Salome Julie Rebecca Zerbib

How to prevent the occurrence of Post Treatment Apical Periodontits:

Narrative Review

Universidade Fernando Pessoa

Faculdade de Ciências de saúde

Mestrado Integrado em Medicina Dentária

PORTO, 2022

Salome Julie Rebecca Zerbib

How to prevent the occurrence of Post Treatment Apical Periodontits:

Narrative Review

Universidade Fernando Pessoa

Faculdade de Ciências de saúde

Mestrado Integrado em Medicina Dentária

PORTO, 2022

Salome Julie Rebecca Zerbib

How to prevent the occurrence of Post Treatment Apical Periodontits:

Narrative Review

"Trabalho apresentado à Faculdade de Ciências de Saúde da Universidade Fernando Pessoa, como parte dos requisitos para a obtenção do Grau de Mestre em Medicina Dentária."

____

Salomé Julie Rebecca Zerbib

RESUMO

As infecções endodônticas são influenciadas pela localização, composição do biofilme e características dos materiais dentários utilizados. Os canais radiculares com periodontite apical associada pós-tratamento, persistente/secundária, indicam que houve uma falha na desinfecção durante o Tratamento Endodôntico Não-Cirúrgico. Neste caso, os microrganismos podem ter tolerado os procedimentos químicos-mecânicos (infecção persistente) ou invadido o espaço canalar por preenchimento radicular tridimensional deficitário (infecção secundária). Este trabalho revê os aspectos microbiológicos da infecção endodôntica, especialmente quando a lesão ocorre após o Tratamento Endodôntico Não-Cirúrgico do canal radicular. Oferece perspectivas para futuras investigações bem como orientações na prevenção desta patologia.

Palavras-chaves: Endodontia, Microbiologia, Prevenção, Infecção Secundária.

ABSTRACT

Endodontic infections are influenced by the location, biofilm composition and features of the dental materials used. Root canals with post-treatment associated apical periodontitis, persistent/secondary, indicate that disinfection failed during Non-Surgical Endodontic Treatment. In this case, the microorganisms may have tolerated the chemico-mechanical procedures (persistent infection) or invaded the pulpal space by deficient three-dimensional root filling (secondary infection). This work reviews the microbiological aspects of endodontic infection, especially when the lesion occurs after non-surgical endodontic treatment of the root canal. It offers prospects for future research as well as guidance in the prevention of this pathology.

Keywords: Endodontic; Microbiology; Prevention; Secondary Infection

INDEX

ACKNOWLEDGEMENTS

I would like to thank, as they deserve, all the people who helped me through this pathway. First of all, I thank Prof. Dra. Ana Moura Teles of Fernando Pessoa University. Thanks for her guidance, her kindness, her availability, her help and teaching.

Thank to Fernando Pessoa University and its teachers.

To my friends who have accompanied and supported me during these five years and more especially to my partner Jade without whom these years would not have has the same flavor.

To my family, the most important of all, because it is only through their merit that one can achieve one's goals and that all this became possible. Thanks to my grandmother for her daily encouragement, "question answer" will have been the motto of my studies. Thanks to my uncle Dan, for his help and support. And thanks to my parents for just being here!

Finally, thanks to this beautiful city of Porto which has allowed me to access all its opportunities to become the person I am today.

Thank you.

LIST OF FIGURES

LIST OF TABLE

LIST OF ABREVIATIONS

AgNP – Silver Nanoparticules

AMP – Anti-microbial peptides

AP - Apical Periodontitis

$Ca(OH)_2$ – Calcium Hydroxide

CN - Chitosan nanoparticles

CHX – Chlorhexidine

CP – Cold Plasma

EDTA - Ethylenediaminetetraacetic Acid

FISH - Fluorescence *in situ* hybridization

MALDI-TOF MS - Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry

MTA - Mineral Trioxide Aggregated

NaOCl - Sodium hypochlorite

NGS - Next-generation sequencing

NiTi - Nickel-titanium

NSRCT – Non Surgical Root Canal Treatment

PCR - Polymerase Chain Reaction

QAMs - Quaternary Ammonium Monomers

RCS - Root canal system

I - INTRODUCTION

Endodontics is a major discipline in Dentistry. It has long been known that endodontic pathology is fundamentally a microbial disease. Rather than one or several bacterial or fungal species, the disease is initiated and propagated by a complex community of microorganisms that are common members of commensal oral microflora (Fouad, 2017). Is one of the most common inflammatory diseases that affect humans (Siqueira and Rôças, 2021). The ultimate goals of Non Surgical Root Canal Treatment (NSRCT) are to prevent and treat Apical Periodontitis (AP) (Ørstavik, 2020). Consequently, this means that treatment should prevent bacteria from infecting or re-infecting the pulp space or the periradicular tissues or, at least, control an already existing root canal infection (Siqueira, and Rôças, 2021). Bacteria develop increased resistance to various treatment strategies, which makes disinfection of the root canal system (RCS) a hard task. The chronic lesion shows the shortcomings of our knowledge of the molecular biology of the oral microbiota. In fact, two phenomena have been identified as sources of failure: bacterial contamination of a canal during NSRCT, and the persistence of microorganisms despite properly performed disinfection procedures.

The whole point of Dentistry is to be the least invasive, the most predictable and to give longevity to our treatments. The presence of a periapical lesion significantly reduces the chances of successful treatment. The overall long-term success rate of endodontic treatment ranges from 82% to 97.8% for teeth without periapical lesions and from 74% to 86% for teeth with periapical lesions. Even an exclusive specialist never has 100% success (Saint-Pierre and al, 2008). According to another study, success rates go from 85 to 94% for primary NRSCT and from 74 to 82% in retreatment cases (Karamifar, 2020). That's why it seems interesting to me to understand the influence of Microbiology in a small space like the RCS. The study of Microbiology allows us to apply our basic theoretical knowledge to concrete behaviour in clinical practice.

This work will focus on the secondary infection. In the first part, the concept of secondary infection will be presented. Subsequently, the factors that contribute to the appearance of the lesion and the methods used to prevent the lesion will be discussed.

1. Materials and Methods

The bibliographic research of the present work was carried out between October 2021 and June 2022, using search engines of scientific publication databases, namely Pubmed, B-On and Information-Dentaire.fr. The following keywords were used: "Endodontic", "Microbiology", "Treatment", "Prevention", "Secundary Infection", "Apical", "Chronic". The research performed with different combinations of these keywords resulted in 661 publications without redundancy. Excluding those published in languages other than English, Portuguese or French, all those that diverged from the theme studied. Initially, the selection was made on the basis of the title and abstract. Also rejected were all those that diverged from the studied theme or whose availability was impossible. Then, the exclusion was determined by the analysis of the integral content of each article, which gives a total of 28 articles. Then, with the help of the bibliographies of respective articles, 5 news articles were added.

