

Antibiotic Resistance of *Enterococcus faecalis* from Persistent Endodontic Infections

Resistencia Antibiótica de *Enterococcus faecalis*
Provenientes de Infecciones Endodónticas Persistentes

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ABSTRACT: Enterococci are important nosocomial pathogens due to their intrinsic multiresistance and the acquisition of new antibiotic resistance genes (ARG). *Enterococcus faecalis* has been shown to be one of the main pathogens in persistent endodontic infections, therefore, the main objective of this study was to evaluate the phenotype and resistance genotype of strains of *E. faecalis* isolated from teeth with persistent endodontic lesions, to the most commonly prescribed antibiotics in dentistry. Thirteen strains of *E. faecalis* of different pulsotype were analyzed to evaluate the susceptibility to antibiotics, amoxicillin, amoxicillin/clavulanic acid, tetracycline, erythromycin and metronidazole, using the Epsilon test (E- test) and the presence of beta-lactamases with nitrocefin test. Finally, the detection of ARG was performed with a molecular polymerase chain reaction (PCR) technique and confirmed by the sequencing of the amplification products. Fisher's exact test was used, using 95 % confidence. Regarding the phenotype of resistance, the evaluated strains, independent of the pulsotype, were totally resistant to the action of metronidazole. Antibiotics with higher minimum inhibitory concentration (MIC) after metronidazole include tetracycline and erythromycin. In contrast, lower MIC are applied to the combination of amoxicillin with clavulanic acid. The nitrocefin test was positive only in one strain. Genotypically, two genetically distant strains isolated from a single patient, presented a genotype of resistance to erythromycin, determined by the presence of the *ermB* gene. No statistically significant relationship was found between phenotypic resistance and the presence of ARG in relation to erythromycin ($p > 0.05$). It was concluded that isolates of *E. faecalis* from persistent endodontic infections showed phenotypes of resistance to several antimicrobial agents, all of which were susceptible to amoxicillin/clavulanic acid. Periodic evaluation of susceptibility to antibiotics is suggested as an important practice for the surveillance of antibiotic resistance in oral strains.

KEY WORDS: *E. faecalis*, antibiotic resistance, persistent endodontic lesions, antibiotic resistance genes.

INTRODUCTION

Enterococcus faecalis (*E. faecalis*) is a bacterial species involved in post-treatment of apical periodontitis due to its capacity to persist after chemical-mechanical treatments during the disinfection process of the root canals. The presence of virulence factors and ARG expression may would produce the failure of conventional endodontic treatments (Duggan & Sedgley, 2007). The

overmedication or inadequate use (use when the etiology is not infectious) of antibiotics have been the key to put a selective pressure in these bacteria and that could be playing a role in persistent endodontic pathologies, through resistance mechanisms encoded in their genome. Some species of the oral cavity can carry, in addition to ARG, genes that encode virulence factors, which facilitate their persistence and survival

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in this complex environment (Stuart *et al.*, 2006; Prazmo *et al.*, 2016). Furthermore, processes of horizontal gene transfer between communities organized in biofilms play an important role in the exchange of genetic information that gives them new traits that allow them to survive (Davies & Davies, 2010).

Apical periodontitis is an inflammatory reaction of the periradicular tissues caused by a microbial infection in the root canals of the teeth (Nair, 2004). In cases of patients with risk of developing bacterial endocarditis, systemic antibiotics become an important complement to the endodontic treatment used as prophylaxis (Anderson *et al.*, 2018). The treatment of acute oral infections is mainly empirical, using different families of antibiotics, among which are beta-lactams, lincosamides, nitroimidazoles, tetracyclines, etc. Beta-lactams (mainly amoxicillin) are used as first-line treatment for oral cavity infections (Oberoi *et al.*, 2015). Bacterial cultures and molecular studies have confirmed that *E. faecalis* is one of the frequent bacteria isolated from the root canal system after endodontic treatment (Pinheiro *et al.*, 2003; Anderson *et al.*). *Enterococcus spp.* have acquired genes that confer resistance to several classes of antibiotics, including clindamycin, erythromycin, tetracycline, chloramphenicol and, more recently, vancomycin (Gomes *et al.*, 2011; Medeiros *et al.*, 2014; Barbosa-Ribeiro *et al.*, 2016; Anderson *et al.*). Although the incidence of resistant strains is more frequent in hospital or systemic infections, studies with bacteria isolated from endodontic infections have shown the emergence of bacterial resistance (Gomes *et al.*), but there are no reports yet in Chilean population. It has been shown that the frequency of *E. faecalis* found in persistent periradicular lesions is higher. In fact, failed cases of root canal treatment are nine fold more likely to contain *E. faecalis* than primary endodontic infections (Barbosa-Ribeiro *et al.*), and studies on teeth with periradicular lesions have shown a prevalence ranging between 24 and 77 % (Stuart *et al.*) depending on the methodology used.

