



Clinical implications of *Enterococcus faecalis* microbial contamination in root canals of devitalized teeth: Literature review

Implicancias clínicas de la contaminación microbiana por Enterococcus faecalis en canales radiculares de dientes desvitalizados: Revisión de la literatura

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ABSTRACT

Dental caries is a highly prevalent disease in the world. Endodontic treatment is an option to treat teeth widely destroyed by dental caries. Even though this type of therapy offers favorable prognosis in most cases, scientific literature suggests there is a possibility for failure. In endodontic treatments, one of the most prevalent causes for treatment failure is permanence of microorganisms within root canals. Among the numerous existing bacterial species, *Enterococcus faecalis* is one of the most frequently found in teeth with pulp necrosis (lacking previous history of endodontic treatment). It is equally the most frequently isolated species in teeth with infection recurrence (teeth exhibiting evidence of recurrent treatment). To achieve successful endodontic treatment in devitalized teeth it is of the utmost importance to study and be aware of endodontic microbiology. This leads then to the need of studying bacterial behavior within the system of root canals as well as their resistance and defense mechanisms in order to best manage their interaction with the tooth and achieve bacterial obliteration. The present article purports to offer a bibliographic review of existing literature on *Enterococcus faecalis*, its main characteristics as well as resistance mechanisms. Bibliographic search was conducted through electronic databases (EBSCO, Cochcrane, Medline and Lilacs), using search engines (Pubmed and Decs). Included articles were written in Spanish and English and published during the last 30 years. Out of a total 52 articles, 26 were selected; they corresponded to experimental studies and literature reviews.

RESUMEN

La caries dental es una enfermedad altamente prevalente en el mundo. Una opción para tratar dientes ampliamente destruidos por caries dental es la realización de tratamientos de endodoncia. Aun cuando estas terapias tienen un pronóstico favorable en la mayoría de los casos, la literatura señala que existe la posibilidad de fracaso. Una de las principales causas de fracaso en los tratamientos de endodoncia es la permanencia de microorganismos en los canales radiculares. Dentro de las numerosas especies bacterianas existentes, una de las más frecuentemente encontradas en dientes con necrosis pulpar (sin historia previa de endodoncia) y la más aislada en aquellos con recidiva de infección (dientes con indicación de re-tratamiento) es *Enterococcus faecalis*. Estudiar y conocer la microbiología endodóntica es requisito fundamental para lograr un tratamiento de endodoncia exitoso en dientes desvitalizados. Surge así la necesidad de conocer el comportamiento de las bacterias dentro del sistema de canales radiculares, así como sus mecanismos de defensa y resistencia, para manejar de la mejor manera posible su interacción con el diente y lograr su eliminación. El siguiente artículo corresponde a una revisión bibliográfica de la literatura existente acerca de *Enterococcus faecalis*, sus características principales y mecanismos de resistencia. La búsqueda bibliográfica se realizó a través de bases de datos electrónicas (EBSCO, Cochcrane, Medline y Lilacs), utilizando motores de búsqueda (Pubmed y Decs); se incluyeron artículos en inglés y español, publicados durante los últimos 30 años. De un total de 52 artículos, se seleccionaron 26, correspondientes a estudios experimentales y revisiones de literatura.

Key words: *Enterococcus faecalis*, endodontics, failed endodontic treatment, endodontic infection.

Palabras clave: *Enterococcus faecalis*, endodoncia, tratamiento de endodoncia fallido, infección endodóntica.

INTRODUCTION

In many countries, oral cavity diseases represent a public health issue. Dental caries is one the most prevalent diseases. In Chile the MINSAL (Chile's Health Ministry) established the fact that 66% of total population was afflicted with dental caries.¹⁻⁴ Endodontic treatment represents one option to treat teeth widely destroyed by this condition. Endodontic treatment's objective is to execute a meticulous

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chemical and mechanical cleansing of root canals to later achieve tri-dimensional seal with inert material and thus prevent re-infection. After this, rehabilitation of the dental crown is undertaken, thus dental caries containment and tooth loss prevention is finally achieved. Even though this kind of therapies, when suitably prescribed, exhibit favorable prognosis, scientific literature indicates there is a possibility of failure. One of the main causes of failure in endodontic treatments is incomplete removal of pulp tissue or micro-organisms present within the root canals. These micro-organisms might have survived biochemical procedures or have invaded root canals through crown filtration in teeth with filled roots.⁵ Several studies have revealed the fact that microbiota present in root canals of endodontically treated roots differ from microbiota normally found in untreated teeth.⁵ When analyzing the micro-organism factor, literature suggests that bacteria most frequently found in first time treatments, as well as treatments with infection recurrence, are limited to a very specific variety of micro-organisms, where gram positive anaerobic facultative species predominate, especially *Enterococcus faecalis*.⁵ This bacteria has been isolated within root canal systems as well as in periapical lesions. It has equally been found in infected teeth that had not been previously endodontically treated. Nevertheless, it is most frequently observed in teeth that have suffered recurrence, that is to say failure of previous endodontic treatment.⁵⁻⁹