II- DEVELOPMENT

1. Definition of Secondary Infection

a. Apical Periodontitis of Infectious Origin

AP has a microbial aetiology and is one of the most common inflammatory diseases that affect humans, caused by biofilm (Costerton et al., 1999 *Cit in* Siqueira and Rôças, 2021). When the pulp is exposed, bacteria present in the caries, oral cavity or periodontal area infect and colonize the previously sterile pulp tissue via the dentinal tubules, apical foramen or secondary root canals. Bacteria, together with the decomposition products of the pulp tissue and the stagnant fluid present in the RCS, then irritate the periapical tissues adjacent to the apical and lateral foramina of infected canals (Ørstavik, 2020). In defence of itself, the host initiates an immunological process to counteract the aggression of the bacteria in the canal, resulting in the formation of an endodontic peri-radicular inflammatory lesion or acute AP. Following this initial acute host response, several evolutions are possible, being one of them, the chronic one (Lasfargues and Machtou, 2001). Sometimes, a balance is established between the host's defences and the bacterial attacks. The periapical lesion then takes the form of an encapsulated lesion in the form of a granuloma or even a cyst. This fragile equilibrium can break down, causing an acute inflammatory outbreak in the chronic phase: the secondary abscess (Lasfargues and Machtou, 2001). In a randomly selected population, from 304 teeth with an apical radiographic lesion on a root-filled tooth, within 6 years 30% of the lesions healed, 60% persisted, and a mere 10% of the teeth were extracted (Zehnder and Belibasakis, 2015). According to another study after 20 years on 2595 treatments, the most frequent was the fracture of the tooth (50.5%); then, 21.3% were lost due to untreatable caries, 17.3% were lost due to periodontal reasons, and 10.9% were lost due to other reasons, including failure of the endodontic treatment (Mareschi and al, 2020).

b. Clinical and Radiological Sings of Chronic Apical Periodontitis

The stage of chronic AP is often asymptomatic. The diagnosis is commonly made by the chance discovery by the presence of a radiolucent image of bone lysis at the apex of the tooth on a periapical radiographic or a change in color of the necrotic tooth. There is a negative response to the sensibility test and, on rare occasions, a very slight positive response to the percussion test. Bone lysis may result in mobility or anteroposterior displacement of the teeth (Ørstavik, 2020). Persistence or appearance of signs and/or symptoms of active infection (e.g., periradicular bone radiolucency, sinus tract, swelling, pain, tenderness to percussion) in root canal-treated teeth means that AP has persisted, emerged or recurred. This condition is referred to as post-treatment AP, caused by persistent or secondary intraradicular infection or, in some cases, by an extraradicular infection (Siqueira, 2001). The causative agents of post-treatment disease have been evaluated in samples taken from the entire extent of the RCS during retreatment or exclusively from the root apices obtained during apical surgery (Siqueira and Rôças, 2022).

Although the development of these radiographic lesions is relatively rapid, complete healing usually takes months to years and may take decades (Fouad, 2017). According to another study, it can take up to 4 years to disappear, sometimes more (Ørstavik, 1996 *Cit in* Zehnder and Belibasakis, 2015; Karamifar, 2020). Changes in the presence and size of the periapical lesions are thought to be fundamentally related to the presence or persistence of microbial irritants. Although cases with primary infections can be easily identified and treated, the situation in cases with pre-existing endodontic treatment are more difficult to discern and provide adequate care, particularly if the history of treatment is not available, because the lesion heals, develops or is stable can't be distinguish. Recently, investigators have sought to measure certain mediators of bone resorption in periapical lesions, to gain insight into this issue (Fouad, 2017). For example, with the study of inflammatory mediator profile, it is more likely to characterize and explain the shift to disease by defining multivariable "signatures" of changes, rather than single parameters alone (Zehnder and Belibasakis, 2015).

2. Factors that Contribute to the Prevention of Lesion

a. Mechanisms of Development

The oral microbiota lives in harmony with the host, until an induced micro-environmental change causes disturbances in this symbiotic relationship. This change may result in selective overgrowth of opportunistic pathogens, or in an insufficient capacity of the host to respond to otherwise commensal microorganisms. The biofilm microbiota is influenced by the local pH, abundance and partial pressure of oxygen, redox potential, availability of selective nutrients, and, last but not least, the state of local host defenses (Marsh and Devine 2011 *Cit in* Zehnder and Belibasakis, 2015, Siqueira, 2001). A high diversity of microorganisms, including bacteria, fungi, viruses, archaea and protozoa belongs to oral microbiota. There are approximately 700 species present in the oral cavity, where 54% have been cultivated and named, 14% have been cultivated but are unnamed, and 32% are known only as uncultivated phylotypes (Bouillaguet and al, 2018 *Cit in* Korona-Glowniak and al, 2021). It was noted a significant decrease in bacterial diversity in secondary infection samples compared with primary ones (Korona-Glowniak and al, 2021). However, other studies reported an increased diversity in secondary infection samples (Tzanetakis and al, 2015 *Cit in* Korona-Glowniak and al, 2021) or no significant difference between the two types of infection (Hong and al, 2013 and Keskin and al, 2017 *Cit in* Korona-Glowniak and al, 2021).

Initially, so-called "pioneer" bacteria invade the RCS. Then, the infection progresses and the root canal environment changes. Presence of oxygen and nutrients decreases, which leads to changes in the composition of the bacterial flora. Obligate anaerobic species are more abundant in the intraradicular bacterial communities of teeth with primary AP, while both anaerobes and anaerobes facultatives dominate the communities in post-treatment AP (Siqueira and Rôças, 2021). At the apical level, there is an aggressive proteolytic, anaerobic flora that can survive with a low nutrient supply and is more difficult to eliminate than the one present in the coronal part of the infected RCS (Lasfargues and Machtou, 2001). The type of species found, their virulence, their number and their synergism are variable from one individual to another. This infection initiates innate or acquired immune reactions in the periradicular periodontium (Siqueira and Rôças, 2021). The biofilm has an organization that makes it highly resistant to antimicrobial treatment (Siqueira and Rôças, 2021).