E. faecalis has several strategies to evade the action of antimicrobials and the immune system. It has various virulence factors including lytic enzymes, aggregation substance, and lipoteichoic acid. *E. faecalis* is able to suppress the action of lymphocytes, potentially contributing to endodontic failure (Prazmo *et al.*). It is small enough to colonize and live properly inside the dentinal tubules (Sun & Song, 2011; Sun *et al.*, 2012). It has the capacity to withstand prolonged periods of starvation until adequate nutritional supply

is available, and to survive in contact with intracanal calcium hydroxide medications for more than 10 days (Rams *et al.*, 2013). *E. faecalis* is able to form a biofilm that helps to resist adverse conditions (Stuart *et al.*; Zhu *et al.*, 2010; Preethee *et al.*, 2012). However, there is limited information about the presence of ARG in strains from endodontic failures and its relationship with the resistance phenotype. The aim of this study was to evaluate the resistant phenotype and resistant genotype of genetically non-related strains of *E. faecalis* isolated from teeth with persistent endodontic lesions.

MATERIAL AND METHOD

Bacterial strains. Thirteen clinical isolates of *E. faecalis* obtained from patients with dental pieces previously treated with indication of endodontic retreatment and with diagnosis of apical periodontitis were included. These strains were isolated from patients treated at the clinic of the Specialty of Endodontics of the Universidad de Concepción, Chile (Sánchez-Sanhueza *et al.*, 2015). The study was carried out in compliance with the protocol approved by the Faculty's Ethical Committee (number C.I.Y.B 08/14). Each strain corresponds to a distinct pulsotype (PFGE patterns) that was previously determined (Sánchez-Sanhueza *et al.*, 2018).

Antibiotic resistance phenotype: The minimum inhibitory concentration (MIC) for each antibiotic was determined by Epsilometer test (Etest®, BioMérieux, Marcy-l'Etoile, France). Each strain was inoculated with a standardized inoculum of $1,5 \times 10^8$ CFU/mL on Mueller Hinton agar plates and cultured for 24 h at 37 °C in aerobic conditions. Strips impregnated with amoxicillin, amoxicillin/clavulanic acid, tetracycline, and erythromycin were used. In the case of metronidazole, the test was carried under anaerobic conditions. The breakpoints to determine susceptibility or resistance were those recommended by CLSI (M100-S27, 2019). Phenotypic detection of beta-lactamase was carried out by the cefinase test (bioMérieux, Marcy-l'Etoile, France). In a sterile Petri dish, the disks were placed, moistened with sterile distilled water, and a one colony was deposited on the disk surface, leaving them at room temperature by 60 minutes. The change of the disk color to red indicates the production of a beta-lactamases (Lopardo & Blanco, 2007). The strains *Staphylococcus aureus* ATCC 43300 was a positive control, because it produces a beta-lactamase.

DNA Extraction: 4-5 colonies of each strain were resuspended in 200 ml of 5 % Chelex® (Bio-Rad, USA) supplemented with 2.5 ml of proteinase K (20 mg/ml) (Thermo Scientific, Massachusetts, USA). It was mixed and incubated at 56 °C for 45 min, homogenized again and boiled for 8 min. Next, it was centrifuged at 14,000 rpm for 1 min. The DNA extracted (template) obtained was stored at 4 °C for later use.

Polymerase chain reaction (PCR): The extracted DNA was diluted with sterile distilled water in a ratio 1:4 (25 µL/ 75µL). For the conventional PCR reaction, the KAPA Taq ReadyMix Polymerase kit (KAPA Biosystems, Massachusetts, USA) was used in the following amounts: KAPA Taq 5 µL, Water 3.8 µL, primer forward 0.1 µL, primer reverse 0.1 µL, DNA template 1 µL. The nucleotide sequences of the primers used are shown in Table I. Amplification was

performed in a Veriti 96-well thermal cycler (Applied Biosystems, Foster City, CA, USA) with the programs described in Table II. The amplification products (amplicons) were separated by agarose gel electrophoresis (1.5 %), to which the SafeView™ reagent (0.5 µg / µL) was added and visualized in a UV transilluminator (Uvitec UVIdoc HD2 - Documentation System from Gels, Warwickshire, UK). Finally, the presence of ARG was corroborated by sequencing the amplicons (Macrogen, Seoul, Korea).

