Diversity of *Enterococcus faecalis* Genotypes from Multiple Oral Sites Associated with Endodontic Failure Using Repetitive Sequence-based Polymerase Chain Reaction and Arbitrarily Primed Polymerase Chain Reaction

Maraísa G. Delboni, DDS, MSc, PhD,[*†] *Brenda P.F.A. Gomes, DDS, MSc, PhD,*[†] *Priscila A. Francisco, DDS,*[†] *Fabrício B. Teixeira, DDS, MSc, PhD,*[‡] *and David Drake, DDS, MSc, PhD*[‡]

Abstract

Introduction: The aim of this study was to evaluate the diversity and similarity of *Enterococcus faecalis* genotype isolates from multiple oral sites using repetitive sequence-based polymerase chain reaction and arbitrarily primed polymerase chain reaction (AP-PCR). **Methods:** Forty-two endodontically treated teeth with apical periodontitis were selected. A total of 126 microbial samples were collected from 3 different sites (saliva, pulp chamber, and root canals, all n = 42) during the nonsurgical retreatment procedures. After growth on m-Enterococcus agar, the colonies were isolated, characterized as gram-positive catalase negative cocci, and identified using an API 20 Strep kit (bioMérieux, Marcy-l'Etoile, France). Seventy-four colonies from 10 patients were confirmed as *E. faecalis* by polymerase chain reaction (16S ribosomal RNA). Repetitive sequence-based polymerase chain reactions using ERIC and AP-PCR using RW3A primers were performed in all 74 colonies. Fingerprints were analyzed and separated into genotypic groups based on the Dice coefficient percentage of similarity (82% or greater) as determined by ERIC reproducibility assays involving *E. faecalis* controls. **Results:** Seven different *E. faecalis* genotypes (GTs) (GT1 = 27%, GT2 = 17.6%, GT3 = 1.3%, GT4 = 18.9%, GT5 = 9.5%, GT6 = 14.9%, and GT7 = 10.8%) were observed in different subjects and oral sites associated with endodontic failure. Remarkably, in 4 of 5 patients, the same GTs present in the infected root canals were also isolated from either the pulp chamber or the saliva samples. In particular, GT6 was detected in all 3 oral sites of patient 37. **Conclusions:** *E. faecalis* GTs isolated from saliva, the pulp chamber, and the root canal were similar using the Rep-PCR and AP-PCR methods. These findings suggest that coronal microleakage is a conceivable cause of endodontic failure. *(J Endod 2016;■:1–6)*

Key Words

Arbitrarily primed polymerase chain reaction, endodontics, *Enterococcus faecalis*, microleakage, microorganisms, repetitive sequence-based polymerase chain reaction, saliva

Significance

This study showed that the multiple *E. faecalis* genotypes present in the saliva or pulp chamber may be the same isolated from root-filled teeth with apical periodontitis. These findings suggest that coronal microleakage may be a potential cause of endodontic failure.

Conventional endodontic therapy usually fails when the treatment is performed inadequately (1). However, there are some instances in which procedures follow the highest standards and still result in nonhealing of apical periodontitis (2).

There is enough evidence in the literature that endodontic failure may take place because of the ability of some microorganisms to survive after current treatment protocols (3, 4). Also, it has been shown that these microorganisms can gain access to the root canal system either during or after treatment because of coronal microleakage (5, 6).

Enterococcus faecalis is a nonmotile, gram-positive, spherical bacterium. It can be observed singly, in pairs, or in short chains and is most often found in the large intestine of humans. It is a facultative anaerobe with a fermentative metabolism (7).

E. faecalis is listed as the first to the third leading cause of nosocomial infections. Most of these infections occur after surgery of the abdomen or a puncturing trauma but can also be linked to the increased use of intravenous lines and catheters, which are considered compromising devices. It is also responsible for urinary tract infections,

From the *College of Dentistry, Facid DeVry University, Teresina, Piauí; †Department of Restorative Dentistry, Endodontic Division, Piracicaba Dental School, State University of Campinas, Piracicaba, São Paulo, Brazil; and ‡Department of Endodontics, Dows Institute for Dental Research, College of Dentistry, University of Iowa, Iowa City, Iowa.

Address requests for reprints to Dr Brenda P.F.A. Gomes, Department of Restorative Dentistry, Endodontic Division, Piracicaba Dental School, State University of Campinas, Av Limeira 901, Bairro Areão, Piracicaba, São Paulo, Brazil 13414-903. E-mail address: bpgomes@unicamp.br

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bacteremia, endocarditis, meningitis, and foodborne disease. It can be found in wound infections and intra-abdominal abscesses, along with many other bacteria (8).

E. faecalis has been frequently recovered from the root canals of teeth with apical periodontitis (9, 10) and is implicated as the major endodontic pathogen in secondary infections (4, 11–17). The epidemiology of enterococcal infections is an important part of dealing with these multiresistant organisms.

