New Bacterial Composition in Primary and Persistent/Secondary Endodontic Infections with Respect to Clinical and Radiographic Findings

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Abstract

Introduction: The aim of the present study was to analyze the microbiota of primary and secondary/persistent endodontic infections of patients undergoing endodontic treatment with respect to clinical and radiographic findings. **Methods:** Samples from the root canals of 21 German patients were taken using 3 sequential sterile paper points. In the case of a root canal filling, gutta-percha was removed with sterile files, and samples were taken using sterile paper points. The samples were plated, and microorganisms were then isolated and identified morphologically by biochemical analysis and sequencing the 16S rRNA genes of isolated microorganisms. **Results:** In 12 of 21 root canals, 33 different species could be isolated. Six (50%) of the cases with isolated microorganisms were primary, and 6 (50%) cases were endodontic infections associated with root-filled teeth. Twelve of the isolated species were facultative anaerobic and 21 obligate anaerobic. Monomicrobial infections were found for *Enterococcus faecalis* and *Actinomyces viscosus*. *E. faecalis* was most frequently isolated in secondary endodontic infections (33%). *Moraxella osloensis* was isolated from a secondary endodontic infection that had an insufficient root canal filling accompanied by a mild sensation of pain. A new bacterial composition compromising *Atopobium rimae*, *Anaerococcus prevotii*, *Pseudoramibacter alactolyticus*, *Dialister invisus*, and *Fusobacterium nucleatum* was recovered from teeth with chronic apical abscesses. **Conclusions:** New bacterial combinations were found and correlated to clinical and radiographic findings, particularly to chronic apical abscesses. *M. osloensis* was detected in root canals for the second time and only in German patients. *(J Endod 2014;40:670–677)*

Key Words

Apical periodontitis, dental trauma, endodontic infection, endodontic microorganisms, root canal treatment

The pulp is a very important tissue in teeth and serves several functions (eg, dentine formation and immune response). Microorganisms can enter the pulp cavity through a deep carious lesion, through cracks in fillings or cracks in the tooth, through blood vessels from the apical region, or from the periodontium. Invasion by bacteria will lead to necrosis of the pulp. In this case, a root canal treatment is indicated. The objectives of root canal treatment are adequate cleaning and shaping of the root canal system and elimination of all portals of entry between the root canal and the periodontium by filling the root canal system using a biologically inert and physically stable material (1). The goal of chemomechanical preparation of root canals is to eliminate the pulp tissue and all microorganisms in the pulp cavity. Because of the complex anatomy of the pulp with its ramifications, isthmi, apical deltas, and accessory canals, it is hard to assess the pulp cavity completely. In the literature, different estimates about the prognosis of root canal treatment range from about 62%–98% depending on pre-, intra-, and postoperative parameters (2). The main cause of endodontic failure is the persistence of microorganisms in the root canal system. Recontamination of the root canal system by insufficient coronal restorations or root canal fillings can also lead to endodontic failure (3, 4).

It is known that microorganisms can gain resistance against disinfecting agents and endodontic medicaments, increasing the challenge to completely eliminate them during root canal treatment (5, 6). In endodontic infections, gram-positive and gram-negative species could be detected, whereas obligate anaerobes dominated (7, 8). During infection, these species can form biofilms, making the microorganisms up to 1,000 times harder to eliminate using disinfecting agents (9). Secondary endodontic infections are dominated by gram-positive species and facultative and obligate anaerobes (10). *Enterococcus faecalis* has gained attention by its ability to persist after root canal treatment and has been isolated from both primary and secondary infections although it has been recovered most frequently from secondary/persistent infections (11). The aim of the present study was to analyze the microbiota of primary and secondary/persistent endodontic infections of patients undergoing endodontic treatment with respect to both clinical and radiographic findings. So far, only 40 German patients with post-treatment apical periodontitis have been studied. However, there is

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0099-2399/$ - see front matter

http://dx.doi.org/10.1016/j.joen.2013.10.005

no previous study correlating isolated microorganisms with clinical and radiographic findings in a German population.

Materials and Methods

Patient Selection

Twenty-one patients (25–75 years of age) who had been referred to the University of Freiburg–Medical Center, Freiburg, Germany, for endodontic treatment took part in this study. Patients were excluded from the study if they showed 1 of the following criteria: severe systemic diseases, pregnancy or lactation, use of any antibiotics within the past 30 days, participation in another clinical study during the previous 3 months, teeth that could not be isolated with a rubber dam, teeth without a coronal seal or with restorations with leaky margins, or teeth with large intraradicular posts. Before the beginning of the study, the patients gave their written informed consent to the study protocol, which was reviewed and approved by the ethics committee of the University of Freiburg (140/09). For each patient, a preassembled data sheet was filled out. For primary endodontic infections, clinical and radiographic findings were assessed. Clinical parameters included the presence of a tissue swelling associated with the tooth, pulp vitality via cold testing using CO_2, percussion sensitivity, and the quality of coronal restoration. The coronal restoration was examined for cracks/fracture lines, caries, or other defects. Radiographic findings included the presence of periradicular radiolucencies and the diameter of periradicular radiolucency; signs of periapical, external, or internal radicular resorption; caries; and cracks/fracture lines of the root. In case of secondary infections, the radiographic quality of the root canal filling (length and homogeneity) was evaluated. Additionally, if the tooth was symptomatic, the following parameters were recorded: onset of pain (time and duration) and quality of pain (throbbing, stabbing, dull, and so on). With all these diagnostic findings a diagnosis of the tooth was made. Table 1 constitutes an overview of primary and secondary/persistent endodontic infections examined.