b. Organization of Bacteria

Infection can spread from the main canal lumen to other areas of the RCS, including apical ramifications, lateral canals, isthmuses, recesses and dentinal tubules, among others. These areas represent a great challenge for proper disinfection during NSRCT, and studies show that bacterial persistence in the RCS before filling is a significant risk factor for post-treatment disease (Sjögren et al., 1997; Waltimo et al., 2005; Zandi et al., 2019, *Cit in* Siqueira and Rocas, 2021). Base on phylogenetic analysis of collected 16S rRNA gene sequences and real-time PCR amplification primers, the oral microbiome comprises more than 1000 individual taxa, most of which are as-yet uncultivated (Zehnder and Belibasakis, 2015). The oral cavity is considered as the second most complex microbiota in human body, following the colon (Korona-Glowniak and al, 2021). It is noted that taxa span across 13 individual phyla (Dewhirst and al, 2010 *Cit in* Zehnder and Belibasakis, 2015). The oral bacterial community is dominated by the six major phyla account for 94% of the taxa detected. *Firmicutes* (36.7%), followed by *Bacteroidetes* (17.1%), *Protobacteria* (17.1%), *Actinobacteria* (16.6%), *Spirochetes* (7.9%) and *Fusobacteria* (5.2%) (Figure 1) (Verma and al, 2018 *Cit in* Korona-Glowniak and al, 2021). More specific to the study of post-treatment infections, the species that have been found to be dominant are gram positive anaerobic facultative such as *Peptostreptoccus, Enterococcus faecalis, Streptococcus species, Actinomyces species, Cutibacterium acnes, Pseudoramibacter alactolyticus, Arachnia propionica (Propionibacterium propionicum), Parvimonas micra* and gram negative, *Dialister species,* Fusobacterium *nucleatum, and Prevotella species* (Siqueira and Rôças, 2021 and Sakko *et al, 2016*). In another study the *species Fusobacterium, Forphyroma, Parvimonas* were also detected (Shin et al. 2018 *Cit in* Siqueira and Rôças, 2021). *E. faecalis* is not more prevalent in root filled teeth with lesions when compared to root filled teeth with no lesions (Kaufman et al. 2005 *Cit in* Pereira and al, 2017). Hence, *E. faecalis* is no longer considered the key pathogenic root canal species (Swimberghe and al, 2019). This species was considered as a secondary invader through coronal leakage or opening for drainage (Siren and al, 1997 *Cit in* Hong and al 2013).Virulence factors of *E. Faecalis* are responsible for resistance against defense mechanisms of the host and production of pathological changes (Kayaoglu and Ørstavik, 2004, Strateva and al, 2016 *Cit in* Zou and al, 2020). The different associations of micro-organisms confer greater virulence on the associated bacteria (Fouad, 2017). An interesting finding was that as-yet-uncultivated bacteria may represent the dominant taxa in some cases, which help explain why culture can fail to detect bacteria in all cases of post-treatment disease (Figure 2) (Siqueira and Rôças, 2021).

c. Exogenous Contamination

Measures are put in place to protect the patient such as instrument sterilization and surface disinfection, as materials remain a source of potential contamination. Even the rubber dam, the method employed to protect the tooth from contamination with saliva directly, can act as a potential source of contamination. For instance, contamination may occur during the manufacturing process, packing and use (Rai and al. 2019 *Cit in* Abusrewil and al, 2020).

To support this claim the processing materials (gutta-percha points, rubber dams, paper mixing pads, caulking agents and endodontic instrument sponges) were tested at the time of opening the manufacturers seal and after clinical storage using both Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) and q Polymerase Chain Reaction (PCR). The results showed a higher contamination after clinical storage except OralSeal (caulking agent). The most common genera identified on the contaminated endodontic materials were *Propionibacterium, Staphylococcus, and Micrococcus* (Saeed and al. 2017). So, it is advised to change gloves frequently after gaining pulp access and after taking the radiographic working length. Make cotton wools out of sterilised cotton. To disinfect gutta-percha points before use. Sodium hypochlorite (NaOCl) is still the chairside disinfectant of choice for this (Rai and al. 2019 *Cit in* Absrewil and al, 2020). It is also advised to do a decontamination of tooth surface, clamp and rubber dam after it is fully applied, were scrubbed with 30% hydrogen peroxide, until no further bubbling of the peroxide occurred followed by a 3% NaOCl or 10% iodine tincture rinse for 1 minute before opening of an access cavity (Teles and al, 2013). According to another study, NaOCl is inactivated with 5% sodium thiosulphate (Marinho and *al*, 2014). It has been reported that bacteria can be sampled from over 40% of the clinical surface in endodontic settings; out of these contaminations, only a small percentage (0.9%) pertains to putative endodontic pathogens such as *E. faecalis*. Nevertheless, the role of other non-pathogenic species in the event of cross-contamination has not been investigated. The increase in the level of contamination may be caused by airborne bacteria (aerosol spray from turbines, water, ambient air and compressed air by hoses connected to equipment) in surgeries that can easily contaminate the endodontic materials, especially when they are stored for a long time in the clinics (Abusrewil and al, 2020).

d. Individual Factors

The presence of bacteria within the endodontium leads to a local inflammatory reaction, but the products of this reaction are found in increased quantities in the circulating blood compared to patients without AP (Georgiou and al, 2019 *Cit in* Saucourt and al, 2021). This chronic inflammatory response may therefore have a systemic impact and many studies try to show a link with various general pathologies such as diabetes, cardiovascular or liver diseases (Aminoshariae and al, 2018 *Cit in* Saucourt and al, 2021). Chronic inflammation seems to imply a bidirectional link with these pathologies, where a deterioration of one leads to a decrease in the prognosis of the other. Thus, there is a longer time to bone remineralisation after endodontic treatment and a poorer prognosis as well as a higher prevalence of AP in diabetics, in primary and secondary infection (Saleh et al, 2020 *Cit in* Saucourt et al, 2021). However, the existence of many confounding factors and common risk factors (smoking, compliance with dietary hygiene rules, etc.) makes this causal link difficult to establish (Jakovljevic et al, 2020, Nagendrabadu et al, 2020 and Segura et al, 2015 *Cit in* Saucourt et al, 2021).

3. How to Prevent?

a. Culture Technique

For analysis in root filled teeth, the apical root specimen can be obtained by root-end resection during endodontic surgery or tooth extraction (Fouad, 2017). To study the collected samples different techniques are possible. Next-generation sequencing (NGS) has been used for profiling endodontic microbial communities (Fouad, 2017). However, this has created more complexity, revealing the need to utilize relevant bioinformatic tools to interrogate the resultant data. These analyses are time-consuming and expensive and are currently reserved for research, with no clinical application. It is important to recognize that NGS alone provides merely a list of implicated microorganisms (Abusrewil and al, 2020).

There is a vast array of deeper understanding, such as metatranscriptomics, proteomics and metabolomics (Abusrewil and al, 2020). The significant breakthrough in the characterization of the endodontic microbiome came with the use of PCR based approaches, which enabled the detection of the uncultivable microbiota. MALDI-TOF MS was used to identify the bacterial colonies isolated from culturing the samples of the tested materials (Saeed et al, 2017). Fluorescence *in situ* hybridization (FISH) would be ideal to reveal the species architecture and arrangements in multispecies biofilms (Siqueira and Rôças, 2021). Last, but not least, microbial shifts are associated with changes of the immune response in the RCS and periapical region. Endodontic infection demonstrates significant microbial diversity, and the properties of such complex biofilms cannot be observed in studies with one species alone; therefore, it must build appropriate multispecies models that reflect with at least some degree of accuracy the clinical situation (Abusrewil, 2020; Neelakantan and al, 2017).

b. Instrumentation, Irrigation and Filling

Despite all the techniques developed, total disinfection of the RCS is a utopia. Therefore, the achievable goal is to reduce bacterial populations to a level below that necessary to induce or maintain disease (Abusrewil and al, 2020). In all cases, where viable bacteria remain in RCS, there is a constant risk that they may perpetuate periradicular inflammation (Siqueira, 2001). If the root canal filling fails to provide a complete seal, seepage of tissue fluids can provide substrate for bacterial growth (Siqueira, 2001). Based on the study of the X-ray images, approximately 30% of the treatments presented at least one major defect (under-filling > 2 mm, sealing vacuities, absence of sealing opacity); therefore, were considered incorrect (Saint-Pierre and al, 2008).

