

Culture and molecular detection of *Enterococcus faecalis* from patients with failure endodontic treatment and antimicrobial susceptibility of clinical isolates

Cultura e detecção molecular de *Enterococcus faecalis* de pacientes com insucesso no tratamento endodôntico e suscetibilidade antimicrobiana dos isolados clínicos

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ABSTRACT

Objective: The aim of this study was to evaluate the minimal inhibitory concentrations (MIC) of different antibiotic agents against to the most prevalent microorganism found in root-filled canals by culture and molecular approaches. **Material and Methods:** The microbial samples were taken either from thirty root-filled canals after removal of gutta-percha. Culture methods and 16s rDNA assay were used to identify the *E faecalis* present in the samples. The antimicrobial susceptibilities of the isolates of *E faecalis* were determined by MIC values using the E test System and interpreted according to the Clinical and Laboratory Standards Institute guidelines. The following antibiotics were used: benzylpenicillin, amoxicillin, amoxicillin-clavulanic acid, erythromycin, azithromycin, vancomycin, chloramphenicol, tetracycline, doxycycline, ciprofloxacin, rifampicin and moxifloxacin. **Results:** *E faecalis* were isolated (7/30) and detected (13/30) by culture and PCR assay, respectively. All tested *E faecalis* (n = 12) were highly sensitive to amoxicillin, moxifloxacin, vancomycin, benzylpenicillin and amoxicillin-clavulanic acid. Isolated *E faecalis* strains were resistant to some antibiotics such as rifampicin (4/12), tetracycline (2/12), doxycycline (1/12), erythromycin (3/12) and azythromycin (8/12). **Conclusion:** Amoxicillin, amoxicillin-clavulanic acid, benzylpenicillin, vancomycin and moxifloxacin were the most active antibiotics, in vitro, against *E faecalis* clinical strains, with all the isolates being susceptible.

RESUMO

Objetivo: O objetivo deste estudo foi avaliar as concentrações inibitórias mínimas (CIM) de diferentes antibióticos contra micro-organismos mais prevalentes em canais radiculares pelos métodos moleculares e de cultura. **Material e Métodos:** As amostras microbianas foram obtidas de trinta canais radiculares após a remoção da gutta-percha. Os métodos de cultura e ensaio 16s rDNA foram utilizados para identificar *E. faecalis* presente nas amostras. As susceptibilidades antimicrobianas dos isolados de *E. faecalis* foram determinadas pelos valores de CIM utilizando o sistema E teste e interpretados de acordo com as diretrizes CLSI (Clinical and Laboratory Standards Institute). Foram utilizados os seguintes antibióticos: benzilpenicilina, amoxicilina, amoxicilina com ácido clavulânico, eritromicina, azitromicina, vancomicina, cloranfenicol, tetraciclina, doxiciclina, ciprofloxacina, moxifloxacina e rifampicina. **Resultados:** *E. faecalis* foram isolados (7/30) e detectados (13/30) por cultura e pelo método PCR, respectivamente. Todos *E. faecalis* (n = 12) foram altamente sensíveis à amoxicilina, moxifloxacina, vancomicina, benzilpenicilina e amoxicilina com ácido clavulânico. Cepas isoladas de *E. faecalis* foram resistentes a alguns antibióticos como a rifampicina (4/12), tetraciclina (2/12), doxiciclina (1/12), eritromicina (3/12) e azitromicina (8/12). **Conclusão:** Amoxicilina, amoxicilina com ácido clavulânico, benzilpenicilina, vancomicina e moxifloxacina foram os antibióticos mais ativos, in vitro, contra cepas clínicas de *E. faecalis*, com todos os isolados sendo suscetíveis. Azitromicina e

Azithromycin and erythromycin were least effective, with none percentage of isolates being susceptible, during laboratory testing. Moreover, *E faecalis* were identified more frequently by PCR assay than by culture technique.

KEYWORDS

Dental pulp cavity; Retreatment; Root canal filling materials; Antibiotics; Antimicrobial susceptibility; *Enterococcus faecalis*; Antibiotic resistance.

eritromicina foram menos eficazes, com nenhuma porcentagem de isolados suscetível durante os testes laboratoriais. E ainda, *E. faecalis* foram identificados mais frequentemente pela técnica PCR do que pela técnica de cultura.

