Microbial Diversity in Persistent Root Canal Infections Investigated by Checkerboard DNA-DNA Hybridization

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Abstract

Introduction: The aim of the present study was to investigate the composition of the root canal microbiota in endodontic failures in order to identify and quantify these microorganisms. **Methods:** Microbiological samples were taken from 36 root canals with persistent endodontic infection. The presence, levels, and proportions of 79 bacterial species were determined by checkerboard DNA-DNA hybridization. The Pearson correlation coefficient was used to investigate the relations between bacterial counts and clinical conditions ($P \leq .05$). **Results:** *Enterococcus faecium* (36%), *Streptococcus epidermidis* (36%), *Eubacterium saburreum* (28%), *Parvimonas micra* (28%), *Streptococcus sanguis* (28%), *Capnocytophaga sputigena* (28%), *Leptotrichia buccalis* (28%), *Enterococcus faecalis* (28%), and *Staphylococcus warneri* (28%) were the most prevalent species; and there was a low prevalence of *Treponema socranskii* (3%), *Fusobacterium periodonticum* (3%), *Capnocytophaga gingivalis* (3%), and *Spiroplasma ixodetis* (3%). The highest mean levels were found for the following species: *E. faecium*, *Dialister pneumosintes*, *Staphylococcus epidermidis* and *Helicobacter pylori*. There was a statistically significant difference between the levels of gram-negative species and gram-positive species (13.5×10^5 vs 6.5×10^5, respectively). A positive correlation was found between the area of the periapical lesion and the levels of gram-negative and rod species ($P < .05$). **Conclusions:** The microbiota from teeth with persistent apical periodontitis presents a mixed and complex profile, hosting *E. faecium* and *S. epidermidis* as the most highly prevalent species. No correlation was found between any of the species tested and clinical findings; however, periapical lesions with the largest areas presented higher counts of gram-negative and rod species. *(J Endod 2014;40:899–906)*

Key Words

Apical periodontitis, bacteria, checkerboard DNA-DNA hybridization, persistent infection

The persistence of microorganisms plays a significant role in endodontic treatment failures (1), which are clinically determined based on radiographic follow-up (eg, appearance, persistence, or increase in size of a periapical lesion) and signs and symptoms of endodontically treated teeth (2–4). Although several factors might be involved with endodontic treatment failure, this usually results from the presence of bacteria in the apical portion of the root canal (1–3, 5). The absence of coronal sealing, microleakage, failures in chemomechanical preparation, and limit and quality of root filling favor the survival of microorganisms after endodontic treatment or reinfection of the canal and may lead to endodontic treatment failure (4–6). There is great variation in the composition of the microbiota associated with endodontic failure and the levels of the bacterial species detected in root canals, which may occur as a result of the different diagnostic techniques used. (7–11). This microbiota is mainly composed of facultative anaerobic gram-positive species. Enterococcus is the genus most frequently isolated, and *Enterococcus faecalis* is the species more commonly found in these lesions (6–10). However, recent studies have questioned the hypothesis that *E. faecalis* is the main species associated with endodontic failures (4, 12–17).

In the 1990s, checkerboard DNA-DNA hybridization emerged as an effective and precise method to identify and quantify oral microorganisms (18, 19). This technique is able to determine the presence and levels of up to 40 bacterial species in 28 samples per test. It has the advantages of not requiring viable microorganisms in order to identify them, thus allowing the detection of bacterial species that are difficult to cultivate. In addition, the method is easy to handle and cost-effective (18, 19). These features allowed the evaluation of 79 bacterial species in a sample consisting of 36 teeth with persistent endodontic infections.

Information about the bacterial diversity in teeth with refractory root canal infection might help to delineate better treatment strategies for eradicating the microorganisms associated with periradicular lesions (3, 4). Thus, the aim of this study was to investigate the microbiota of endodontic treatment failure-related cases and to correlate these findings with clinical symptoms.

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Materials and Methods

Subject Population and Inclusion and Exclusion Criteria

Patients referred to the Dental Service of the Military Police of the State of Rio de Janeiro, Rio de Janeiro, Brazil, requiring nonsurgical endodontic retreatment participated in this study. This work was approved by the Ethics Committee of the Pedro Ernesto University Hospital, Rio de Janeiro, Brazil. All patients were informed about the nature and goals of the study and signed a consent form before entering the study.

For this study, 36 patients were selected who presented at least 1 tooth that was endontically treated at least 1 year previously and showed radiographic evidence of a periapical lesion. The patient was excluded if the tooth presented advanced mobility, periodontal pockets >4 mm, intracanal posts, or radicular fractures. Patients who had received antibiotics, corticosteroids, or systemic anti-inflammatory drugs in the last 6 months or those who presented with systemic conditions that needed antibiotic coverage for routine dental therapy or systemic diseases were also excluded from the study.