Before disinfection, preparation of the RCS is essential. Root canal instrumentation with Nickel-titanium (NiTi) instruments using NaOCl as the irrigant, show that 60% of the cases still had detectable bacteria (Rôças and Siqueira, 2013). NiTi rotary instrumentation (Roças and al, 2013 *Cit in* Fouad, 2017) and the self-adjusting file (Neves and al, 2014 *Cit in* Fouad 2017) were shown to be more efficacious than hand NiTi instruments. In addition, latter, it was reshown that rotary NiTi instrumentation was equivalent from a microbiological perspective to instrumentation activated by reciprocation (Neves and al, 2016 *Cit in* Fouad, 2017). Root canal preparation with rotary instruments in the full extension of the root canal was capable to achieve a removal of 90% of the LPS content against a removal of 50 % using hand K-files (Marinho and *al*, 2014), which are detected in 100% of necrotic pulp, with significantly higher levels in symptomatic teeth (Gomes and Herrera, 2018).

In spite of development of “space” for irrigant placement, penetration is impaired by many factors. The needle does not always go deep enough into the RCS (Ram 1977 *Cit in* Abusrewil and al, 2020) and vapour lock effect that forms when air entrapment occurs ahead of the irrigant within the limited and closed RCS (Tay et al. 2010 *Cit in* Abusrewil and al, 2020). A flexible polymer needle rather than a metallic one can increase the penetration of the irrigant (Siquet and al, 2021 *Cit in* Abusrewil and al, 2020). Improvement was seen when the needle tip was closer to the apex, when the irrigation volume was greater, and when the needle gauge was smaller. However, this increases the risk of apical extrusion, slow irrigant delivery in combination with continuous hand movement will minimize NaOCl accidents (Gu and al, 2009). Currently, NaOCl and *Ethylenediaminetetraacetic Acid* (EDTA) are the most commonly used irrigants for eradication of the biofilm and disruption of inorganic material resulting from canal instrumentation. No differences were found among the irrigants use (Marinho and *al*, 2014). NaOCl may be associated with several risks such as tissue toxicity, emphysema, allergy and undesirable taste and smell (Mohammadi 2008 *Cit in* Abusrewil and al, 2020). Irrigation can be achieved manually or by sonic or ultrasonic activation, photoactivated disinfection or laser/led activated irrigation (Gu et al. 2009; Nagendrababu et al. 2018 *Cit in* Abusrewil and al, 2020). The efficiency of the diode laser depends on its application time and the power of the laser from 2 W, the laser is more efficient than the NaoCl, with a temperature lower than 7° (safety threshold) (Zou and a, 2020). The results of the present study suggest that photo activated disinfection along with 3% NaOCl can be an alternative and better option for root canal disinfection (Sarda and al, 2019). It is widely accepted that irrigant activation via agitation results in greater microbial load reduction when compared to conventional needle irrigation (passive irrigation) (Van der Sluis et al. 2007; Mohmmed et al. 2017, 2018; Bryce et al. 2018; AlObaida et al. 2019 *Cit in* Abusrewil and al, 2020). But offers no benefits over needle irrigation in terms of healing rate of periapical tissue (Abusrewil and al, 2020).

One other study examined several sizes of apical preparation in infected canals. The results showed significant improvement in healing when preparation was performed to 3 file sizes larger than the initial file, and a dose-response improved healing for larger apical sizes (Saini and al, 2012 *Cit in* Fouad, 2017). It is assumed that the larger sizes created more disruption of apical bacterial biofilm, and elimination of more bacteria through disinfectant irrigation (Fouad, 2017). It has been reported that hand-held syringe needle irrigation is less effective when the canal is enlarged to less than size ISO 40 at the apex (Wu and Wesselink, 1995 and Falk and Sedgley *Sint in* Gu and al, 2009).

Conventionally used gutta-percha filling material has no antimicrobial activity (Sakko and al, 2016). For optimal filling, cement must also have an active role in maintaining asepsis. For example, EDTA, NaOCl or Mineral Trioxide Aggregated (MTA) allows to release dentin growth factors (Fouad 2017). Some epoxy resin based, polymethacrylate resin-based and calcium silicate-based sealers and MTA may have antimicrobial properties (Sakko and al, 2016). Overfilling should be prevented as often as possible since undesirable postoperative complications such as flare-ups can develop – usually when a large amount of filling material extrudes through the apical foramen (Siqueira, 2001).

c. Coronal sealing

The coronal restoration is essential to complete this seal (AAE 2009 *Cit in* Abusrewil and al, 2020). When living conditions change due to coronal leakage during or after root canal treatment, bacterial growth can occur (Korona-Glowniak and al, 2021), microorganisms may invade and recolonize the RCS (Siqueira, 2001). There are some situations in which obturated root canals may be contaminated from the oral cavity: leakage through the temporary or permanent restorative material; breakdown, fracture or loss of the temporary/permanent restoration; fracture of the tooth structure; recurrent decay exposing the root canal filling material; or delay in the placement of permanent restorations (Siqueira et al. 1999 *Cit in* Siqueira, 2001). If microbial cells and their products reach the periradicular tissues, they can induce and/or perpetuate periradicular disease (Siqueira, 2001).

Therefore, from a clinical standpoint, coronal exposure of the root canal obturation to saliva for a relatively short period of time (30 days or more) might be considered an indication for retreatment (Siqueira, 2001). It is therefore important to choose materials with antibacterial properties such as, for example, Quaternary Ammonium Monomers (QAMs). QAM-based dental materials such as resin composites, dental adhesives, glass ionomer cements (GICs), and resin-modified GICs, pit-and-fissure sealants, pulp capping materials, root canal sealers and acrylic resins exhibit “contact inhibition” effects on bacteria that contact their surfaces (Imazato and al, 2014 *Cit in* Jiao and al, 2019). The improved antimicrobial activities are attributed to the incorporation of more antibacterial resin monomers in the resin composites (18 wt%) and adhesives (10 wt%) (Cheng and al, 2012 and Antonucci and al, 2012 *Cit in* Jiao and al, 2019).

d. Antibiotics

Infection may remain localized, have a systemic impact without the bacteria having left the primary site of infection, but they may also translocate to cause focal infections and/or an inflammatory syndrome with a systemic response (Saucourt and al, 2021). Unless there are general signs or loco-regional spread, antibiotic prescription is meaningless in endodontics and will not provide analgesic relief. Antibiotic prescription can even lead to a modification of the phenotypes found within the endodontic tissue and thus favour the selection of resistant bacteria (Moraes and al, 2020 *Cit in* Saucourt and al, 2021). However, it can be justified as a complement to an anti-inflammatory prescription which would aim to reduce the symptomatology to avoid dissemination of the infection with the weakening of the inflammatory reaction linked to the prescription (Saucourt and al, 2021). Culture and antibiotic susceptibility testing of anaerobic bacteria provide results in about 7–14 days, which is usually too late (Rôças and Siqueira, 2013). Antibiotics are, therefore, in most cases, prescribed based on the empiric knowledge of endodontic infections (Segura-Egea JJ and al, 2017). However, situations like abscesses rapidly disseminating to facial and/or neck anatomic spaces may require rapid diagnosis (Rôças and Siqueira, 2013). Most cases positive for antibiotic resistance genes were rendered negative after chemomechanical debridement. This confirms that endodontic treatment is effective in eliminating a possible reservoir of antibiotic resistance gene in the majority of cases. However, in about 30% of the previously positive cases, resistance genes were still detected (Rôças and Siqueira, 2013).