Statistical analysis: The data was processed with the statistical software IBM SPSS Statistics v.25.0 where parameters of descriptive statistics such as frequency and percentage were used for the analysis and presentation of the results. To analyze a possible relationship between the resistance / susceptibility phenotype and the presence of ARG of each strain, Fisher's exact test was used, using 95 % confidence.

Table I. List of primers.

Genes	Resistance to	5' → 3' nucleotide sequence	Amplicon size (bp)	Tm (C°)	Reference
<i>erm(a)</i>	erythromycin	F: TCTAAAAAGCATGTAAAAGAA R: CTTCGATAGTTTATTAATATTAGT	645	50	(Lins, 2013)
<i>erm(b)</i>	erythromycin	F: GAAAAGGTA CTCAACCAATA R: AGTAACGGTACTTAAATTGTTTC	639	52	(Lins, 2013)
<i>tet(W)</i>	tetracycline	F: GAGAGCCTGCTATATGCCAGC R: GGGCGTATCCACAATGTTAAC	168	64	(Jungermann, 2011)
<i>tet(Q)</i>	tetracycline	F: TTATACTTCCTCCGGCATCG R: ATCGGTTTCGAGAATGTCCAC	904	55	(Jungermann, 2011)
<i>bla_{TEM-1}</i>	β-lactams	F: CCAATGCTTAATCAGTGAGG R: ATGAGTATTCAACATTTCCG	858	55	(Jungermann, 2011)
<i>bla_Z</i>	β-lactams	F: CAGTTCACATGCCAAAGAG R: TACACTCTTGGCGGTTTC	846	54	(Lins, 2013)
<i>cfxA</i>	β-lactams	F: GCAAGTGCAGTTTAAGATT R: GCTTTAGTTTGCATTTTCATC	802	58	(Lins, 2013)
<i>nim</i>	metronidazole	F: ATGTTTCAGAGAAATGCGGCGTAAGCG R: GCTTCCTTGCCGTGCATGTGCTC	458	62	(Koukos, 2016)

F: forward; R: reverse

Table II. PCR Programs.

Gen	Initial denaturation	n° Cycles	Amplification			Final Extension	Ref
			Denaturation	Alignment	Extension		
<i>erm(a)</i>	95°C - 1min	26	94°C - 45s	50°C - 45s	72°C - 90s	72°C - 15min	(Lins <i>et al.</i> , 2013)
<i>erm(b)</i>	95°C - 1min	26	94°C - 45s	50°C - 45s	72°C - 90s	72°C - 15min	(Lins <i>et al.</i> , 2013)
<i>tet(W)</i>	94°C - 5min	30	94°C - 30s	64°C - 30s	72°C - 30s	72°C - 7min	(Aminov <i>et al.</i> , 2001)
<i>tet(Q)</i>	94°C - 5min	35	94°C - 60s	55°C - 60s	72°C - 60s	72°C - 10min	(Ng <i>et al.</i> , 2001)
<i>bla_{TEM-1}</i>	95°C - 2min	30	96°C - 30s	60°C - 30s	72°C - 30s	72°C - 10min	(Chouchani <i>et al.</i> , 2012)
<i>bla_Z</i>	95°C - 10min	50	94°C - 12s	55°C - 15s	72°C - 20s	72°C - 10min	(Pereira <i>et al.</i> , 2014)
<i>cfxA</i>	94°C - 5min	25	94°C - 60s	58°C - 60s	72°C - 90s	72°C - 10min	(Fosse <i>et al.</i> , 2002)
<i>nim</i>	94°C - 10min	32	94°C - 30s	62°C - 60s	72°C - 60s	72°C - 10min	(Koukos <i>et al.</i> , 2016)

RESULTS

The results obtained in the E-test, summarized in Table III, show that all strains were susceptible to amoxicillin (MIC, 6 - 0.38 µg/mL) and to the amoxicillin/clavulanic acid combination (MIC, 6 - 0.025 µg/mL), but resistant to metronidazole (MIC >256 µg/mL). The higher MIC were observed in metronidazole, tetracycline and erythromycin. Three strains (3.3; 6.5 and 9.2) were resistant to these latest antibiotics simultaneously, and belongs to three different PFGE pulsotypes. However, it should be noted that strains 3.1 and 3.3 (90.3 % similarity), as well as 10.3 and 9.2 (94.5 % similarity), despite being considered closely related, exhibited a completely different resistant phenotype, except for strains 4.3 and 7.3 (96.8 % similarity), which only had in common that they were resistant to metronidazole. The detection of beta-lactamases was performed by the nitrocefin test, where only the strain 9.1 showed the production of beta-lactamases.