PALAVRAS-CHAVE

Cavidade Pulpar; Retratamento; Materiais obturadores do canal radicular; Antibióticos; Susceptibilidade antimicrobiana; *Enterococcus faecalis*; Resistência aos antibióticos.

INTRODUCTION

Endodontic infections are polymicrobial involving a combination of Gram-positive, Gram-negative, facultative anaerobes and strict anaerobic bacteria [1]. The bacteria remaining in the root canal system after endodontic treatment cause secondary, or persistent, infections [2]. These microorganisms may have survived to the biomechanical procedures or invaded the canal via coronal leakage of the root filling. Bacterial cultures and molecular studies have confirmed that *Enterococcus faecalis* is one of the most prevalent bacteria found in the root canal after endodontic treatment [3,4].

The general interest for enterococci and treatment of enterococcal infections has increased due to the appearance of antibiotic multiresistant strains. Enterococci frequently cause a wide variety of infections in humans and it has also been implicated in endodontic infections [5]. *E faecalis* are frequently isolated from obturated root canals of teeth that exhibit chronic periapical pathology [6]. *E faecalis*, intrinsically or via acquisition, may be resistant to a wide range of antibiotics [7,8], which, if used, may shift the microbiota in favor of *E faecalis*.

The role of systemic antibiotic therapy in endodontics is limited. Antibiotics are not generally used to treat chronic infections, such as apical periodontitis, in root-filled teeth [9]. Usually, it is applied when patients present

with progressive, diffuse swelling and systemic signs of infection including fever, malaise, and lymphadenopathy. Systemic antibiotics may also be used as a prophylactic measure for medically compromised patients. Despite these treatment guidelines, dental practitioners tend to overprescribe antibiotics in their practice, often without sufficient rationale for choosing a particular drug [10].

The resistance of oral microbiota to antibiotics has increased during the past decades, possibly because of the empiric use of antibiotics for a variety of pathologies and the use of antibiotics for minor infections, or in some cases in patients without infections [11]. The intensive use of antibiotics in medicine and dentistry has selected for antibiotic resistant bacteria. When bacteria become resistant to antibiotics, they gain the ability to exchange this resistance, making other microorganisms nonsusceptible to antibiotics prescribed [12]. The increasing resistance of bacteria to some widely used antibiotics ensures the need of monitoring susceptibility patterns periodically by using susceptibility tests. Therefore, the Epsilometer test (E test), an agar diffusion susceptibility test, holds the promise of being accurate and flexible enough to be performed in the most clinical laboratories [13]. Thus, it is prudent to study changes in the antimicrobial susceptibilities of endodontic pathogens to facilitate the choice of an appropriate antibiotic when indicated for the treatment of infections.

The aim of the present study was to evaluate the minimal inhibitory concentrations (MIC) of different antibiotic agents against to the most prevalent microorganism found in post-treatment apical periodontitis patients associated with failed root canals.

METHODS

Patient selection

Thirty patients were selected from those who attended the Piracicaba Dental School, SP, Brazil, with a need for nonsurgical endodontic retreatment. The Human Volunteers Research and Ethics Committee of the Piracicaba Dental School approved a protocol describing the specimen collection for this investigation, and all patients signed an informed consent to participate. A detailed medical and dental history was obtained from each patient. Patients who had received antibiotic treatment during the last 3 months or had a general disease were excluded from the study. The age of the patients ranged from 19 to 65 years. Preoperative radiographs were taken to ensure the presence of a single filled root canal and evidence of apical periodontitis. Failure of root canal treatment was determined on the basis of clinical and radiographical examinations. All teeth had been root canal treated filled more than 2 years ago and the patients presented asymptomatic. All teeth had enough crown structure for adequate isolation with a rubber dam, and showed an absence of periodontal pockets deeper than 4 mm.