Polymerase chain reaction (PCR)-based DNA fingerprinting of microorganisms has been developed using a wide variety of techniques and primer designs. The basis of PCR-based DNA fingerprinting is that the primers can bind to specific regions of the DNA, and when this binding occurs in the proper orientation and within an optimum distance, species- or strain-specific amplification products may be generated. Primers such as REP1R and REP2, which are derived from the repetitive extragenic palindromic sequences found primarily in gram-negative bacteria (18), have been used in the technique known as repetitive sequence-based PCR (Rep-PCR) (18, 19) for studying DNA fingerprints of many bacterial species.

Arbitrarily primed PCR (AP-PCR) is any form of PCR that uses primers of arbitrary sequence and that amplifies random but discrete sequences of chromosomal DNA. AP-PCR has been used for typing bacteria. PCR is initially performed under low stringency, and the primers bind at various sites to each strand of heat-denatured chromosomal DNA; the binding of primers occurs at "best-fit" sequences and may include mismatches (20).

Because persistent root canal infection has been suggested as 1 of the factors responsible for failure, particularly in well-treated teeth, the literature has investigated the most likely sources of coronal leakage by detection of *Enterococcus* spp. Although unsatisfactory coronal restorations are associated with lower rates of complete apical healing (21), the biological aspects of coronal microleakage have not been clearly investigated. It is critical to evaluate this species occurrence in the patient's saliva and under the restorative materials compared with the root canal samples of previously endodontically treated teeth.

The literature has indicated that the overall success rate of nonsurgical retreatment of teeth with apical periodontitis is 76.7% (22). This poor prognosis may be associated with difficulties in the elimination of the particular resistant microbiota, particularly *E. faecalis*.

It would be of interest to know *in vivo* the pathways of the root canal reinfection, starting from the saliva and then entering the internal surface of the pulp chamber and spreading through the root-filled canal system. Therefore, the goals of this study were

1. To identify and locate *E. faecalis* in each clinical case using *in vivo* sampling protocols and culture techniques;
2. To analyze DNA fingerprints from the saliva, pulp chambers, and root canals of endodontically treated teeth with apical periodontitis using 2 molecular methods; and
3. To determine the diversity and similarity of genotypes present within the oral cavity.

The tracking using their genotypes may prove by DNA fingerprinting that saliva could really be a potential source of *E. faecalis* that could invade the root canal via coronal microleakage.

Materials and Methods

Clinical Examination

All the protocols and the specimen collection methods for this investigation were approved by the institutional review board of the Piracicaba Dental School, State University of Campinas, Piracicaba, São Paulo, Brazil. Informed consent forms were provided and signed by the patients involved in the study. Forty-two teeth of 20 patients were included. The patients were screened and scheduled to receive nonsurgical endodontic retreatment because of radiographic evidence of apical periodontitis. Medical history and dental records were obtained.

The inclusion criteria used to select teeth and patients were as follows:

1. Previously endodontically treated teeth with radiographic evidence of apical periodontitis
2. Previously endodontically treated teeth with persistence of symptoms
3. No evidence of longitudinal fractures
4. No antibiotic therapy provided 3 months before the consultation visits
5. Absence of systemic disease and periodontal disease

Sampling Procedure

Microbial sampling of the saliva followed by the pulp chamber and the root canals was collected during the first dental appointment. Aseptic techniques were used throughout the nonsurgical root canal retreatment and before sample collection. The sampling protocols used in this study were previously detailed by Gomes et al (4, 23) and were adapted for this study as described.

Saliva Sampling

Patients were informed to not brush their teeth or eat anything 2 hours before the appointment. One milliliter of whole saliva was collected from the patients and placed in a sterile plastic receptacle in the beginning of each section (24). Forty-two saliva samples were collected from 20 patients. The number of saliva samples was related to the number of teeth treated per patient, even if their saliva samples had already been previously collected.

Pulp Chamber Sampling

Rubber dam isolation was placed in all teeth. The external surfaces of the crowns were then disinfected with 30% hydrogen peroxide followed by 2.5% sodium hypochlorite. These solutions were inactivated with 5% sodium thiosulfate in order to avoid interference with bacteriological sampling. Afterward, the internal surfaces of the coronal restorations or posts were collected using a sterile swab.

Root Canal Sampling

The access cavities were disinfected using the same protocol as described previously. The root canal filling materials were then removed with Gates-Glidden drills and K-files (Dentsply Maillefer, Ballaigues, Switzerland) without the use of endodontic solvents. Sampling was performed during all retreatment procedures. Not only the gutta-percha but also debris was collected. All the canals were kept moist with sterile saline solution before the final sampling was taken with sterile paper points. The paper points were placed to the working length of the canals determined by an electronic apex locator (Novapex; Forum Engineering Technologies, Rishon Lezion, Israel). The sterile paper points were maintained inside the root canals for 60 seconds in order to absorb as much fluid as possible.

A total of 126 microbial samples were collected, 42 from each of the 3 sites (ie, the saliva, pulp chamber, and root canal of previously treated teeth), during the retreatment procedures. The microbial evaluation of the single-canal ecological environment was evaluated. The root with radiographic evidence of apical periodontitis was selected in teeth with multiple canals. In teeth in which all the roots showed periapical lesions, the largest canals were then chosen.