Molecular analysis with PCR only evidenced the presence of two *ermB* genes for strains 6.4 and 6.5 (Fig. 1). Sequencing of both *ermB* genes was performed, which was over 600 base pairs (bp). A good quality of the signal was obtained, observing separated peaks, with the

G signal above 500 as observed in Figure 2. Both genes showed a similarity of 99 % identity with the reference in Gene Bank (ID: AP018546. 1), using the BLASTN® computer tool ("Gene Bank," 2019), which shows that the gene is located in the plasmid pKUB3007-3 KUB3007 (Kuroda *et al.*, 2018). The results indicate that there is no statistically significant relationship between the presence of the gene and the antibiotic resistance to erythromycin ($p = 0.077$).

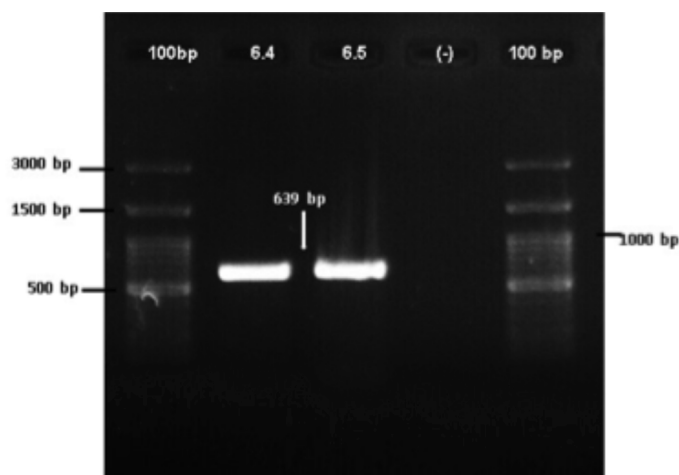


Fig. 1. PCR identification of the *ermB* gene for strains 6.4 and 6.5.

Table III. E-test based antibiotic resistance profiles.

Strain	PFGE pulsotype	Antimicrobials				
		AMX	AMC	TET	ERY	MTZ
3.1	K1	2	1.5	1.5	0.5	>256
3.3	K2	6	6	128	192	>256
4.3	I1	2	2	6	6	>256
6.4	M1	0.75	0.75	4	>256	>256
6.5	L1	0.5	0.38	64	>256	>256
7.3	I2	1.5	1	4	4	>256
8.6	M2	0.75	0.75	1	0.75	>256
9.1	C1	0.5	0.025	0.75	0.25	>256
9.2	A1	3	2	>256	>256	>256
10.3	A2	0.75	0.75	24	1	>256
12.4	H1	0.75	0.5	0.75	1.4	>256
15.3	F1	0.75	0.5	32	2	>256
16.3	L2	0.38	0.38	192	0.064	>256
Resistant strains		0	0	6	4	13
Susceptible		13	13	7	9	0
% Resistant		0%	0%	46.15%	30.77%	100%

AMX: amoxicillin, AMC: amoxicillin + ac. Clavulanic, ERY: erythromycin, TET: tetracycline, MTZ: metronidazole. N: resistant strains. * Interpretation based on the cut-off points established by the CLSI 2017



whether patients were exposed to beta-lactam antibiotics prior to the 3 months requested as inclusion criteria, nor at the time the endodontic treatment failed was performed. Amoxicillin/clavulanic acid combination and amoxicillin were the antibiotics that exhibited the lowest MIC, in agreement with other studies in the literature, which indicate that penicillins are the first-choice treatment for oral pathologies (Barbosa-Ribeiro *et al.*; Komiyama *et al.*, 2016). However, the presence of penicillin-resistant enterococcus strains has been reported in endodontic infections (Koukos *et al.*, 2016), which underscores the need for periodic susceptibility testing of these isolates. TEM-type beta-lactamases have been found in a wide variety of bacteria, generally Gram positive (Rôças & Siqueira, 2012). In the literature, unlike our study, some authors have found the presence of the *bla*_{TEM} gene in strains of *E. faecalis* isolated from endodontic pathology (Jungermann *et al.*; Rôças & Siqueira, 2013) and in particular one of them that reported in 30 % of the samples (Anderson *et al.*, 2018). However, emphasize that enterococci are not often producers of beta-lactamase (Fontana *et al.*, 1992), which could be related to the high susceptibility to amoxicillin in this study. Strain 9.1 showed evidence of beta-lactamase the nitrocefin test; however, when the same strain was assayed with the E-test, it was totally susceptible to amoxicillin and amoxicillin/clavulanic acid. This may be due to a low beta-lactamase activity and the MIC value is less than necessary to generate resistance. It has also been described that the nitrocefin test can generate false positives or negatives because many times the molecular reactions are incomplete (Pitkälä *et al.*, 2007), although an effectiveness greater than 90 % has been described (Vergis *et al.*, 2018). It would be interesting to sequence the genome of this strain to identify the beta-lactamase involved.