Microbial sampling

The teeth were isolated with a rubber dam. The crown and the surrounding rubber dam were disinfected with 30% H₂O₂ (v/v) for 30 s followed by 2.5 NaOCl for an additional 30 s. Subsequently, 5% sodium thiosulphate was used to inactivate the disinfectant agents [6,9,14]. A swab sample was taken from the surface and streaked on blood agar plates to test for disinfection. An access cavity was prepared

with sterile high-speed diamond burs under irrigation with sterile physiological solution. Before entering the pulp chamber, the access cavity was disinfected with the same protocol as above and the sterility again checked by taking a swab sample of the cavity surface and streaking onto blood agar plates. Aseptic techniques were used throughout root canal treatment and sample acquisition. The initial samples were collected with three sterile paper points, which were consecutively placed into each canal to the total length calculated from the pre-operative radiograph, kept in place for 60 s and then pooled in a sterile tube containing 1 mL VMGA III transport medium [14]. The samples were transported to the microbiology laboratory within 15 min to an anaerobic workstation (DonWhitley Scientific, Bradford, UK).

Clinical procedures

The same endodontic specialist performed all retreatments and sampling procedures. The tooth was anesthetized and after accessing the pulp chamber, the root filling materials were removed using crown-down technique. No solvent was used at any time to avoid a negative effect on microbial viability. Radiographs performed in bucco-lingual and mesio-distal directions for each tooth were taken to confirm gutta-percha removal.

The canal filling material was removed using MTwo R files (VDW, Munique, Germany). A K-file (Dentsply Maillefer, Ballaigues, Switzerland) size #15 was used to negotiate the root canal. MTwo R file size 15, 0.05 (21 mm) taper was first used to working length (at apical foramen) followed by MTwo R size 25, taper 0.05 (21 mm) also to working length, both in a brushing action with a lateral pressing movement. All instrumentation of the MTwo R was performed using an electric motor (VDW, Munique, Germany) operated according to the manufacturer's instructions. The working length was established radiographically and with the aid of an electronic apex locator (Novapex, Forum Technologies, Rishon le-Zion, Israel)

at apical foramen. Progression of the rotary files was performed by applying slight apical pressure and frequently removing the files to inspect the blade and clean the debris from the flutes. Furthermore close inspection under high magnification with the dental operating microscope (D F Vasconcellos S/A, São Paulo, Brazil) showed complete removal of gutta-percha. After removal gutta-percha, the sample was taken with three paper points in VMGA III.

Microbial identification

Microbial samples, isolation and speciation were done using advanced microbiologic techniques for anaerobic species.

Inside the anaerobic workstation, the tubes containing the transport medium were shaken in a mixer for 60 s (Agitador MA 162-MARCONI, São Paulo, SP, Brazil). Serial 10-fold dilutions were made up to $1/10^4$ in pre-reduced Fastidious Anaerobe Broth (FAB, Lab M, Bury, UK) and 50 μL of each serial dilution were plated onto several media, as follows: 5% defibrinated sheep blood-FAA Agar (FAA, Laboratory M, Bury, UK) alone, and supplemented with 600 μL of hemin and 600 μL of menadione added to 500 mL of medium. The plates were incubated at 37 °C in an anaerobic atmosphere for up to 48 h to allow anaerobic or facultative microorganisms growth. In addition, 50 μL of initial sample was plated onto m-Enterococcus agar (Difco, Maryland, USA) and Mitis salivarius agar (Difco, Maryland, USA) to increase the chance of finding *Enterococcus faecalis*.

Preliminary characterization of microbial special was based on the features of the colonies (i.e. size, color, shape, high, lip, surface, texture, consistency, brightness and hemolysis), visualized under a stereoscopic lens (Lambda Let 2, Atto instruments Co., Hong Kong) at 16x magnification. Isolates were then purified by subculture, Gram-stained, tested for catalase production, and their gaseous requirements established by incubation for 2 days aerobically and anaerobically. Based on this information it was possible to select appropriate procedures

for identification of *E faecalis* using API 20 Strep (BioMérieux SA, Marcy-l'Etoile, France) for streptococci (Gram-positive cocci, catalase-negative). The detection system API 20 Strep is based on fermentative and biochemical properties of facultative anaerobe microorganisms being identified by standardized enzymatic reactions.

Antimicrobial Susceptibility Test

The antimicrobial susceptibility of isolates was investigated by means of the E test System (AB Biodisk, Solna, Sweden).