MATERIALS AND METHODS

Bibliographic review was undertaken through internet search of different scientific articles collected from electronic databases such as EBSCO, Cochcrane, Medline and Lilacs, using electronic search engines such as Pubmed and Decs.

To undertake the search, the following key words were used: *Enterococcus faecalis*, endodontics, failed endodontic treatment, endodontic infection.

Experimental studies, written in Spanish and English as well as literature reviews published during the preceding 30 years (1985 to 2014) were used. The search was not limited to search by country or by type of study. Once the articles were downloaded, they were stored in pdf format to preserve their originality.

In order to proceed to selection, abstracts were reviewed, and when needed, full articles were examined, so as to decide whether their information was related (or unrelated) to the target of the present work.

After initial search, 52 articles were located, out of which 22 were discarded due to lack of relevance

to the target of this review. 26 articles were finally selected.

CAUSES FOR ENDODONTIC TREATMENT FAILURE

Bacteria and their by-products are considered primary etiological agents for pulp necrosis and periapical lesions. For this reason, their complete elimination is one of the most important steps of endodontic treatment.¹⁰

In most cases, treatment failure sets in when procedures undertaken within root canals do not achieve a satisfactory control and infection elimination. Incomplete removal of pulp tissue and microorganisms present in the root canal systems have been proposed as main causes for endodontic treatment failure.^{11,12} In many cases, full debridement of infected canals can be unattainable due to inaccessibility of infection sites, for example, when there is presence of accessory canals which are totally unreachable to instruments, medication or irrigants.¹³ In most cases, endodontic treatment failures are due to the activity of microorganisms which are surviving within the apical portion of root canal systems, even in well-treated teeth. It has been demonstrated that parts of the root canal frequently remain intact during the process of chemical and mechanical preparation, regardless of technique employed or instruments used. Areas untouched by instruments may contain bacteria or rest of necrotic tissue, even though root canals filling might appear radiographically adequate.¹⁴

Within the canal system, bacteria are found located in areas such as isthmus ramifications, deltas, canal irregularities and dentine tubules.¹⁴⁻¹⁶ In those cases when use of bio-mechanical instruments, irrigator materials or medication are unable to reach those sites, it is probable that nutrient supply for bacteriae placed therein remain unaltered after root therapy. Nevertheless, bacteria present in areas such as dentin tubules might have a drastically reduced substrate. In those anatomical regions, bacteria isolated by root filling normally die. Nevertheless, some bacterial species might survive for relatively long periods. Thus, in cases when root filling materials fail, and provide incomplete sealing, fluid filtration from the periapical tissue might provide a substrate suitable for bacterial growth.¹⁴ The same situation might take place in cases when there are filtrations from the crown.

Depending on the circumstances present in root canals, some bacteria might be more capable to survive and multiply than others. Thus, even though it is possible to find facultative anaerobes in teeth

with pulp necrosis, it is more frequent to find strict anaerobes in primarily infected canals (that is to say, without previous endodontic treatment and with necrotic pulp), and facultative anaerobes in cases of failed endodontic treatment.^{6,8} Microbial flora present in canals after endodontic treatment failure is limited to a small number of predominantly Gram-positive microbial species. Facultative anaerobes, especially *Enterococcus* spp, are the most frequently⁶ isolated species.^{10,13,14} Several studies have shown that frequency of *Enterococcus* species found in filled roots which exhibit apical periodontitis can even reach 70%.^{6,15-18}