The clinical importance of tetracyclines in endodontics is their use in various intracanal medications. A study had previously reported the presence of *tetW* and *tetQ* genes in endodontic infections (Jungermann *et al.*); however, the main genes found in strains of tetracycline-resistant enterococci were the *tetS* and *tetM* (Roberts *et al.*, 2006; Jungermann *et al.*). In previous studies have been reported a variable percentage of resistance to tetracycline (between 5 % and 75 %) (Lins *et al.*, 2013; Rams *et al.*; Komiyama *et al.*), which agrees to the phenotypic resistance to tetracycline although no tet genes were found in this study. It is suggested that the search for other tet genes be extended in those strains with a tetracycline resistant phenotype.

In reference to metronidazole, its antibacterial activity preferentially is focused upon anaerobic Gram-positive bacilli and Gram-negative cocci, some protozoa and *Helicobacter pylori*. In the dental field it is used for mainly maxillofacial or periodontal infections, due to the predominance of anaerobic bacteria in this kind of pathology (Poveda Roda *et al.*). In this case, phenotypic resistance to metronidazole was expected because it has an antimicrobial spectrum limited to strict anaerobic bacteria and protozoa (Rams *et al.*).

Four isolates were phenotypically resistant to erythromycin, which agrees with previous reports showing different degrees of resistance to this macrolide, so that the percentage obtained in the study around 30 %, keeps close relationship with what was found previously (Skucaite *et al.*, 2010; Rams *et al.*; Komiyama *et al.*). Two of the isolates were positive for the *ermB* gene, which is described as one of the most common genes in oral isolates with prevalence in strains highly resistant to erythromycin (Jain *et al.*, 2016). The *erm* gene confers resistance to the antibiotics macrolide-lincosamide-streptogramin B (MLS_B), three groups of antimicrobials of different chemical structures, but with similar mechanisms of action. A methylase encoded by *erm* results in a specific adenine base dimethylation in the 23 S subunit of ribosomal RNA which affects the binding of antibiotics to their ribosome site of action (Min *et al.*, 2008). On the other hand, four strains were phenotypically resistant to erythromycin, and only two of them identified the *ermB* gene. However, the statistical study concludes that there is no relationship between the presence of the gene and antibiotic resistance ($p > 0.05$), so it could be other genes or resistance mechanisms that were not included in this study.

One of the principal limitations of this study was to demonstrate the location of the *ermB* and a possible relation with mobile genetic elements. For the case of *ermB*, it was identified that the gene is located in the plasmid pKUB3007-3 KUB3007 (Kuroda *et al.*; Yin *et al.*, 2018). The importance of this lies in that being a plasmid a mobile element within the genome of the bacteria could contribute to the processes of horizontal genes transfer with other microorganisms in the community, which could transform the root canal into a reservoir of genes that could help the propagation to other anatomical areas where these drugs are routinely used as treatments (Davies & Davies).

E. faecalis shows fairly high levels of resistance to many antimicrobial agents, presumably due to the

presence of efflux pumps (Davis *et al.*, 2001). In the present study it did not include them, so it could be related to phenotypic resistance to antibiotics. The *mefA* and *msrA/msrB* efflux pumps (Chouchani *et al.*, 2012), associated with the production of beta-lactamase TEM-1, among them resistant to erythromycin and ampicillin (Rôças & Siqueira, 2012).