Antibiotics should be considered in patients having systemic diseases with compromised immunity or in patients with a localized congenital or acquired altered defence capacity (Segura-Egea JJ and al, 2017). Penicillin v potassium, possibly combined with metronidazole to cover anaerobic strains, is still effective in most cases. Amoxicillin (alone or together with clavulanic acid) is recommended because of better absorption and lower risk of side effects. In case of confirmed penicillin allergy, lincosamides, such as clindamycin and erythromycin, are the drug of choice (Segura-Egea JJ and al, 2017). Metronidazole has been suggested as a supplemental medication for amoxicillin (AAE, 1999 *Cit in* Segura-Egea and al 2017)[1] because of its excellent activity against anaerobes. However, high doses of clindamycin increase the probability of serious side effects, such as pseudomembranous colitis (Trexler and al, 1997 *Cit in* Segura-Egea and al 2017) and neutropenia (Bubalo and al, 2003 *Cit in* Segura-Egea and al 2017).

III- DISCUSSION

1. Ability of Microbiology

Despite the progress, only about half of oral bacteria are cultivable. The oral cavity and the endodontic environment contain many microorganisms that are not cultivable but may contribute to a significant degree in the pathogenesis of disease and resistance to treatment. Moreover, some bacteria that are ordinarily readily cultivable in the oral environment may be rendered uncultivable despite being viable if the environment contains materials or conditions that interfere with growth in the laboratory (Fouad, 2002 *Cit in* Fouad, 2017). Metagenomic approaches are unable to determine viability unless appropriate methodologies are applied. Therefore, unless studies are designed to exclude sequencing from dead bacteria or fungi then it is possible for example that our understanding of the microbiome in post-treatment disease may be founded on erroneous data (Abusrewil and al, 2020). Presence of bacteria does not always mean treatment failure. It has been found that some periapical lesions can heal despite the presence of bacteria in the RCS at the time of obturation. To maintain the infection, the presence of bacteria alone is not enough (Abusrewil and al, 2020).

For more clinical reality, chairside tests can be developed. They could predict the treatment outcome or the risk of flare-ups and other complications. Treatment times may be reduced, or numbers of visits minimized and give a green light for filling (Abusrewil and al, 2020). With the advances in molecular microbiologic diagnosis, rapid, sensitive and accurate assays are now available to identify bacteria and other microorganisms in a matter of minutes to a few hours. The tests that can be used are autofluorescence (provides results in less than 10 minutes) or Adenosine Triphosphate detection (results in 5 minutes) (Abusrewil and al, 2020). In cases of disseminating and complicated abscess infection of endodontic origin, the clinician and the patient might benefit from a rapid microbiologic diagnosis to adhere to the best therapy. However, they have yet to be introduced in the clinical routine, and there is a need for them to be validated by long-term longitudinal follow-up studies showing a correlation between the rapid chairside results and treatment outcome (Siqueira and Rôças, 2021). Rapid detection of antibiotic resistance genes can also guide the prescription of the best antibiotic for each individual case (Fouad, 2017).

2. New Research

New research focuses on the possibility of improving the techniques used daily at all steps of the protocols. For example with regard to irrigation: the major disadvantage of EDTA comes from its annihilation power of NaOCl. Recently, a new chelating agent has been proposed: etidronic acid, which has the advantage of being compatible with NaOCl (Siquet and al, 2021). These findings have stimulated searches for alternatives. Amongst the large number of the tested products, only few showed antimicrobial efficacy comparable to that of NaOCl, but none was superior for exemple Propolis (bee glue). The studies concluded that propolis was as effective as NaOCl against C. albicans but less effective than Chlorhexidine (CHX) (Carbajal Mejia 2014; Mattigatti and al. 2012 *Cit in* Abusrewil and al, 2020).

The antimicrobial effects can be enhanced by incorporating different compounds into it. For instance, it was shown that a combination of intracanal medicaments of Calcium Hydroxide ($Ca(OH)_2$) and CHX (Sinha and al. 2013 *Cit in* Siquet and al, 2021), a mixture of Chitosan nanoparticles (CN) and $Ca(OH)_2$ paste (del Carpio-Perochena et al. 2017 *Cit in* Siquet and al, 2021) and a combination of $Ca(OH)_2$ and silver nanoparticles (AgNP) (Afkhami and al. 2015 *Cit in* Siquet and al, 2021) produce additive antimicrobial effects to the $Ca(OH)_2$ alone. $Ca(OH)_2$ has been shown to be ineffective against biofilms of E. fecalis even after 24 hours of treatment (Upadya and al, 20172017 *Cit in* Neelakantan and al, 2017). While this lack of effectiveness was also true for multi-species biofilms, addition CN to $Ca(OH)_2$ appears to enhance the bacterial killing in a multi-species model over a 7 and 14 day period (Del Carpio-Perochena and al, 2017 *Cit in* Neelakantan and al, 2017). Nevertheless, it is unknown if this strategy per se can remove biofilms from within the radicular space. Furthermore, research is lacking in terms of the ability of intracanal medicaments to penetrate the EPS matrix of biofilms (Neelakantan and al, 2017).

CN are biopolymers that results from deacetylation of naturally occurring chitin. They have attributed to its high antibacterial, antifungal and antiviral properties with less tendencies to induce microbial resistance compared with antibiotics (Rabea and al, 2003 *Cit in* Abusrewil and al, 2020). The rate of killing by nanoparticles is dependent on the concentration and treatment time. A maximum reduction in bacterial adhesion (97%) was achieved when radicular dentine treated with CHX was followed by nanoparticle treatment (Kishen and al, 2008 *Cit in* Abusrewil and al, 2020). The ability of CN to remove smear layer was comparable of that of EDTA (Silva and al. 2012; Silva and al. 2013; del Carpio-Perochena and al. 2015 *Cit in* Abusrewil and al, 2020). It has been reported that photosensitizer functionalized bioactive nanoparticles resulted in significant elimination and higher penetration of a multispecies biofilm (Shrestha and Kishen 2014 *Cit in* Abusrewil and al, 2020). Similar findings against a multispecies biofilm were found when CN were mixed with $Ca(OH)_2$ (Carpio-Perochena and al. 2017 *Cit in* Abusrewil and al, 2020). It is clear that although CN show promise, their true value may lie, not as a replacement for existing endodontic disinfectants, but as a supplement to what is currently used, either used simultaneously or sequentially (Table 1) (Abusrewil and al, 2020).