The strains isolated of *E faecalis* (n = 12), facultative anaerobic Gram-positive cocci from root-filled teeth with apical periodontitis sampled, were tested for their susceptibility/resistance against 12 antibiotics: benzylpenicillin, amoxicillin, amoxicillin + clavulanic acid, erythromycin, azithromycin, vancomycin, chloramphenicol, tetracycline, doxycycline, moxifloxacin, ciprofloxacin and rifampicin. The E test consists of strips containing different concentration of an antimicrobial agent which can be placed directly on the agar plate. This test was evaluated by using Mueller-Hinton agar plates (Oxoid, Basingstoke, UK) 4 mm thick. Inocula were prepared by suspending growth on plates in Fastidious Anaerobe Broth (Lab M, Bury, UK) to a McFarland turbidity of 0.5. Sterile cotton swabs were used to inoculate plates, to which E test strips were then applied within 20 min of inoculation. The surface of the plate was swabbed in three directions to ensure a complete distribution of the inoculum over the entire plate. Plates were incubated in an aerobic incubator for aerobic bacteria (36 °C for 24 h). All the tests were completed in duplicate. The susceptibility result was interpreted by comparing the minimum inhibitory concentrations (MICs) of isolates with MIC interpretive standards established for National Committee for Clinical Laboratory Standards (NCCLS). After 24 hours of incubation in aerobic condition and 10% CO₂ the concentration of the drug that inhibits 90% of bacterial growth in-vitro (MIC) could be easily read from the strip [15].

The E test is based on the diffusion of a continuous, exponential concentration gradient of the antimicrobial from a plastic strip containing the antibiotic. After incubation of the E test strip on agar media with a lawn of bacteria, an ellipse of inhibition is formed around the strip. The point where the ellipse intersects the strip is where the MIC is read from the interpretive scale.

Table 1 shows the MIC interpretative standards for each antimicrobial agent. The MICs were read from the intercept where the ellipse inhibition zone intersected with the scale. The MICs including 90 and 50% of the strains were calculated. The diameter of the inhibition zone of each strain was measured and the strains were graded as sensitive (S), intermediate (I) and resistant (R) according to the guidelines of NCCLS (2002) [16].

***Enterococcus faecalis* detection and confirmation (Polymerase chain reaction - PCR 16S rDNA)**

DNA extraction

Microbial DNA from all samples and control sample from *Enterococcus faecalis* (ATCC 4034) were extracted and purified by using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The DNA concentration (absorbance at 260 nm) was determined with a spectrophotometer (Nanodrop 2000; Thermo Scientific, Wilmington, DE, USA).

PCR Assay

The oligonucleotide species-specific primers for *Enterococcus faecalis* were 5'-CCG AGT GCT TGC ACT CAA TTG G-3' (forward primer) and 5'- CTC TTA TGC CAT GCG GCA TAA AC-3' (reverse primer), producing a PCR amplicon of 138 bp [17]. The PCR reaction was performed in a thermocycler (GenePro, Bioer Technology, Hangzhou, China) with a total volume of 25 μ L containing 2.5 μ L of 10X Taq buffer (1x) (MBI Fermentas, Mundolsheim,

France), 0.5 μ L of dNTP mix (25 μ mol/L of each deoxyribonucleoside triphosphate – dATP, dCTP, dGTP, and dTTP) (MBI Fermentas, Hanover, MD, USA), 1.25 μ L of 25 μ mol/L MgCl₂, 0.25 μ L of forward and reversal universal primers (0.2 μ mol/L) (Invitrogen, Eugene, OR, USA), 1.5 μ L of sample DNA (1 μ g/50 μ L), 1.5 μ L of Taq DNA polymerase (1 U) (MBI Fermentas), and 17.25 μ L of nuclease-free water. Polymerase chain reaction amplification was performed in a DNA thermocycler. The temperature profile for the universal reaction included an initial denaturation step at 95 °C for 2 min followed by 36 cycles of a denaturation step at 95 °C for 1 min, a primer annealing step at 57 °C for 1 min, an extension step at 72 °C for 1 min, and a final step at 72 °C for 7 min.