ROLE OF *ENTEROCOCCUS FAECALIS* IN ENDODONTIC TREATMENT FAILURE

The gender *Enterococcus* corresponds to gram-positive, facultative anaerobe type coccus bacteriae; they are part of the habitual flora found in the mouth, human gastrointestinal tract and female genital tract. Moreover, they are a well-known cause of endodontic treatment failure as well as some systemic diseases such as urinary tract infections, surgical wounds infections, bacteremia and bacterial endocarditis.^{5,19} They have developed high resistance to antimicrobial agents and possess numerous virulence factors such as aggregation substances, surface protein, gelatinase, extracellular superoxide production, capsular polysaccharides and antibiotic resistance determinant. *Enterococcus* are recognized as potential human pathogens, causing 12% of all hospital infections. In recurrent endodontic infections, the *Enterococcus* species most frequently isolated is *Enterococcus faecalis*.⁸

The frequent presence of *Enterococcus faecalis* in root canals where endodontic treatment has failed suggests that this is an opportunist pathogen, whose persistence in the canals represents a significant therapeutic problem.

Once it is installed within the canals system, *Enterococcus faecalis* faces several challenges in order to ensure survival, including the ability to withstand the action of antimicrobial agents used during endodontic treatment as well as surviving lack of nutrients in canals which have been cleansed and filled.²⁰ Two potential causes emerge when analyzing the causes which lead to finding this bacteria in teeth which require secondary endodontic treatment. One indicates that *Enterococcus faecalis* possesses the ability to colonize and infect dentin tubules, this fact complicates its removal through chemical and

mechanical cleansing, given the small diameter of these anatomical structures,^{20,21} along with the ability exhibited by these bacteriae to bond to collagen.⁸ Another possible cause is the potential resistance these bacteria might possess against calcium hydroxide, which is the antibacterial medication most commonly used within the root canal system during endodontic therapy²² which would allow these micro-organisms to remain in quiescent state.^{7,8,13,20,21} *Enterococcus faecalis* persistence has been attributed to its ability to resist the high pH of calcium hydroxide, material which is frequently introduced in the canals, and preserved in that location for at least a week.²³ The resistance of this microorganism might be influenced by the effects of dentin plugging, so that pH increase cannot be achieved within dentin tubules¹³ where this bacteria might be lodged. Besides this fact, research on the possible causes which produce *Enterococcus faecalis* resistance to calcium hydroxide, suggests that the expression of certain genes of this bacteria as well as the operation of a proton bomb, play an important role in this phenomenon.^{13,20}

ENTEROCOCCUS FAECALIS RESISTANCE MECHANISMS TO ANTIMICROBIAL AGENTS

Antimicrobial activity of calcium hydroxide is based upon release of hydroxide ions²⁴ which are able to generate numerous alterations in the cell's aqueous environment and generate harmful changes in the membrane's structure, finally affecting the bacterial DNS. When the *Enterococcus faecalis* cells enter into their latency phase, they acquire greater resistance to the calcium hydroxide's antibacterial effect, through mechanisms which have not yet been fully ascertained. Even though it has been documented that *Enterococcus faecalis* can withstand the high pH of calcium hydroxide, relatively little is known about the survival mechanisms that enable it to tolerate such exposition. Curiously, the resistance to calcium hydroxide destruction exhibited by *Enterococcus faecalis* is shared with other microorganisms associated with endodontic treatment failures, such as species of *Candida* and *Actinomyces*.²⁰

The ability to survive in adverse circumstances is important for most bacteriae, due to the long starvation periods they normally experience. Several regulation systems play essential roles in the ability of bacteriae to withstand nutrient depletion. These systems are under the control of certain genes whose transcription is activated under starvation circumstances.¹⁴

In general when bacteriae are confronted to an adverse or potentially lethal agent, a stress response is activated which allows them to withstand the threat, survive and recover.^{25,26} When this response is elicited, said status can confer general protection against a variety of other stress factors, for example, a stress response triggered by lack of nutrients can afford protection against heat exposition. *Enterococcus faecalis* is able to synthesize a wide variety of proteins when exposed to adverse environmental circumstances, such as an environment with high pH or exposure to sodium hypochlorite (irrigator material used in endodontic procedures). If nutrient depletion, exposure to sodium hypochlorite solution or calcium hydroxide induce *Enterococcus faecalis* to trigger a stress response, then said response could confer crossed protection for this bacteria at later exposures, for example, exposure to a new medication with calcium hydroxide;²⁰ thus this mechanism could partly explain its resistance.