To reach the bacteria that are still resistant, it can be used pre and probiotics. Probiotics are micro-organisms that will inhibit the growth of potentially pathogenic bacteria and/or modulate the mucosal immune response within the microbiome, thus reducing gingival inflammation, plaque formation and alveolar bone loss (Agnello and al, 2017 *Cit in* Prêcheur and al, 2021). An in vitro study showed the inhibitory power of *Lactobacilli* and *Bifidobacterium* on *Enterococcus faecalis* and *Candida albicans* (Bohora and al, 2019 *Cit in* Siquet and al, 2021). Probiotics can be carried by nanoparticles, and would inhibit microorganisms that remain unaffected by the chemo-mechanical preparation (Kumar and al, 2021 *Cit in* Siquet and al, 2021). Prebiotics are dietary substances that promote the establishment of a eubiotic microbiota. For example, arginine has been shown to prevent caries disease by neutralising the acids produced by cariogenic bacteria and promoting the growth of microorganisms compatible with a eubiotic microbiota (Jayaram and al, 2016 *Cit in* Prêcheur and al, 2021).

The use of anti-microbial peptides (AMPs) seems to be also one such promising avenue. AMPs are defence biomolecules produced in response to infection (Lima and al, 2015), synthesised by bacteria in order to kill or limit the growth of other prokaryotes in the same environment (Morales and al, 2018, Oldak and Zielinska, 2017 *Cit in* Prêcheur and al, 2021). One of the advantages of these AMPs is their broad spectrum of activity and some molecules could in the future become a new generation of antibiotics (Prêcheur and al, 2021). There are a considerable number of AMPs that exhibit excellent antimicrobial activity against endodontic microbiota at a small inhibitory concentration and modulate an exacerbated immune response, down-regulating bone resorption. All these reasons indicate AMPs endodontic treatment as an emerging and promising option (Lima and al, 2015).

3. Protocol Update

The Cochrane database of systematic reviews found no evidence to suggest that one treatment regimen (one-visit or multiple-visit) is better than other (Siquet and al, 2021, Saint-Pierre and al, 2008). The healing of AP may be better with interappointment $Ca(OH)_2$ medication (Wang and al, 2015 *Cit in* Sakko and al,2016). In fact, "neither can prevent 100% of short- and long-term complications." (Abusrewil and al, 2020). The use of combined antimicrobial techniques is, therefore, an additional guarantee of the resolution of AP. Under the term antimicrobial techniques, it may include: antimicrobial enzymes (AMEs), AMPs, Cationic compounds (CN /CHX /QAMs), Metal and metal oxides (Ag NPs), other non-cationic compounds (nitric oxides (NO) donors), Natural products (tea), Amino acids (Argine), Antioxidants (N-acetylcysteine (NAC). However, its usage must be carefully monotorated as they present disadvantages like reservoir depletion problems, short duration of action and surface biofouling, among others (Jiao and al, 2019). Performing NSRCT with the combination of antimicrobial techniques and standard irrigation protocol increased the treatment success. Moreover, it is necessary to improve antibiotic-prescribing habits in the treatment of endodontic infections, as well as to introduce educational initiatives to encourage the coherent and proper use of antibiotics (Segura-Egea and al 2017).

It was concluded that the best available clinical evidence does not support the prescription of antibiotics for treatment of endodontic diseases unless the spread of infection is systemic, the patient is febrile, or both (Aminoshariae and Kulild, 2016 *Cit in* Segura-Egea and al 2017).

Using antibiotics in a healthy individual may contribute to the global antibiotic-resistance problem (AAE, 1999 *Cit in* Segura-Egea and al 2017). Penicillin has two main drawbacks: its poor absorption from the intestinal tract, meaning that more than 50% of an oral dose is wasted; and its short-acting effect, with half of the amount circulating being removed every half hour (US NLM, 2006 *Cit in* Segura-Egea and al 2017).

In addition to the use of the combination of different microbial agents, an additional or alternative tool to the antibiotic can be added to the treatment protocol, Cold plama (CP) with argon/oxygen (Ar 98%/O2 2%). Its use has been compared to that of $Ca(OH)_2$ and CHX gel 2% using a CP flow of 5 L/min, at a distance of 10mm. Results show there were no detectable live bacteria after 8 minutes (Jiao and al, 2019) to 12 minutes (Li and al, 2015) of CP treatment. Atmospheric pressure CP is an effective therapy in endodontics for its strong sterilization effect within a few minutes, it reduce 94% of the regular bacterial skin flora (Lademann and al, 2011 *Cit in* Jiao and al, 2019) and significantly reduced bacterial load in chronic wounds (Isbary and al, 2012 *Cit in* Jiao and al, 2019). After CP treatment, 15 mL sterile normal saline was injected into the RCS (Li and al, 2015). Although there is great potential for CP to be adopted for clinical dental applications, several potential barriers to its clinical translation have been brought out: as the effects of gas phase interactions; a more precise definition of the plasma dose; tissue-specific effects on the flux of species delivered; and modulation of the host's immune responses (Pei and al, 2018 *Cit in* Jiao and al, 2019).

IV. CONCLUSIONS

To prevent the occurrence of post treatment AP review clinical protocol is necessary. This requires establishing a better education for asepsis protocol in order to prevent exogenous contamination. It would be interesting to develop chairside test in clinical practice to increase the quality of disinfection. While using complementary tools for root canal disinfection such as ultrasound, laser, led, CN, CP in addition to the classical irrigation protocol. The chairside test will allow us to know when to stop the disinfection. Therefore we will have less lost of time in consultation and a NRCT more efficient. Despite many promising techniques, it would still be interesting to carry out other comparative studies to obtain an international consensus. It is only by starting from this healthy base that the correct filling at apical and coronal levels can guarantee better prevention of the occurrence of post-treatment AP.