RESULTS

Neither microbial growth nor bacterial DNA was observed in any of the sterility check samples. *Enterococcus faecalis* were found in seven cases after root canal filling removal by culture technique, while in thirteen cases *E faecalis* were detected by using 16S rDNA PCR. Furthermore, it was isolated twelve strains of *E faecalis* from seven initial samples.

The values of MIC50 and MIC90 refer to the minimal inhibitory concentration that was effective against 50% and 90% of the tested strains. Table 1 also show the range of MIC for each antibiotic against the *E faecalis* strain tested (n = 12), as well as the susceptibility rate of the strains against each antibiotic according to the susceptibility breakpoints previously determined by the NCCLS criteria. All strains were susceptible to amoxicillin, moxifloxacin, vancomycin, benzylpenicillin and amoxicillin-clavulanic acid. Chloramphenicol and ciprofloxacin was effective against 83.3% and 50% of the strains, respectively. About 33% of the isolates were resistant to rifampicin, 16.7% to tetracycline and 8.3% to doxycycline. *E faecalis* strains were resistant to azithromycin (66.7%) and erythromycin (25%).

Table 1 - Antimicrobial susceptibility of *Enterococcus faecalis* (n = 12) strains isolated from root-filled canals to twelve antibiotics

Antibiotics	MIC ($\mu\text{g/mL}$)			Susceptibility rate (%)		
	MIC ₅₀	MIC ₉₀	Range of MIC ($\mu\text{g/mL}$)	S	I	R
Amoxicillin	0.315	0.75	0.094-1	100.0%	-	-
Rifampicin	2	12	1-32	16.7%	50.0%	33.3%
Moxifloxacin	0.38	15	0.094-15	100.0%	-	-
Vancomycin	15	3	0.25-3	100.0%	-	-
Tetracycline	0.38	24	0.125-32	83.3%	-	16.7%
Ciprofloxacin	15	4	0.38-4	50.0%	50.0%	-
Chloramphenicol	6	8	3-12	83.3%	16.7%	-
Benzylpenicillin	1	2	0.75-3	100.0%	-	-
Amoxicillin-Clavulanic Acid	0.5	1	0.25-1	100.0%	-	-
Doxycycline	0.22	12	0.094-16	83.3%	8.3%	8.3%
Erythromycin	2	16	1-16	-	75.0%	25.0%
Azithromycin	12	>256	3->256	-	33.3%	66.7%

* S = susceptible; I = intermediate; R = resistant.

DISCUSSION

E faecalis dominated in cases of secondary endodontic infection compared with the cases of primary endodontic infection ($p < 0.001$) [18]. The ability of *E faecalis* to tolerate or adapt to harsh environmental conditions may act as an advantage over other species. It may explain its survival in root canal infections, where nutrients are scarce and there are limited means of escape from root canal medicaments. *E faecalis* is resistant to the antimicrobial effects of calcium hydroxide [19], probably partly due to an effective proton pump mechanism which maintains optimal cytoplasmic pH levels [20]. *E faecalis* is a well-recognised cause of endocarditis. In compromised patients, antibiotic prophylaxis to prevent endocarditis that occurs following endodontic retreatment of root-filled teeth should be also directed against these microorganisms. Emerging antibiotic resistance in *Enterococcus* spp. has been shown in recent studies [21], especially against penicillin, the drug of choice. Enterococci have acquired genetic determinants that confer intrinsic resistance to many classes of antimicrobials,

including tetracycline, erythromycin, and chloramphenicol [7,8]. *E faecalis* has also been shown to possess multiple antibiotic resistance, including resistance to vancomycin and macrolides. [22,23] Dahlén et al. [24] have described enterococcal isolates resistant to benzylpenicillin, ampicillin, clindamycin, metronidazole and tetracycline.

In the present study the occurrence of *E faecalis* in root-filled teeth associated with periradicular lesions was detected by culture and PCR (23.3% and 43.3%). Others studies found *E faecalis* range from 0 to 70% by culture and 0 to 90% by PCR [25]. The differences in findings between the present study and the previously studies may be caused by geographic differences, different dietary intake, variations in clinical sampling, and sample analysis methods. The sensitivity of culture is approximately 10^3 to 10^5 cells for target species using nonselective media, whereas for PCR it varies from 10 to 10^2 cells depending on the technique used [26]. Thus, in the present study *E faecalis* were found more frequently by PCR [27]. It is worth to mention that the major advantage of the culture

procedure is its ability to enable the detection of viable cells, while molecular procedures enable the detection of only target microbial species.

