, inside the tooth.

Prada *et al.* (2019) carried out a literature review to identify the microbiota associated with endodonetic failure, as well as the reasons why these microorganisms are capable of surviving basic disinfection procedures. A systematic search for scientific articles was performed in PubMed database, based on the following meshes: "Endodontic Infections", "Endodontic Microbiology", "Endodontic Failure", "Enterococcus Faecalis", "Endodontics Retreatment". Case reports and articles published before 2000 were not included in the review. Most authors highlight E. faecalis as the main microorganism associated with endodontic failure, although there are recent studies that mostly isolated other bacteria, such as Fusobacterium nucleatum and Propioni bacterium. They have concluded that these microorganisms have the following properties – which can form biofilm, and place themselves in areas that are inaccessible to root canal instrumentation techniques - in common: synergism, ability to express survival genes and to activate alternative metabolic pathways.

Restrepo *et al.* (2019) retrospectively assessed association among several prognostic variables related to outcomes recorded for endodontic treatments applied to patients with pre-therapeutic apical periodontitis after time lapse ranging from 1 to 12 years. It was done to find compliance among predictors assessed through Cone Beam Computed Tomography (CBCT) and digital periapical radiography. The study sample included 125 teeth from 84 individuals. Post-operative clinical signs/symptoms, as well as estimates, were used to determine healing results. Results were dichotomized in fully healed periapical structures vs. persistent disease cases for the statistical analysis - success rate reached 53.6%. Mandibular tooth location, periapical lesion size > 10 mm, poor-quality coronal restoration, lack of magnification/illumination, lack of gutta-percha disinfection, time elapsed for definitive coronal restoration > 1 week, and low density of root canal filling, remained as failure predictors after the logistic regression analysis. These authors have concluded that several predictive variables related to tooth and treatment, including tooth location, periapical radiolucency size, coronal restoration quality, magnification/illumination, gutta-percha disinfection, elapsed time for definitive coronal restoration and filling material density, can influence outcomes of endodontic treatments applied in teeth with preoperative apical periodontitis.

Xu *et al.* (2019) investigated instrumentation and irrigation effects on the initial adhesion of E. faecalis to root canal dentine and explored the initial microbial adhesion to root filling materials. Samples were prepared with instrumented and non-instrumented dentin for the study, which also followed different irrigation protocols and root filling materials. It was done to register and measure the number of E. faecalis cells adhered to the dentine, the adhesion power of E. faecalis cells to different materials and the roughness of different surfaces, as well as the contact angle of surfaces. The instrumented dentine samples presented significantly higher adherence to E. faecalis than the non-instrumented one. There was a larger number of bacteria adhering to dentine when EDTA was used, in separate, in comparison to other irrigants. Thus, these authors have concluded that instrumentation and irrigation have changed the initial adherence of E. faecalis to root canal dentine and dentine's surface properties.

Lukic *et al.* (2020) investigated the role played by different substrates in biofilm formation, as well as the separate and collective addition of six endodontic pathogens to the "basic biofilm" of nine species, namely: Enterococcus faecalis, Staphylococcus aureus, Prevotella nigrescens, Selenomonas sputigena, Parvimonas micra and Treponema denticola. This biofilm was formed in vitro as standard sub-gingival biofilm, which encompassed Actinomyces oris, Veillonella dispar, Fusobacterium nucleatum, Streptococcus anginosus, Streptococcus oralis, Prevotella intermedia, Campylobacter rectus, Porphyromonas gingivalis and Tannerella forsythia. Biofilms similar to the resulting endodontics were cultivated on hydroxyapatite and dentin discs, for 24h, under the same conditions. Bacterial growth was determined through quantitative PCR at real time after endodontic biofilm collection; they were marked by fluorescent in situ hybridization (FISH) and analyzed through confocal laser scanning microscopy (CLSM). The addition of six endodontic pathogens to the "basic biofilm" induced reduction in the number of cells belonging to the "basic species". Interestingly, C. rectus counting increased in biofilms with E. faecalis, S. aureus, P. nigrescens and S. sputigena, respectively, either in hydroxyapatite or in dentin discs, whereas P. intermedia counting only increased in dentine discs due to E. faecalis addition. E. faecalis growth in hydroxyapatite discs, and of E. faecalis and S. aureus in dentine discs, was significantly higher in biofilm with all the species than in the "basic biofilm". On the other hand, P. nigrescens, S. sputigena and P. micra counting in hydroxyapatite discs, as well as the counting of P. micra and T. denticola in dentine discs, decreased in the biofilm with all species. Overall, all bacterial species associated with endodontic infections were successfully added to the standard multi-species biofilm model in hydroxyapatite and dentin discs. These authors have concluded that their study shows the successful addition of six endodontic bacteria to the existing sub-gingival biofilm model of nine species. The counting of five among nine strains in the "basic biofilm" tended to decrease due to the addition of some endodontic pathogens in the HA discs. Only C. rectus counting increased due to the addition of E. faecalis, S. aureus, P. nigrescens and S. sputigena. C. rectus and P. intermedia counting in dentine discs increased due to the addition of the aforementioned strains or of E. feacalis, alone, respectively.