Glycopeptides have seen an increase in resistance to last-line antibiotics, such as vancomycin, due to the expression of *van*-like genes (Miller *et al.*, 2014) To emphasize that, although the present study identified the presence of genes of resistance to erythromycin in 2 strains of *E. faecalis*, this information does not indicate that these bacteria are functionally or phenotypically resistant to antibiotics. The mechanisms of antibiotic resistance are linked to gene regulation, mediating all the functions associated with the evasion of antibiotic action and have described processes of: mutation of the gene, activation of specific regulatory genes and of global transcription (Troncoso *et al.*, 2017). The regulation of gene expression in a cell starts at the level of transcription of DNA into mRNA. The circumstances under which a particular gene is positively- or negatively-regulated provide important clues about the function of genes (Jungermann *et al.*; Troncoso *et al.*).

Studies related to antimicrobial susceptibility are based on culture dependent microorganisms. Of the microorganisms of oral microbiota only have this condition less than 1 %, so the study is limited (Wade, 2011). Metagenomics is a new field in which you can obtain the genome sequences of the different microorganism communities, extracting and analyzing their DNA globally, without the need to carry out cultures. This is important to study the interaction between the different microorganisms, their organization and the identification of new structures that could be related to the presence of ARG. In this case, the structures that generate the resistance phenotype have been called resistome, composed of the total set of antibiotic resistance factors (ARD) (Sukumar *et al.*, 2016).

Finally, during endodontic treatments the use of rubber dam isolation in the procedure is a basic standard to prevent the entry of microorganisms into the root canal system. There is clear evidence of the effectiveness of endodontic treatment when rubber dam is used (Anabtawi *et al.*, 2013; Goldfein *et al.*, 2013). This study incorporated strains of patients, which in many cases did not use tooth isolation, so it could be a cause of the nosocomial contamination.

CONCLUSION

Within the limitations of this study, it can be concluded that *E. faecalis* isolates of persistent endodontic infections showed varying degrees of resistance to several antibacterial agents commonly used in dentistry, with beta-lactams showing the lowest MICs, where only a strain showed beta-lactamase action. Two strains not genetically related, evidenced the presence of *ermB* genes related to the phenotypic resistance to erythromycin. Finally, regarding the relationship between the phenotype/genotype of antibiotic resistance, in this study there was no relationship between both parameters. The periodic evaluation of antibiotic susceptibility is an important practice to establish the best therapy if its use is necessary.

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RESUMEN: Los enterococos son importantes patógenos nosocomiales debido a su multi resistencia intrínseca y la adquisición de nuevos genes de resistencia a los antibióticos (ARG). *Enterococcus faecalis* es uno de los principales patógenos en infecciones endodónticas persistentes, por lo tanto, el objetivo principal de este estudio fue evaluar el fenotipo y el genotipo de resistencia de cepas de *E. faecalis* aisladas de dientes con lesiones endodónticas persistentes, a los antibióticos comúnmente recetados en odontología. Se analizaron 13 cepas de *E. faecalis* de diferentes pulsotipos para evaluar la susceptibilidad a los antibióticos, amoxicilina, amoxicilina / ácido clavulánico, tetraciclina, eritromicina y metronidazol, utilizando la prueba de Epsilometría (E-test) y la presencia de beta-lactamasas con prueba de nitrocefina. Finalmente, la detección de ARG se realizó con una técnica molecular de reacción en cadena de la polimerasa (PCR) y se confirmó mediante la secuenciación de los productos de amplificación. Se utilizó la prueba exacta de Fisher, con un 95 % de confianza. En cuanto al fenotipo de resistencia, las cepas evaluadas, independientes del pulsotipo, fueron totalmente resistentes a la acción del metronidazol. Los antibióticos con los valores más altos de concentración mínima inhibitoria (CMI) después del metronidazol incluyen tetraciclina y eritromicina. En contraste, las CMI más bajas se aplican a la combinación de amoxicilina con ácido clavulánico. La prueba de nitrocefina fue positiva solo en una cepa. Genotípicamente, dos cepas distantes genéticamente, aisladas de un mismo paciente fueron positivas para el gen *ermB*. No se encontró una relación estadísticamente significativa entre la resistencia fenotípica

y la presencia de ARG en relación con la eritromicina ($p > 0,05$). Se concluyó que los aislamientos de *E. faecalis* de infecciones endodónticas persistentes mostraron fenotipos de resistencia a varios agentes antimicrobianos, todos los cuales fueron susceptibles a amoxicilina / ácido clavulánico. Se sugiere una evaluación periódica de la susceptibilidad a los antibióticos como una práctica importante para la vigilancia de la resistencia a los antibióticos en las cepas orales.

PALABRAS CLAVE: *E. faecalis*, resistencia a antibióticos, lesiones endodónticas persistentes, genes de resistencia a antibióticos.

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