The E test method was used in the present study because it provides a simple and a rapid method for quantitative susceptibility testing. Moreover, the MICs obtained with this test are generally in very good agreement with those obtained by agar dilution methods, which is the reference method of the NCCLS [15]. It is important to periodically obtain culture and susceptibility data to monitor possible changes in the types and antibiotic resistance of microorganisms responsible for failure endodontic treatment.

All *E faecalis* strains studied were susceptible to benzylpenicillin, amoxicillin, amoxicillin-clavulanic acid, vancomycin and moxifloxacin in accordance with Skucaite et al. [18] and Pinheiro et al. [9]. The MICs of amoxicillin and amoxicillin-clavulanic acid were lower than for benzylpenicillin. These findings are in agreement with previous studies [6] which have found that enterococci are more sensitive to amoxicillin than to benzylpenicillin. The results indicated that *E faecalis* strains isolated from canals of root filled canals with periapical lesions remain susceptible, in vitro, to amoxicillin. However the presence of enterococcal strains resistant to penicillin has been reported in endodontic infections [24] which underlines the need to perform susceptibility tests of these isolates.

The MIC of erythromycin varied between 1 and 16 $\mu\text{g}/\text{mL}$ and resistance was verified with three isolates. Eight strains were found to be resistant to azithromycin (3 to $> 256 \mu\text{g}/\text{mL}$). The number of resistant strains was higher than values found by Pinheiro et al. [6]. In this study, 75% of the isolates showed an intermediate pattern against erythromycin. Similar results have been reported by Sedgley et al. [28] who have found, amongst 12 oral enterococci, eight (66.6%) with an intermediate pattern. Pinheiro et al. [9] found that 28.5% and 14.2% of *E faecalis* strains were susceptible to erythromycin

and azithromycin respectively. Nevertheless in the present study none of the *E faecalis* strains studied demonstrated to be susceptible to either erythromycin or azithromycin. Bacterial resistance to these drugs has been increasing over time; which suggests that oral enterococci have become less susceptible. It has been noted that erythromycin is not effective against *E faecalis*. Kuriyama et al. [29] have suggested that erythromycin may be effective against mild or moderate infections in people with penicillin allergies, but it may not be suitable in cases of more severe infection. Azithromycin was tested as a substitute for erythromycin and was found to be less effective against enterococci than erythromycin. Furthermore, the present study showed lower percentage of susceptibility against chloramphenicol, tetracycline and ciprofloxacin when compared with Pinheiro et al. [9].

Antibiotics are often prescribed for the adjunctive treatment of acute endodontic infections. The choice of antibiotic is usually based on previously published susceptibility testing and previous clinical success [30]. It would be ideal if susceptibility testing could always be undertaken before the prescription of antibiotics. Unfortunately, it usually takes from several days to weeks to cultivate and to do susceptibility tests on bacteria [31]. Bacterial resistance to antimicrobials has been an ongoing challenge for clinicians ever since the discovery of antimicrobial agents because bacteria have succeeded in developing resistance to all antibacterial agents shortly after they had been marketed. We have now entered an era where some bacterial species, including those involved in endodontic infections, are resistant to the full range of antibiotics presently available. Recently, Rodriguez-Núñez et al. [10] have reported that, with regards to irreversible pulpitis and necrotic pulps with no systemic involvement, endodontists were overprescribing antibiotics. The use of antibiotics for minor infections, or in some cases in patients without infections, could be a major contributor to the world problem of antimicrobial resistance. Then, these empirical and inappropriate prescriptions lead to selection

of resistant strains which is potentially damaging to the community.