Studies have shown that at a pH level of 11.5 or over, *Enterococcus faecalis* cannot survive, nevertheless it can survive at lesser concentrations.^{20,27} Due to the dentin's buffer effect, it is unlikely that the high pH of calcium hydroxide reaches dentin tubules, location where *Enterococcus faecalis* has the ability to deeply penetrate into.²⁸ Although pH value of calcium hydroxide pastes used in endodontic treatments is 12.3, in the root dentin achieved alkalinity does not exceed 10.3 pH. After canals are covered with calcium hydroxide²¹ in the root canal system, this value can fall to even pH 8.5-9.0 due to the dentin plugging effect. This value is not sufficiently high to eradicate *Enterococcus faecalis*.²⁹ Furthermore, since endodontic treatment generally includes alternate use of drugs at the different stages of root instrumentation procedures. *Enterococcus faecalis* is frequently exposed to a sub-lethal alkaline pH and this could induce bacterial cells to generate a stress response that might improve survival. Thus, repeated exposure of *Enterococcus faecalis* to calcium hydroxide and sodium hypochloride solution could trigger resistance mechanisms at subsequent exposures, even at levels which could be lethal.²⁰

In addition to the adaptive response in an alkaline pH and stress-induced protein synthesis, in these bacteriae a proton bomb has also been described, with the ability to acidify cytoplasm. This mechanism would be vital for the survival of *Enterococcus faecalis* at high pH levels, and could be considered more important than the aforementioned adaptive mechanisms.²⁰ The operating mechanism of this proton bomb basically

consists on a bacterial response to penetration of hydroxyl ions into the bacterial cytoplasm, which could increase intracellular pH. In that situation, the proton bomb is activated and responds sending (positively charged) potassium ions to the bacterial cytoplasm, thus achieving their acidification and preventing enzymatic inhibition.²⁹

Microorganisms remaining within root canals can die or remain viable depending on the nutrient availability in the canal system and the ability to survive in nutrient-depleted circumstances. In those cases when they remain viable, their proliferation can be inhibited or reduced. In spite of aforementioned resistance mechanisms, failure of endodontic treatment attributed to residual microorganisms will only take place in cases when they possess pathogenicity, reach sufficient numbers and have access to periapical tissues in order to induce or preserve the disease around the root.¹⁴ Furthermore, variation of resistance to alkaline pH can be related to existence of variable genotypes among *Enterococcus faecalis* strains, that is to say, genetic variations of *Enterococcus faecalis* strains can take place in the time they generate phenotypes of greater resistance to alkaline pH.³⁰

CONCLUSIONS

The fact of frequently finding *Enterococcus faecalis* in the root canals of teeth having received failed endodontic procedures means that this species is intimately involved in the pathogenesis and persistence of apical periodontitis. Thus, this bacteria is frequently isolated in cases of primary and secondary endodontic infections. It is thus necessary to conduct further research which might allow to lead to the preparation of more efficient antimicrobial agents to be used in endodontic treatments.

It is important to point out that *Enterococcus faecalis*'s survival in root canals is due to the fact that irrigator materials or drugs used during the instrument-phase of the treatment are not able to fully reach the canal system, or the fact that root filling might not achieve full tri-dimensional seal, leaving thus spaces where these microorganisms might hide and survive, this would be the case of dentin tubules. Therefore in addition to finding better and more effective endodontic drugs, emphasis must be placed on searching for more efficient and tri-dimensionally effective filling systems.

An important point to assess would be the clinical importance of *Enterococcus faecalis* antibacterial resistance mechanisms. Is has been

documented that this bacteria is resistant to the antimicrobial effect of several drugs, including calcium hydroxide. Therefore, when *Enterococcus faecalis* is established within the canal system its eradication with conventional means can be extremely difficult. Thus, it would be interesting to further the search for new drugs that might be used by themselves, or associated to calcium hydroxide, or else determine minimal inhibitory concentrations of different drugs so as to achieve improved clinical results. It is worth mentioning that in order to prevent microbial re-infection of the canal system, how important it is to disinfect root canals and completely fill them as well as to place a suitable crown seal immediately after endodontic treatment, and its timely substitution for a permanent restoration.

Although it has been proven that *Enterococcus faecalis* is present in most cases of infected canals, (mainly those which have been endodontically re-treated) its role in the pathogenesis of periapical diseases associated to necrotic pulp or endodontic failure has not yet been clearly established. Even though *Enterococcus faecalis* might possess the ability to resist medication used in endodontic treatments, the possibility that some strains are more resistant than others or might be better adapted to surviving in extreme circumstances must not be overlooked. Furthermore, identification of genes expressed by cells of this bacteria during the stationary phase could further the development of treatment protocols which might permanently eradicate *Enterococcus faecalis* from infected root canals.

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