ANNEX

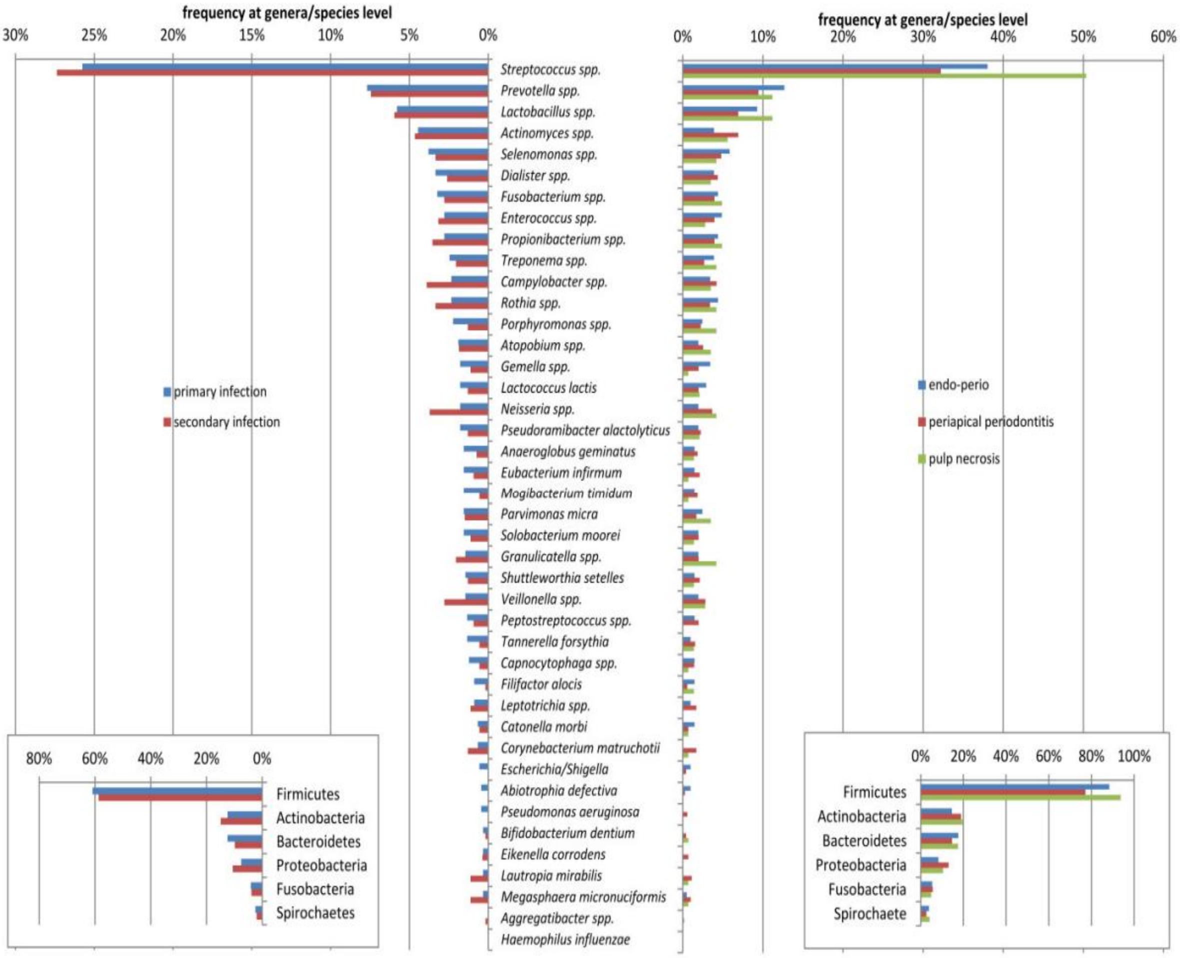

Figure 1: The distribution of microorganisms in the root canal samples from patients with primary and secondary endodontic infections and with different entities of apical periodontitis obtained by molecular methods (Korona-Glowniak and al, 2021).

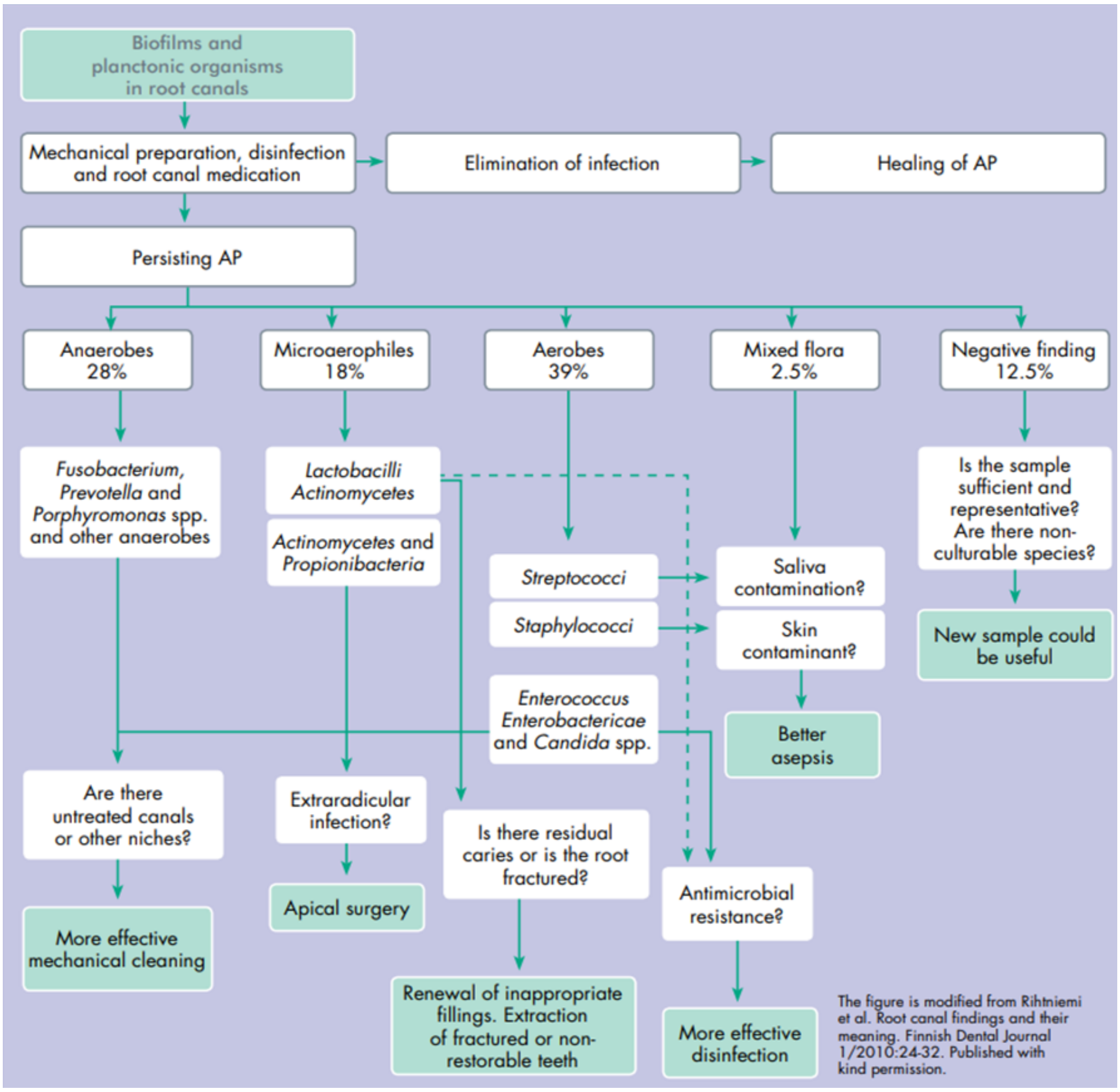

Figure 2: Root canal microbiological findings and what they mean (Sakko and al, 2016).

Antimicrobial Agent	Chemical Type	Concentration Used/Recommended in Root Canal Disinfection	Activity on Bacterial Suspensions (Root Canal Models Only)	ACTIVITY on Mono-Species or Multi-Species Biofilms (Endodontic Taxa Only)
Sodium hypochlorite (NaOCl)	Halogen releasing agent	1–6%	Yes	Yes
Chlorhexidine (CHX)	Bisbiguanide	2%	Yes	Unclear
Alexidine (ALX)	Bisbiguanide	1–2%	Yes	Unclear
Octenidine (OCT)	Bisbiguanide		Yes	Unclear
Iodine Potassium Iodide (IKI)	Halogen releasing agent	2–5%	Yes	Insufficient evidence
Ethylene diamine tetraacetic acid (EDTA)	Polyprotic acid	15–17%	No	No
Maleic acid	Diprotic acid	7%	Yes	Insufficient evidence
Peracetic acid	Organic peroxide	2.25%	Yes	Yes
MTAD	Mixture of antibiotic, organic acid (citric acid), detergent		Yes	Unclear
QMix	Mixture of CHX and EDTA		Yes	Yes
Etidronic acid (with 6% NaOCl)	Bis-phosphonate	18%	Yes	Yes
Curcumin	Phyto-polylphenol	—	Yes	Yes
Chitosan with Rose Bengal	Polysaccharide with photosensitiser		Yes	Yes
Silver nanoparticles	Metallic nanoparticle		Yes	Yes
Trypsin and Proteinase K	Enzymes	1%	Yes	Yes (Trypsin)
D-leucine	Amino acid		Yes	Yes

Table 1: Summary of effect of commonly used root canal disinfectants on bacterial suspensions or biofilms in an endodontic disinfection model (Neelakantan and al, 2017).