In relation with antibiotic therapy, an endodontic infection must be persistent or systemic to justify the need for antibiotics, i.e. fever, swelling, lymphadenopathy, trismus or malaise in a healthy patient [10,32]. Antibiotics are also more likely to be needed in an immunocompromised [30] patient or a patient in poor health. Endodontic infections typically have a rapid onset and short duration, 2–7 days or less, particularly if the cause is treated or eliminated [33]. The proper dose and duration of an antibiotic are enough when there is sufficient evidence that the patient host defenses have gained control of the infection. When the infection is resolving or has resolved, then the drug should be terminated [32,33].

CONCLUSION

Based on the study results, amoxicillin, amoxicillin-clavulanic acid, benzylpenicillin, vancomycin and moxifloxacin were the most active antibiotics, in vitro, against *E faecalis*, with all the isolates being susceptible. Azithromycin and erythromycin were least effective, with none of isolates being susceptible, during laboratory testing. Moreover, *E faecalis* was identified more frequently by PCR assay than by culture technique.

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REFERENCES

- Siqueira JF Jr, Roças IN. Exploiting molecular methods to explore endodontic infections: part 2—redefining the endodontic microbiota. *J Endod.* 2005 Jul;31(7):488-98.
- Bystrom A, Happonen RP, Sjogren U, Sundqvist G. Healing of periapical lesions of pulpless teeth after endodontic treatment with controlled asepsis. *Endod Dent Traumatol.* 1987 Apr;3(2):58-63
- Foschi F, Cavrini F, Montebugnoli L, Stashenko P, Sambri V, Prati C. Detection of bacteria in endodontic samples by polymerase chain reaction assays and association with defined clinical signs in Italian patients. *Oral Microbiol Immunol.* 2005 Oct;20(5):289-95.
- Peciuliene V, Reynaud AH, Balciuniene I, Haapasalo M. Isolation of yeasts and enteric bacteria in root-filled teeth with chronic apical periodontitis. *Int Endod J.* 2001 Sep;34(6):429-34.
- Dahlén G, Blomqvist S, Almståhl A, Carlén A. Virulence factors and antibiotic susceptibility in enterococci isolated from oral mucosal and deep infections. *J Oral Microbiol.* 2012;4.
- Pinheiro ET, Gomes BP, Ferraz CC, Teixeira FB, Zaia AA, Souza Filho FJ. Evaluation of root canal microorganisms isolated from teeth with endodontic failure and their antimicrobial susceptibility. *Oral Microbiol Immunol.* 2003 Apr;18(2):100-3.
- Murray BE. The life and times of the enterococcus. *Clin Microbiol Rev.* 1990 Jan;3(1):46-65.
- Shepard BD, Gilmore MS. Antibiotic-resistant enterococci: the mechanisms and dynamics of drug introduction and resistance. *Microbes Infect.* 2002 Feb;4(2):215-24.
- Pinheiro ET, Gomes BP, Drucker DB, Zaia AA, Ferraz CC, Souza-Filho FJ. Antimicrobial susceptibility of *Enterococcus faecalis* isolated from canals of root filled teeth with periapical lesions. *Int Endod J.* 2004 Nov;37(11):756-63.
- Rodríguez-Núñez A, Cisneros-Cabello R, Velasco-Ortega E, Llamas-Carreras JM, Torres-Lagares D, Segura-Egea JJ. Antibiotic use by members of the Spanish Endodontic Society. *J Endod.* 2009 Sep;35(9):1198-203.
- Segura-Egea JJ, Velasco-Ortega E, Torres-Lagares D, Velasco-Ponferrada MC, Monsalve-Guil L, Llamas-Carreras JM. Pattern of antibiotic prescription in the management of endodontic infections amongst Spanish oral surgeons. *Int Endod J.* 2010 Apr;43(4):342-50.
- Sedgley CM, Lee EH, Martin MJ, Flannagan SE. Antibiotic resistance gene transfer between *Streptococcus gordonii* and *Enterococcus faecalis* in root canals of teeth ex vivo. *J Endod.* 2008 May;34(5):570-4.
- Sanches ML, Jones RN. E test, an antimicrobial susceptibility testing method with broad clinical and epidemiologic application. *Antimicrob Newslett.* 1993;8(1):1-7.
- Möller AJ. Microbiological examination of root canals and periapical tissues of human teeth. *Methodological studies. Odontol Tidskr.* 1966 Dec 20;74(5):Suppl:1-380.
- Citron DM, Ostovari MI, Karlsson A, Goldstein EJ. Evaluation of the E test for susceptibility testing of anaerobic bacteria. *J Clin Microbiol.* 1991 Oct;29(10):2197-203.
- National Committee for Clinical Laboratory Standards. Performance standards for antimicrobial disk susceptibility tests. Approved standard M2–A9. Wayne (PA): Clinical and Laboratory Standards Institute; 2008.
- Sedgley CM, Molander A, Flannagan SE, Nagel AC, Appelbe OK, Clewell DB, et al. Virulence, phenotype and genotype characteristics of endodontic *Enterococcus* spp. *Oral Microbiol Immunol.* 2005 Feb;20(1):10-9.
- Skucaite N, Peciuliene V, Vitkauskienė A, Machiulskienė V. Susceptibility of endodontic pathogens to antibiotics in patients with symptomatic apical periodontitis. *J Endod.* 2010 Oct;36(10):1611-6.
- Ørstavik D, Haapasalo M. Disinfection by endodontic irrigants and dressings of experimentally infected dentinal tubules. *Endod Dent Traumatol.* 1990 Aug;6(4):142-9.