Pereira *et al.* (2021) investigated the anti-biofilm effectiveness of irrigation based on the simulated root canal model, the chemical effect of irrigants against biofilm cultivated in dentine discs, and their impact on biofilm viscoelasticity, the effectiveness of irrigants in decontaminating infected tooth tubules and bacteria's capacity to grow back. Biofilm was removed, the viscoelasticity of remaining biofilms was analyzed and bacterial viability was assessed based on the root canal simulated model, which presented lateral morphological features, dentine discs and a tooth tubule model, respectively. The experiments were conducted based on a two-phase irrigation protocol. Phase 1: modified saline solution (RISA) and sodium hypochlorite (NaOCl) were used at low flow to assess irrigants' chemical action. Ultrasonic activation (US) of a chemically inert solution (buffer) was used to assess irrigation's mechanical effectiveness. Phase 2: final irrigation with high flow buffer was carried out in all groups. Optical coherence tomography (OCT), low load compression test (LLCT) and scanning laser confocal microscopy analysis were used in different models. The following results were recorded: US and high flow rate have significantly removed more biofilm from the artificial side canal; there were no significant differences between groups. The intra-group analysis showed significant differences among experimental stages, except for NaOCl. With respect to dentine discs, there were no differences in biofilm removal and viscoelasticity. NaOCl presented the highest anti-biofilm efficacy. These authors have concluded that irrigation's mechanic effect is relevant for biofilm removal. Extra high-flow irrigation rate led to higher biofilm removal than US in the artificial isthmus. US mechanical effect seemed to be effective when the surface of the biofilm-contact irrigant was small. The remaining biofilm could survive for 5 days after the irrigation procedure. RISA and NaOCI seemed to have changed the remaining post-treatment biofilms.

Zhang *et al.* (2021) have featured the bacterial community found in extraradicular biofilm and periradicular lesions associated with persistent apical periodontitis. They selected 18 adult patients who presented periradicular lesions after root canal treatment and who were scheduled for endodontic surgery. Extraradicular biofilm and periradicular lesion samples were collected during surgery. Ten pairs of periradicular lesions and extraradicular biofilm samples were randomly selected for 16S ribosomal rRNA cloning and sequencing purposes. Wilcoxon rank sum test was used to compare total bacterial counts, as well as genera and individual species levels, between the two groups ($P < 0.05$). Overall, 73 phyla types belonging to 6 different phyla were identified from 1,000 sequenced clones. M. timidum, S. intermedius and E. faecalis prevailed in both the extraradicular biofilm and periapical lesions. P. propionicus, A. adiacens, P. prevotii, C. gracilis and P. aeruginosa were found at significantly higher levels in the extraradicular biofilm than in periapical lesions, whereas P. micra and A. rimae were more abundant in periapical lesions ($P < 0.05$). The aforementioned authors have concluded that the microbial profile of extraradicular biofilms differed from that of periapical lesions, and it indicated the incidence of different bacterial populations in these regions. Many bacterial genera and species were significantly associated with extraradicular biofilm formation.

4. Final Considerations

Based on the literature review carried out in the current study, it was possible concluding that persistent apical periodontitis has polymicrobial profile, whose main representatives comprise the following microorganism species: E. faecalis, C. albicans, Streptococcus spp., and Actinobacteria spp. Treatments applied to persistent disabilities can include wider apical third preparation or even endodontic microsurgery.

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