Clinical Monitoring

The clinical symptoms, tooth type, presence or absence of permanent coronal restoration, cavity, sinus tract, swelling, pain on percussion, mobility, periodontal parameters, presence or absence of exudates, and the radiographic quality of root canal filling were recorded for each patient (Table 1). The preoperative periapical radiographs were scanned, and the area of interest was selected and subsequently calculated using the software ImageTool (UTHSCSA, San Antonio, TX).

Microbiological Monitoring

Sample Collection. Samples were collected under strict aseptic conditions as previously described by Sassone et al (20). Briefly, the tooth was cleaned with pumice and isolated with a rubber dam. After isolation, the tooth and the rubber dam were cleaned with 3% hydrogen peroxide, disinfected with 5.25% sodium hypochlorite, and subsequently inactivated with 5% sterile sodium thiosulphate. Coronal access was performed with sterile burs without water. The pulp chamber and the operating field were again disinfected with 5.25% sodium hypochlorite and subsequently inactivated with 5% sodium thiosulphate. Disinfection of the operating field was checked by swabbing the access cavity with a sterile cotton pellet, which was incubated in a blood agar plate at 37°C under aerobic and anaerobic conditions, serving as the control. The root canal filling was removed with Gates-Glidden drills and endodontic files without the use of chemical solvents. Irrigation with sterile saline solution was used to remove the remaining filling material and moisten the root canal before sample collection.

Root canal samples were taken as follows: a #15 H-type file (Dentsply Maillefer, Ballaigues, Switzerland) with the handle cut off was introduced up to 1 mm short of the apical foramen, and a slight grinding movement was made. Subsequently, 2 sterile paper points were consecutively introduced into the canal at the same level and were used to soak up the fluid in the canal. Each paper point remained

TABLE 1. Preclinical Data

Case	Tooth	Sex	Age	Cavity	Size of lesion (area mm^2)	Radiographic quality of canal filling	Quality of coronal restoration
1	21	Female	43	Absent	4.90	Poor	Acceptable
2	21	Male	42	Absent	7.77	Poor	Acceptable
3	24	Female	38	Absent	2.04	Poor	Acceptable
4	23	Male	32	Present	6.64	Poor	Poor
5	25	Male	40	Absent	3.77	Poor	Poor
6	22	Male	52	Absent	11.24	Poor	Acceptable
7	46	Female	35	Absent	0.79	Acceptable	Acceptable
8	11	Female	57	Absent	1.06	Poor	Acceptable
9	21	Female	47	Absent	8.88	Poor	Poor
10	25	Female	44	Absent	8.66	Acceptable	Acceptable
11	21	Female	34	Absent	11.10	Poor	Poor
12	11	Male	48	Absent	18.36	Poor	Acceptable
13	24	Female	44	Absent	2.91	Poor	Acceptable
14	15	Female	36	Absent	7.19	Poor	Poor
15	11	Female	48	Absent	50.95	Poor	Acceptable
16	25	Female	27	Present	3.70	Poor	Poor
17	15	Female	58	Absent	1.50	Poor	Poor
18	22	Female	31	Absent	17.39	Poor	Acceptable
19	21	Female	42	Absent	22.95	Poor	Acceptable
20	45	Male	51	Absent	0.87	Poor	Poor
21	36	Male	34	Absent	9.19	Acceptable	Acceptable
22	35	Male	44	Present	0.88	Poor	Poor
23	11	Male	66	Absent	20.66	Acceptable	Acceptable
24	14	Female	63	Absent	0.82	Acceptable	Poor
25	11	Male	43	Absent	7.12	Acceptable	Acceptable
26	24	Male	43	Absent	1.08	Poor	Poor
27	11	Male	38	Absent	1.29	Poor	Acceptable
28	45	Male	49	Present	2.00	Poor	Poor
29	41	Female	43	Absent	1.59	Acceptable	Acceptable
30	15	Female	31	Absent	13.52	Acceptable	Acceptable
31	11	Male	38	Absent	50.22	Acceptable	Acceptable
32	12	Female	37	Absent	20.00	Acceptable	Acceptable
33	22	Male	38	Absent	47.80	Poor	Acceptable
34	14	Female	37	Absent	10.93	Poor	Acceptable
35	22	Male	28	Absent	2.41	Poor	Poor
36	11	Female	40	Absent	8.63	Acceptable	Acceptable