Sampling Procedure

All samples were taken by the same endodontic specialist under strictly aseptic conditions. Each tooth was isolated with a rubber dam. The tooth and the surrounding area were then disinfected with 30% hydrogen peroxide (H_2O_2) and decontaminated with a 3% sodium hypochlorite (NaOCl) solution. Endodontic access was achieved with a sterile high-speed carbide bur until the pulp cavity or the root filling was exposed. After access was achieved, the tooth and the adjacent rubber dam were disinfected with 30% H_2O_2 and 3% NaOCl again. NaOCl was then inactivated by swabbing the cavity with 5% sodium thiosulfate solution. To assess the efficacy of the disinfection procedure, 2 sequential sterile foam pellets were moistened in sterile saline solution (0.9% NaCl) and used to swab the access cavity and the tooth surface. They were then transferred into a vial containing 0.75 mL reduced transport fluid (RTF) (12) and sampled for bacterial growth (quality control). If growth occurred, the patient sample was disqualified from the study.

The working length was established radiographically using an electronic apex locator (Raypex 5; VDW, Munich, Germany). The canal was enlarged up to 1–2 mm from the radiographic apex with a minimum size of ISO 35 nickel-titanium K-type file. Teeth that could not be instrumented to this length were excluded from the study. No solvent was used at any time. Approximately 40 μL sterile saline solution was introduced to the canal with a sterile syringe. A sterile Hedstrom file corresponding to the last instrument used was placed in the canal at the working length and pumped vigorously with minimal reaming motion to disrupt the canal contents. Three sequential sterile paper points were placed at the working length and used to soak up the fluid in the canal. Each paper point was retained in position for 1 minute and transferred into a vial containing 0.75 mL RTF.

In cases in which the root canal treatment had been previously initiated, the root canals contained an intracanal dressing. The intracanal dressing was either a calcium hydroxide slurry or Ledermix (RIEMSER Pharma GmbH, Greifswald, Germany). The intracanal dressing was removed using 5 mL sterile Ringer solution and sampled as described previously.

In root-filled teeth, coronal gutta-percha was removed by using Gates-Glidden drills; the apical material was retrieved with Hedstrom files and, if possible, was then transferred to a sterile tube containing 0.75 mL RTF. After removing gutta-percha, the working length was determined radiographically using an electronic apex locator (Raypex 5; VDW, Munich, Germany). Root canals were enlarged apically and sampled as described earlier. Finally, conventional root canal treatment was finished after root canal disinfection, and the root canal system was filled using the vertical compaction technique.

Isolation of Microorganisms

The microorganisms originating from the samples were isolated and identified morphologically by biochemical analysis and sequencing the 16S rRNA genes of the pure isolates as described in earlier studies (12, 13). The undiluted samples were plated. This corresponds to a dilution of 10^{-1} of the original root canal bacteria referring to sampling using 3 paper points and stored in 0.75 mL RTF. Additionally, serial dilutions (10^{-1}–10^{-3}) were prepared in peptone yeast medium containing cysteine hydrochloride. Each dilution was plated on yeast-cysteine blood agar plates, Columbia blood agar plates, and bile esculin plates. The yeast-cysteine blood agar plates were used to cultivate anaerobic bacteria at 37°C for 10 days (anaerobic jar, Anaerocult A; Merck, Darmstadt, Germany). The Columbia blood agar plates were incubated at 37°C and 5%–10% CO_2 atmosphere for 3 days to cultivate facultative anaerobic bacteria. The bile esculin agar plates were incubated at 37°C and 5%–10% CO_2 atmosphere for 3–5 days to cultivate *E. faecalis*. The colonies were differentiated by morphology, color, size, and hemolytic reaction, and colonies were counted to

TABLE 1. Analyzed Cases and Diagnoses of Endodontic Infections

Type of infection	Diagnosis		Cases
Primary infection	Asymptomatic apical periodontitis		1
Primary infection	Crown fracture with exposed pulp		2
Primary infection	After intracanal dressing	Ledermix	2
		Calcium hydroxide	4
Primary infection	Chronic apical abscess		1
Primary infection	Symptomatic apical periodontitis		1
Secondary/persistent infection	Insufficient root canal filling		1
Secondary/persistent infection	Asymptomatic apical periodontitis with insufficient root canal filling		9