Note: The term “unclear” has been used when methods other than confocal laser scanning microscopy have been used to detect the effect on biofilms.

REFERENCES

Abusrewil, and al. (2020). *Detection, treatment and prevention of endodontic biofilm infections: what's new in 2020?, Critical Reviews in Microbiology*.

Fouad, F. A. DDS, MS (2017). *Endodontic Microbiology and pathobiolofy Current State of Knowledge*, Dent Clin N Am, 61, pp.1-15.

Gomes, B.P.F. de A. and Herrera, D.R. (2018). *Etiologic role of root canal infection in apical periodontitis and its relationship with clinical symptomatology*, Brazilian Oral Research, 32(suppl 1).

Gu, L. and al. (2009). *Review of Contemporary Irrigant Agitation Techniques and Devices*, Journal of Endodontics, 35(6), pp. 791–804.

Hong, B.-Y. and al. (2013). *Microbial Analysis in Primary and Persistent Endodontic Infections by Using Pyrosequencing*, Journal of Endodontics, 39(9), pp. 1136–1140.

Jiao, Y. and al. (2019). *Advancing antimicrobial strategies for managing oral biofilm infections, International Journal of Oral Science,* 11(3), p. 28.

Karamifar, K. (2020). *Endodontic Periapical Lesion: An Overview on Etiology, Diagnosis and Current Treatment Modalities,* European Endodontic Journal.

Korona-Glowniak and al. (2021) *A. Patterns of Oral Microbiota in Patients with Apical Periodontitis.* J. Clin. Med. 10, 2707.

Lasfargues J-J and Machtou P. (2001). Pathogenèse des lésions périapicales. Réalités cliniques, 12(2), pp. 139-48.

Li, Y. and al. (2015). *Evaluation of Cold Plasma Treatment and Safety in Disinfecting 3-week Root Canal Enterococcus faecalis Biofilm In Vitro,* Journal of Endodontics, 41(8), pp. 1325–1330.

Lima, S.M. de F. and *al.* (2015). *Antimicrobial peptide-based treatment for endodontic infections — Biotechnological innovation in endodontics,* Biotechnology Advances, 33(1), pp. 203–213.

Mareschi, P. Taschieri, S. and Corbella, S. (2020*). Long-Term Follow-Up of Nonsurgical Endodontic Treatments Performed by One Specialist: A Retrospective Cohort Study about Tooth Survival and Treatment Success, International Journal of Dentistry. Edited* by T. Lombardi, 2020, pp. 1–8.

Marinho, A.C.S. and al. (2014). *Monitoring the effectiveness of root canal procedures on endotoxin levels found in teeth with chronic apical periodontitis,* Journal of Applied Oral Science, 22(6), pp. 490–495.

Neelakantan, P.and al. (2017). *Biofilms in Endodontics—Current Status and Future Directions,* International Journal of Molecular Sciences, 18(8), p. 1748.

Ørstavik, D.(2020). *Essential endodontology: prevention and treatment of apical periodontitis*. 3rd edition. Hoboken, NJ: Wiley-Blackwell, p.243

Pereira R. S. and al. (2017). *Microbial analysis of root canal and periradicular lesion associated to teeth with endodontic failure, Anaerobe.*

Prêcheur Isabelle, Blasco-Baque Vincent, Roger-Leroi Valérie. (2021). *Microbiote buccal : quelles avancées peuvent modifier les pratiques de soins du chirurgien-dentiste ?,* L'Information Dentaire, 26, pp. 64-71.

Rôças, I.N, José F. Siqueira Jr. (2013). *Detection of antibiotic resistance genes in samples from acute and chronic endodontic infections and after treatment, Department of Endodontics and Molecular Microbiology Laboratory,* Faculty of Dentistry, Estácio de Sá University, Rio de Janeiro, RJ, Brazil.

Saeed and al. (2017). Bacterial *Contamination of Endodontic Materials before and after,* Clinical Storage Media.

Saint-Pierre, F. and al. (2008). *Service évaluation des actes professionnels,* Haute Autorité de Santé, p. 46.

Sakko, M., Tjaderhane, L. and Rautemaa-Richardson, R. (2016). *Microbiology of Root Canal Infections,* Primary Dental Journal, 5(2), pp. 84–89.

Sarda, R.A. and al. (2019). *Antimicrobial efficacy of photodynamic therapy, diode laser, and sodium hypochlorite and their combinations on endodontic pathogens, Photodiagnosis and Photodynamic Therapy*, 28, pp. 265–272.

Saucourt, M. Pelletier, P. Diemer, F. (2021). Physiopathologie et impact systémique du microbiote endodontique, L'Information Dentaire, 26, pp. 26-30.

Segura-Egea J. J. and al. (2017). *Antibiotics in Endodontics: a review.* International Endodontic Journal, 50, 1169–1184.

Segura-Egea, J.J. and al. (2017). *Worldwide pattern of antibiotic prescription in endodontic infections,* International Dental Journal, 67(4), pp. 197–205.

Siqueira Jr, J. F. (2001). Ae*tiology of root canal treatment failure: why well-treated teeth can fail,* International Endodontic Journal, 34(1), pp. 1–10.

Siqueira, J.F. Jr. and Rôças, I.N. (2021). *Present status and future directions: Microbiology of endodontic infections.* International Endodontic Journal, 00, pp. 1–19.

Siqueira, J.F. Jr. & Rôças, I.N. (2022) *A critical analysis of research methods and experimental models to study the root canal microbiome. International Endodontic Journal.*

Siquet J.P. and al. (2021). La gestion clinique du microbiote intracanalaire dans les pathologies endodontiques, L'Information Dentaire 26, pp. 32-37.

Swimberghe, R.C.D. and al. (2019). *Biofilm model systems for root canal disinfection: a literature review,* International Endodontic Journal, 52(5), pp. 604–628.

Teles AM and al. (2013). *Analysis of Endodontic Pathogens by Classical Microbiological and Nucleic Acid Approaches.* Abstracts of the ESE Wladimir Adlivankine Research Prize and Original Scientific Posters, pp. 82-83.

Zehnder M. and Belibasakis G. N. (2015). *On the dynamics of root canal infections—what we understand and what we don't*, Virulence, 6:3, pp. 216-222.

Zou, Z. and al, (2021). *Effect of using diode laser on Enterococcus faecalis and its lipoteichoic acid (LTA) in chronic apical periodontitis, Lasers in Medical Science*, 36(5), pp. 1059–1066.