20. Evans M, Davies JK, Sundqvist G, Figdor D. Mechanisms involved in the resistance of *Enterococcus faecalis* to calcium hydroxide. *Int Endod J*. 2002 Mar;35(3):221-8. Heath CH, Blackmor TK, Gordon DL. Emerging resistance in *Enterococcus* spp. *Med J Aust*. 1996 Jan 15;164(2):116-20.
21. Coleri A, Cokmus C, Ozcan B, Akcelik M, Tukul C. Determination of antibiotic resistance and resistance plasmids of clinical *Enterococcus* species. *J Gen Appl Microbiol*. 2004 Aug;50(4):213-9.
22. Lee EW, Huda MN, Kuroda T, Mizushima T, Tsuchiya T. EfrAB, an ABC multidrug efflux pump in *Enterococcus faecalis*. *Antimicrob Agents Chemother*. 2003 Dec;47(12):3733-8.
23. Dahlén G, Samuelsson W, Molander A, Reit C. Identification and antimicrobial susceptibility of enterococci isolated from the root canal. *Oral Microbiol Immunol*. 2000 Oct;15(5):309-12.
24. Schirrmeister JF, Liebenow AL, Braun G, Wittmer A, Hellwig E, Al-Ahmad A. Detection and eradication of microorganisms in root-filled teeth associated with periradicular lesions: an in vivo study. *J Endod*. 2007 May;33(5):536-40.
25. Spratt DA. Significance of bacterial identification by molecular biology methods. *Endod Topics*. 2004;9(1):5-14.
26. Gomes BP, Pinheiro ET, Sousa EL, Jacinto RC, Zaia AA, Ferraz CC, et al. *Enterococcus faecalis* in dental root canals detected by culture and by polymerase chain reaction analysis. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2006 Aug;102(2):247-53.
27. Sedgley CM, Lennan SL, Clewell DB. Prevalence, phenotype and genotype of oral enterococci. *Oral Microbiol Immunol*. 2004 Apr;19(2):95-101.
28. Kuriyama T, Karasawa T, Nakagawa K, Saiki Y, Yamamoto E, Nakamura S. Bacteriologic features and antimicrobial susceptibility in isolates from orofacial odontogenic infections. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2000 Nov;90(5):600-8.
29. Baumgartner JC, Xia T. Antibiotic susceptibility of bacteria associated with endodontic abscesses. *J Endod*. 2003 Jan;29(1):44-7.
30. Khemaleelakul S, Baumgartner JC, Pruksakorn S. Identification of bacteria in acute endodontic infections and their antimicrobial susceptibility. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2002 Dec;94(6):746-55.
31. Yingling NM, Byrne BE, Hartwell GR. Antibiotic use by members of the American Association of Endodontists in the year 2000: report of a national survey. *J Endod*. 2002 May;28(5):396-404.
32. Pallasch TJ. How to use antibiotics effectively. *J Calif Dent Assoc*. 1993 Feb;21(2):46-50.

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